

Systematic Review

# The Prevalence of *TET2* Gene Mutations in Patients with *BCR-ABL*-Negative Myeloproliferative Neoplasms (MPN): A Systematic Review and Meta-Analysis

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**Simple Summary:** Many molecular biology techniques have been widely used to study the pathogenesis of different diseases, particularly haematologic malignancies which are generally caused by abnormalities in the genome. *TET2* gene is one of the commonly found mutated genes in *BCR-ABL*-negative myeloproliferative neoplasms. However, the prevalence of *TET2* gene mutations in the disease remains unclear. Therefore, this study aims to estimate the prevalence of *TET2* gene mutations in myeloproliferative neoplasms. The findings may be helpful for future research, diagnoses and the identification of better therapeutic strategies to manage the diseases.

**Abstract:** Multiple recurrent somatic mutations have recently been identified in association with myeloproliferative neoplasms (MPN). This meta-analysis aims to assess the pooled prevalence of *TET2* gene mutations among patients with MPN. Six databases (PubMed, Scopus, ScienceDirect, Google Scholar, Web of Science and Embase) were searched for relevant studies from inception till September 2020, without language restrictions. The eligibility criteria included *BCR-ABL*-negative MPN adults with *TET2* gene mutations. A random-effects model was used to estimate the pooled prevalence with 95% confidence intervals (CIs). Subgroup analyses explored results among different continents and countries, WHO diagnostic criteria, screening methods and types of MF. Quality assessment was undertaken using the Joanna Briggs Institute critical appraisal tool. The study was registered with PROSPERO (CRD42020212223). Thirty-five studies were included ( $n = 5121$ , 47.1% female). Overall, the pooled prevalence of *TET2* gene mutations in MPN patients was 15.5% (95% CI: 12.1–19.0%,  $I^2 = 94%$ ). Regional differences explained a substantial amount of heterogeneity. The prevalence of *TET2* gene mutations among the three subtypes PV, ET and MF were 16.8%, 9.8% and 15.7%, respectively. The quality of the included studies was determined to be moderate–high among 83% of the included studies. Among patients with *BCR-ABL*-negative MPN, the overall prevalence of *TET2* gene mutations was 15.5%.

**Keywords:** essential thrombocythaemia; meta-analysis; myelofibrosis; myeloproliferative neoplasms; polycythaemia vera; *TET2*

## 1. Introduction

Myeloproliferative neoplasms (MPN) are a group of rare blood cancers characterised by the clonal expansion of a large number of abnormal haematopoietic stem cells. Classic Philadelphia-negative (*BCR-ABL*-negative) MPN can be divided into three categories: (i)

polycythaemia vera (PV), (ii) essential thrombocythaemia (ET) and (iii) primary myelofibrosis (PMF). MPN can transform into acute myeloid leukaemia (AML) and may be associated with an elevated risk of thrombotic and haemorrhagic events [1,2]. Thrombosis and haemorrhage are the major causes of mortality and morbidity amongst patients with MPN and occur in about 34–39% of cases with PV, 10–29% with ET and 7.2–13.2% of patients with PMF [3].

Three main driver gene mutations, Janus kinase 2 (*JAK2*), Thrombopoietin receptor (*MPL*) and Calreticulin (*CALR*), have been identified in association with MPN and may have an important role in assisting the diagnosis of MPN [4]. In addition, epigenetic modification genes such as *TET2*, *ASXL1*, *DNMT3A* and *EZH2* are also commonly mutated in cases of MPN with a frequency of 1–30% [5–8].

*TET2* participates in one of the crucial steps in gene regulation, and mutations in this gene have been identified in 5–20% of people diagnosed with MPN [9]. Somatic missense mutations, somatic nonsense mutations and insertion–deletion mutations are detected in the *TET2* gene among MPN patients. All of these mutations are loss-of-function mutations. Malfunction of *TET2* protein may lead to the development of MPN and contributes to the disease progression [10,11]. However, some disagreement still exists about the relative significance of these *TET2* gene mutations to MPN. Some researchers suggest that *TET2* gene mutations are not important for MPN [12,13], whereas others have concluded that these mutations significantly contribute to their phenotype [14,15].

The prevalence of *TET2* gene mutations among MPN has not yet been established. This meta-analysis aims to estimate the prevalence of *TET2* gene mutations among all *BCR-ABL*-negative MPN and its three main subtypes.

## 2. Materials and Methods

PRISMA guidelines [16] were followed, and a study protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42020212223.

### 2.1. Data Sources and Searches

PubMed, Scopus, ScienceDirect, Google Scholar, Web of Science and Embase databases were searched from their inception till September 2020, without any language restrictions. Detailed search strategies are presented in Table S1. Any published studies or preprints with relevant data were included. Review articles, case reports and opinion articles were excluded. Data presented on websites or reported by press releases and news reports were not considered. Snowball searching was employed to review the references of included studies. Endnote X8 software was used to remove duplicate studies.

### 2.2. Study Selection

Study eligibility was determined by screening the title and abstract of the articles of interest. Two authors (Y.C.C. and M.A.I.) independently examined full-text reports of potentially relevant studies for inclusion. Any disagreements were resolved by consensus.

### 2.3. Extraction of Data

Data were independently extracted by two authors (Y.C.C. and M.A.I.). The following data were obtained from each eligible study and inserted into a customised Excel spreadsheet: author surname, publication year, study design, study location, type of MPN, number of patients with MPN, demographic characteristics of patients including age and sex, clinical characteristics of the MPN patients including haemoglobin level, leucocyte and platelet counts, the total number of mutated *ASXL1* and the screening method used to identify *TET2* gene mutations and diagnostic criteria employed for MPN diagnoses.

#### 2.4. Quality Assessment

A random-effects model was used to estimate the pooled prevalence of the *TET2* gene mutations amongst patients with MPN, including 95% confidence intervals (Cis). Two authors (Y.C.C. and M.A.I.) independently assessed the quality of included studies using the Joanna Briggs Institute critical appraisal tools [13]. Study quality was categorised into three groups: low-quality or high risk of bias, moderate quality or moderate risk of bias, and high-quality or low risk of bias with overall scores of <50%, 50–69% and  $\geq 70\%$ , respectively [17].

#### 2.5. Publication Bias

Funnel plots presenting estimates of prevalence plotted against standard error measures were used to assess the likelihood of publication bias. When a minimum of 10 studies were available, an Egger's test was conducted to assess publication bias based on funnel plot asymmetry.

#### 2.6. Data Synthesis and Sensitivity Analysis

The  $I^2$  statistic was used to gauge the heterogeneity between studies, with  $I^2 > 75\%$  indicating substantial heterogeneity. The statistical significance of study heterogeneity was also assessed using Cochran's Q test;  $p < 0.05$  was considered statistically heterogeneous. To help identify the outlier studies and the sources of heterogeneity, a Galbraith plot was constructed. Prevalence estimates were explored with sensitivity analyses. Three strategies were followed for these analyses: (i) studies with small sample sizes (<100) were excluded, (ii) low-quality studies were excluded and (iii) outlier studies were excluded. In each case, the results were then compared to the overall prevalence estimate. Metaprop codes in meta (version 4.15-1) and metaphor (version 2.4-0) packages of R (version 3.6.3) and RStudio (version 1.3.1093) were used for the analyses and graphs [18].

### 3. Results

#### 3.1. Study Selection

The search generated 758 potentially relevant studies. After excluding 558 studies (duplicates  $n = 450$ ; review articles  $n = 67$ ; non-human studies  $n = 31$ ; and case reports,  $n = 10$ ), 200 full-text studies were examined and 35 studies met the inclusion criteria and were included in the review (Figure 1).

#### 3.2. Characteristics of Included Studies

Table 1 presents the main characteristics of the 35 included studies. Overall, the meta-analysis includes data from 5121 patients with MPN (47.1% female). Study participants were located in four continents: Europe ( $n = 1758$ ), Asia ( $n = 301$ ), North America ( $n = 3019$ ) and Australia ( $n = 43$ ), and 12 countries (Australia, China, Denmark, France, Germany, Italy, Korea, Spain, Sweden, Switzerland, the United Kingdom and the United States of America). Most (27/35) studies used a version of the World Health Organization classification and diagnostic criteria (WHO 2016 7 studies, WHO 2008 17 studies and WHO 2001 3 studies) to determine MPN diagnoses. Many studies confirmed *TET2* gene mutations with either next-generation sequencing (NGS) or Sanger sequencing, which have higher sensitivity in detecting mutations compared with other methods, such as high-resolution melting (HRM) analysis [19]. One study was published in Chinese Mandarin and was translated into English (Y.C.C.).

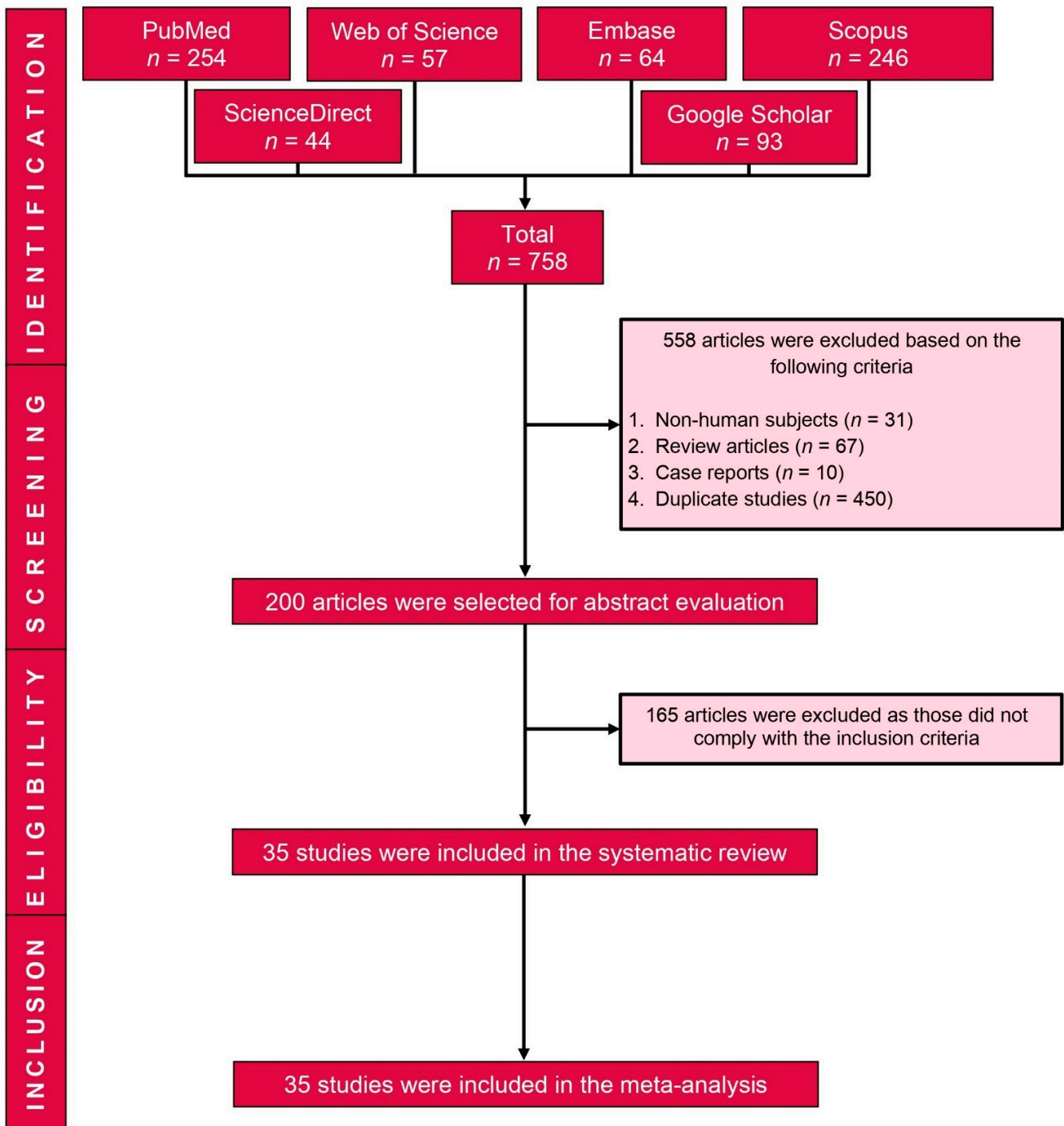


Figure 1. PRISMA flow diagram of study selection.

**Table 1.** Major characteristics of the included studies.

No	Study ID [References]	Study Design	Country	Type of MPN	Total Number of MPN Patients (Female)	Age (Years) [Mean $\pm$ SD/Median (IQR)/Range]	Haemoglobin (g/dL) [Mean $\pm$ SD/Median (IQR)/Range]	Leucocyte Count ( $10^9/L$ ) [Mean $\pm$ SD/Range/Median (IQR)]	Platelet Count ( $10^9/L$ ) [Mean $\pm$ SD/Range/Median (IQR)]	Total Number of Mutated ASXL1 (%)	Screening Method for TET2 Gene Mutations	Diagnostic Criteria
1	Andreasson 2020 [20]	Cross-sectional	Sweden	PV	85 (41)	71.0 (37.0–94.0)	NR	NR	NR	8.2	NGS	2008 WHO
2	Barraco 2017 [21]	Cross-sectional	USA	PV	267 (125)	64.0 (17.0–94.0)	18.0 (14.8–24.3)	11.5 (4.3–59.3)	439.0 (37.0–2747.0)	8.1	NR	2016 WHO
3	Bartels 2019 [22]	Case-control	Germany	MF	104 (53)	NR	NR	NR	NR	9.6	NGS	2016 WHO
4	Brecqueville 2012 [23]	Cross-sectional	France	PV, ET & MF	127 (57)	NR (29.0–97.0)	NR	NR	NR	11.0	SS	2008 WHO
5	Brecqueville 2014 [24]	Cross-sectional	France	MF	68 (NR)	69.0 (30.0–86.0)	11.4 (5.8–17.8)	8.9 (1.3–120.0)	256.0 (5.0–1188.0)	26.5	SS	2008 WHO
6	Carbuccia 2009 [25]	Cross-sectional	France	PV, ET & MF	NR	NR	NR	NR	NR	7.3	SS	NR
7	Cerquozzi 2017 [26]	Cross-sectional	USA	PV	587 (302)	60.0 (17.0–94.0)	NR	NR	476.0 (41.0–2747.0)	10.5	NGS	2016 WHO, ELN
8	Delhommeau 2009 [11]	Cross-sectional	France	PV, ET & MF	203 (41)	NR	NR	NR	NR	NR	SS, SNP array, CGH	2001 WHO
9	Delic 2016 [27]	Cross-sectional	Germany	PV, ET & MF	100 (NR)	69.0 (28.0–87.0)	NR	NR	NR	21.0	NGS	2008 WHO
10	Gill 2018 [28]	Cross-sectional	China	MF	101 (39)	60.0 (26.0–89.0)	10.3 (3.0–18.5)	12.1 (1.5–177.4)	344.0 (19.0–1720.0)	30.7	NGS	2016 WHO, IWG-MRT
11	Guglielmelli 2011 [29]	Cross-sectional	Italy	MF	518 (303)	NR	NR	NR	NR	22.2	HRM	2008 WHO, IWG-MRT
12	Ha 2014 [14]	Cross-sectional	Korea	PV, ET & MF	99 (50)	63.7 $\pm$ 13.0	13.7 $\pm$ 3.8	16.5 $\pm$ 15.4	825.4 $\pm$ 490.0	NR	SS, SNP array, CGH	2008 WHO
13	Huang 2020 [30]	Cross-sectional	China	PV, ET & MF	65 (32)	62.0 (NR)	NR	NR	NR	10.8	NGS	2016 WHO
14	Hussein 2010 [31]	Cross-sectional	USA	PV, ET & MF	199 (96)	58.0 (19.0–93.0)	NR	NR	NR	NR	NGS	2001 WHO
15	Kröger 2017 [32]	Cross-sectional	Germany	MF	169 (73)	58.0 (18.0–75.0)	NR	NR	NR	29.0	SS	NR

Table 1. Cont.

No	Study ID [References]	Study Design	Country	Type of MPN	Total Number of MPN Patients (Female)	Age (Years) [Mean $\pm$ SD/Median (IQR)/Range]	Haemoglobin (g/dL) [Mean $\pm$ SD/Median (IQR)/Range]	Leucocyte Count ( $10^9$ /L) [Mean $\pm$ SD/Range/Median (IQR)]	Platelet Count ( $10^9$ /L) [Mean $\pm$ SD/Range/Median (IQR)]	Total Number of Mutated ASXL1 (%)	Screening Method for TET2 Gene Mutations	Diagnostic Criteria
16	Leibundgut 2020 [33]	Cross-sectional	Switzerland	ET	18 (10)	59.5 (21.0–83.0)	NR	7.8 (3.0–14.6)	788.0 (521.0–1359.0)	11.1	NGS	2016 WHO
17	Magor 2016 [34]	Cross-sectional	Australia	PV, ET & MF	43 (16)	61.0 (24.0–91.0)	NR	NR	NR	9.3	Targeted exon resequencing	2008 WHO
18	Martínez-Avilés 2012 [35]	Cross-sectional	Spain	PV, ET & MF	62 (43)	NR	NR	NR	NR	4.8	HRM, SS	2008 WHO
19	Nielsen 2017 [36]	Case-control	Denmark	MF	16 (3)	66.0 (52.0–80.0)	10.3 (7.9–13.4)	5.9 (2.3–64.4)	155.5 (56.0–357.0)	50.0	PCR-DGGE	NR
20	Nischal 2013 [37]	Cross-sectional	USA	PV, ET & MF	25 (14)	68.0 (54.0–72.0)	NR	NR	NR	24.0	SS	NR
21	O'Sullivan 2019 [38]	Cross-sectional	UK	ET	NR	NR	NR	NR	NR	NR	NGS	NR
22	Pardanani 2010 [39]	Cross-sectional	USA	PV, ET & MF	78 (34)	64.0 (22.0–95.0)	NR	NR	NR	NR	NGS	2008 WHO
23	Patel 2015 [40]	Cross-sectional	USA	MF	95 (44)	66.0 (40.0–84.0)	10.7 (7.2–16.9)	25.0 (2.5–159.0)	339.0 (13.0–969.0)	21.1	NGS	IWG-MRT
24	Patriarca 2013 [41]	Cross-sectional	Italy	PV, ET & MF	97 (44)	NR	NR	NR	NR	NR	NGS	2008 WHO
25	Saint-Martin 2009 [42]	Cross-sectional	France	PV, ET & MF	NR	NR	NR	NR	NR	NR	SS	2008 WHO
26	Schlenk 2016 [43]	Cross-sectional	Germany	MF	96 (33)	NR	NR	NR	NR	30.2	SS	2008 WHO, IWG-MRT
27	Schnittger 2012 [44]	Cross-sectional	Germany	ET & MF	NR	NR	NR	NR	NR	NR	SS, HRM	NR
28	Segura-Díaz 2020 [45]	Cross-sectional	Spain	PV, ET & MF	68 (40)	68.0 (43.0–90.0)	NR	NR	NR	8.8	NGS	2016 WHO
29	Song 2017 [46]	Cross-sectional	USA	PV, ET & MF	135 (64)	NR	NR	NR	NR	21.2	NGS	2008 WHO
30	Tefferi 2009 [47]	Cross-sectional	USA	PV, ET & MF	227 (111)	NR	NR	NR	NR	NR	NGS	2001 WHO

Table 1. Cont.

No	Study ID [References]	Study Design	Country	Type of MPN	Total Number of MPN Patients (Female)	Age (Years) [Mean ± SD/Median (IQR)/Range]	Haemoglobin (g/dL) [Mean ± SD/Median (IQR)/Range]	Leucocyte Count (10 <sup>9</sup> /L) [Mean ± SD/Range/Median (IQR)]	Platelet Count (10 <sup>9</sup> /L) [Mean ± SD/Range/Median (IQR)]	Total Number of Mutated ASXL1 (%)	Screening Method for TET2 Gene Mutations	Diagnostic Criteria
31	Tefferi 2010 [48]	Cross-sectional	USA	PV, ET & MF	908 (487)	NR	NR	NR	NR	NR	NGS	2008 WHO, IWG-MRT
32	Tefferi 2016 [49]	Cross-sectional	USA	MF	182 (64)	63.0 (22.0–87.0)	10.1 (5.8–16.0)	10.5 (1.9–219.0)	224.0 (11.0–1493.0)	35.7	NGS	2008 WHO
33	Tefferi 2016a [50]	Cross-sectional	USA	PV & ET	316 (177)	NR	NR	NR	NR	11.4	NGS	2008 WHO
34	Verger 2014 [51]	Cross-sectional	France	PV, ET & MF	27 (NR)	NR	NR	NR	NR	NR	SS	NR
35	Zhang 2015 [52]	Cross-sectional	China	MF	36 (15)	65.0 (46.0–93.0)	10.9 (3.0–16.0)	22.3 (1.4–54.5)	215.0 (3.0–1157.0)	11.1	WGS	2008 WHO

aCGH: array-comparative genomic hybridisation; ASXL1: Additional sex combs-like 1; CGH: comparative genomic hybridisation; ELN: European Leukemia Net; ET: essential thrombocythaemia; HRM: high-resolution melting analysis; IQR: interquartile range; IWG-MRT: International Working Group for Myelofibrosis Research and Treatment; MF: myelofibrosis; MPN: myeloproliferative neoplasms; SS: Sanger sequencing; NGS: next-generation sequencing; NR: not reported; PCR-DGGE: polymerase chain reaction-denaturing gradient gel electrophoresis; PV: polycythaemia vera; SD: standard deviation; SNP: single nucleotide polymorphism; TET2: Ten–eleven translocation 2; UK: United Kingdom; USA: United States of America; WGS: whole-genome sequencing; WHO: World Health Organization.



### 3.3. Meta-Analysis

The overall pooled prevalence of *TET2* gene mutations in patients with MPN was 15.5% (95% CI: 12.1–19.0%,  $I^2 = 94%$ , Figure 2A). The prevalence of *TET2* gene mutations in PV, ET and MF patients was 16.8% (95% CI: 13.2–20.5%,  $I^2 = 60%$ , Figure 2B), 9.8% (95% CI: 7.0–12.7%,  $I^2 = 62%$ , Figure 2C) and 15.7% (95% CI: 11.2–20.2%,  $I^2 = 89%$ , Figure 2D), respectively. In other subgroup analyses, the pooled prevalence of *TET2* gene mutations was compared between four continents: Europe (13.0%; 95% CI: 8.8–17.2%,  $I^2 = 92%$ ), North America (17.4%; 95% CI: 14.0–20.9%,  $I^2 = 74%$ ), Asia (20.8%; 95% CI: 10.5–31.1%,  $I^2 = 80%$ ) and Australia (7.0%; 95% CI: 0.0–14.6%,  $I^2 = \text{NA}$ ). The prevalence of *TET2* gene mutations were further analysed based on countries: China (23.9%; 95% CI: 9.6–38.1%,  $I^2 = 82%$ ), France (13.6%; 95% CI: 10.6–16.7%,  $I^2 = 0%$ ), Germany (14.2%; 95% CI: 9.2–19.1%,  $I^2 = 61%$ ), Italy (1.9%; 95% CI: 0.0–5.7%,  $I^2 = 71%$ ), Spain (10.7%; 95% CI: 0.0–23.2%,  $I^2 = 82%$ ) and the United States (17.4%; 95% CI: 14.0–20.9%,  $I^2 = 74%$ ). Assessments of PV, ET and MF prevalence across the four continents (Figure S1) and in relation to different countries were also examined (Figure S2). Three forms of WHO criteria were used and the prevalence of *TET2* gene mutations was highest in the 2016 version (WHO 2001 criteria 12.9%, 95% CI: 10.2–15.5%,  $I^2 = 0%$ , WHO 2008 criteria 14.5%, 95% CI: 9.7–19.3%,  $I^2 = 95%$  and WHO 2016 criteria 20.1%, 95% CI: 14.7–25.4%,  $I^2 = 61%$ ) (Figure S3). A higher prevalence of *TET2* gene mutations were observed while using NGS (17.2%, 95% CI: 14.0–20.4%,  $I^2 = 80%$ ) and Sanger sequencing (12.7%, 95% CI: 9.6–15.9%,  $I^2 = 52%$ ), but not in HRM analysis (7.7%, 95% CI: 0.0–16.6%,  $I^2 = 88%$ ) (Figure S4). The MF subgroup was further divided into two subgroups (PMF and SMF), and the prevalence of *TET2* gene mutations were studied in both and found to be similar (PMF 16.7%, 95% CI: 13.6–19.8%,  $I^2 = 24%$  and SMF 14.8%, 95% CI: 9.3–20.2%,  $I^2 = 0%$ ) (Figure S5). Various levels of heterogeneity were observed in the main analyses (ranging from 60% to 94%) (Figure 2) and subgroup analyses (ranging from 0% to 93%) (Table 2, Figures S1–S5).

**Table 2.** The pooled prevalence of *TET2* gene mutations in different subgroups of MPN.

Subgroups	Prevalence [95% CIs] (%)	Number of Studies Analysed	Total Number of Patients	Heterogeneity		Publication Bias, Egger's Test ( $p$ -Value)
				$I^2$	$p$ -Value	
<b>Overall myeloproliferative neoplasms</b>						
Europe	13.0 [8.8–17.2]	19	2010	92%	<0.0001	0.004
North America	17.4 [14.0–20.9]	11	1976	74%	<0.0001	0.0005
Asia	20.8 [10.5–31.1]	4	291	80%	0.001	NA
Australia	7.0 [0.0–14.6]	1	43	NA	NA	NA
China	23.9 [9.6–38.1]	3	200	82%	0.003	NA
France	13.6 [10.6–16.7]	5	480	0%	0.67	NA
Germany	14.2 [9.2–19.1]	5	510	61%	0.03	NA
Italy	1.9 [0.0–5.7]	2	607	71%	0.06	NA
Spain	10.7 [0.0–23.2]	2	130	82%	0.01	NA
USA	17.4 [14.0–20.9]	11	1976	74%	<0.0001	0.0005
WHO criteria reported	15.7 [11.8–19.7]	27	3782	95%	<0.0001	0.0002
WHO criteria not reported	13.1 [8.9–17.3]	8	538	47%	0.06	0.005
WHO 2001 criteria	12.9 [10.2–15.5]	3	613	0%	0.93	NA
WHO 2008 criteria	14.5 [9.7–19.3]	17	2594	95%	<0.0001	0.0004
WHO 2016 criteria	20.1 [14.7–25.4]	7	575	61%	0.01	0.40
NGS method	17.2 [14.0–20.4]	18	2604	80%	<0.0001	0.0007
SS method	12.7 [9.6–15.9]	11	965	52%	0.02	0.001
HRM method	7.7 [0.0–16.6]	3	621	88%	0.0002	NA
<b>Polycythaemia vera</b>						
Europe	14.6 [8.0–21.1]	10	343	63%	0.01	0.58
North America	18.2 [14.2–22.5]	9	839	57%	0.01	NA
Asia	29.6 [14.1–45.2]	2	39	17%	0.27	NA
Australia	0.0 [0.0–15.0]	1	8	NA	NA	NA
France	12.5 [7.6–17.5]	4	172	0%	0.90	NA
Spain	12.7 [0.0–37.2]	2	21	61%	0.28	NA
USA	18.2 [14.0–22.5]	9	839	57%	0.01	NA
WHO 2001 criteria	13.7 [9.6–17.9]	3	260	0%	0.40	NA
WHO 2008 criteria	16.9 [11.3–22.6]	12	685	69%	0.0009	0.77



Table 2. Cont.

Subgroups	Prevalence [95% CIs] (%)	Number of Studies Analysed	Total Number of Patients	Heterogeneity		Publication Bias, Egger's Test ( <i>p</i> -Value)
				<i>I</i> <sup>2</sup>	<i>p</i> -Value	
<b>Polycythaemia vera</b>						
WHO 2016 criteria	21.4 [15.6–27.3]	4	256	16%	0.31	NA
NGS method	19.8 [15.1–24.6]	12	922	67%	0.0005	0.009
SS method	13.0 [8.4–17.7]	7	203	0%	0.71	NA
HRM method	0.0 [0.0–22.1]	1	5	NA	NA	NA
<b>Essential thrombocythaemia</b>						
Europe	8.8 [5.7–12.0]	12	531	39%	0.08	0.002
North America	8.7 [3.8–13.6]	7	507	69%	0.003	NA
Asia	25.1 [0.0–56.9]	2	100	93%	0.0002	NA
Australia	6.2 [0.0–18.1]	1	16	NA	NA	NA
France	9.7 [5.3–14.2]	4	166	0%	0.44	NA
Spain	12.1 [0.0–21.2]	2	46	74%	0.04	NA
USA	8.7 [3.8–13.6]	7	507	69%	0.003	NA
WHO 2001 criteria	5.3 [1.1–9.6]	3	180	44%	0.16	NA
WHO 2008 criteria	9.4 [6.1–12.6]	11	700	49%	0.03	0.06
WHO 2016 criteria	20.3 [0.0–43.7]	3	81	89%	<0.0001	0.41
NGS method	10.2 [6.1–14.4]	12	787	75%	<0.0001	0.003
SS method	10.4 [6.2–14.6]	8	316	31%	0.18	NA
HRM method	14.9 [0.0–35.2]	2	82	83%	0.01	NA
<b>Myelofibrosis</b>						
Europe	13.7 [7.9–19.5]	15	1127	85%	<0.0001	0.008
North America	16.8 [12.3–23.7]	9	640	52%	0.09	NA
Asia	17.4 [11.4–23.5]	4	152	0%	0.82	NA
Australia	10.5 [0.0–24.3]	1	19	NA	NA	NA
China	17.4 [11.2–23.6]	3	141	0%	0.63	NA
France	17.6 [9.9–25.3]	5	142	20%	0.51	NA
Germany	11.0 [8.0–14.0]	5	410	0%	0.61	NA
Italy	0.4 [0.0–0.9]	2	527	0%	0.50	NA
Spain	14.0 [0.0–39.4]	2	32	70%	0.21	NA
USA	17.7 [13.8–21.6]	8	631	35%	0.15	NA
WHO 2001 criteria	17.5 [11.9–23.3]	3	173	0%	0.52	NA
WHO 2008 criteria	14.4 [8.1–20.7]	15	1210	90%	<0.0001	0.04
WHO 2016 criteria	16.5 [11.8–21.2]	4	238	0%	0.39	0.20
NGS method	16.5 [13.2–19.8]	13	896	38%	0.17	0.053
SS method	13.3 [9.1–17.5]	11	446	24%	0.35	0.01
HRM method	5.0 [0.0–18.2]	3	534	50%	0.10	NA
<b>Different types of myelofibrosis</b>						
PMF	16.7 [13.6–19.8]	20	853	24%	0.41	0.06
SMF	14.8 [9.3–20.2]	9	158	0%	0.95	NA

CIs: confidence intervals; HRM: high-resolution melting analysis; NA: not applicable; NGS: next-generation sequencing; PMF: primary myelofibrosis; SMF: secondary myelofibrosis; SS: Sanger sequencing; WHO: World Health Organization.

### 3.4. Quality Assessment

Detailed quality assessments of the included studies are presented in Tables S2 and S3. Most studies were judged to be of high quality (68.6%), while the remainder were considered to be of either moderate (14.3%) or low quality (17.7%).

### 3.5. Publication Bias

The results from the funnel plots and Egger's tests suggest that there is only a small likelihood of publication bias (Figures 3 and S6).

### 3.6. Sensitivity Analyses

In the sensitivity analyses, only minor differences (ranging from 4.0% lower to 1.8% higher) were observed in the pooled prevalence estimates of *TET2* gene mutations among cases of MPN compared to the main findings (Table 3 and Figure S7). A Galbraith plot was performed, and four outlier studies [29,30,37,39] were identified (Figure 4).

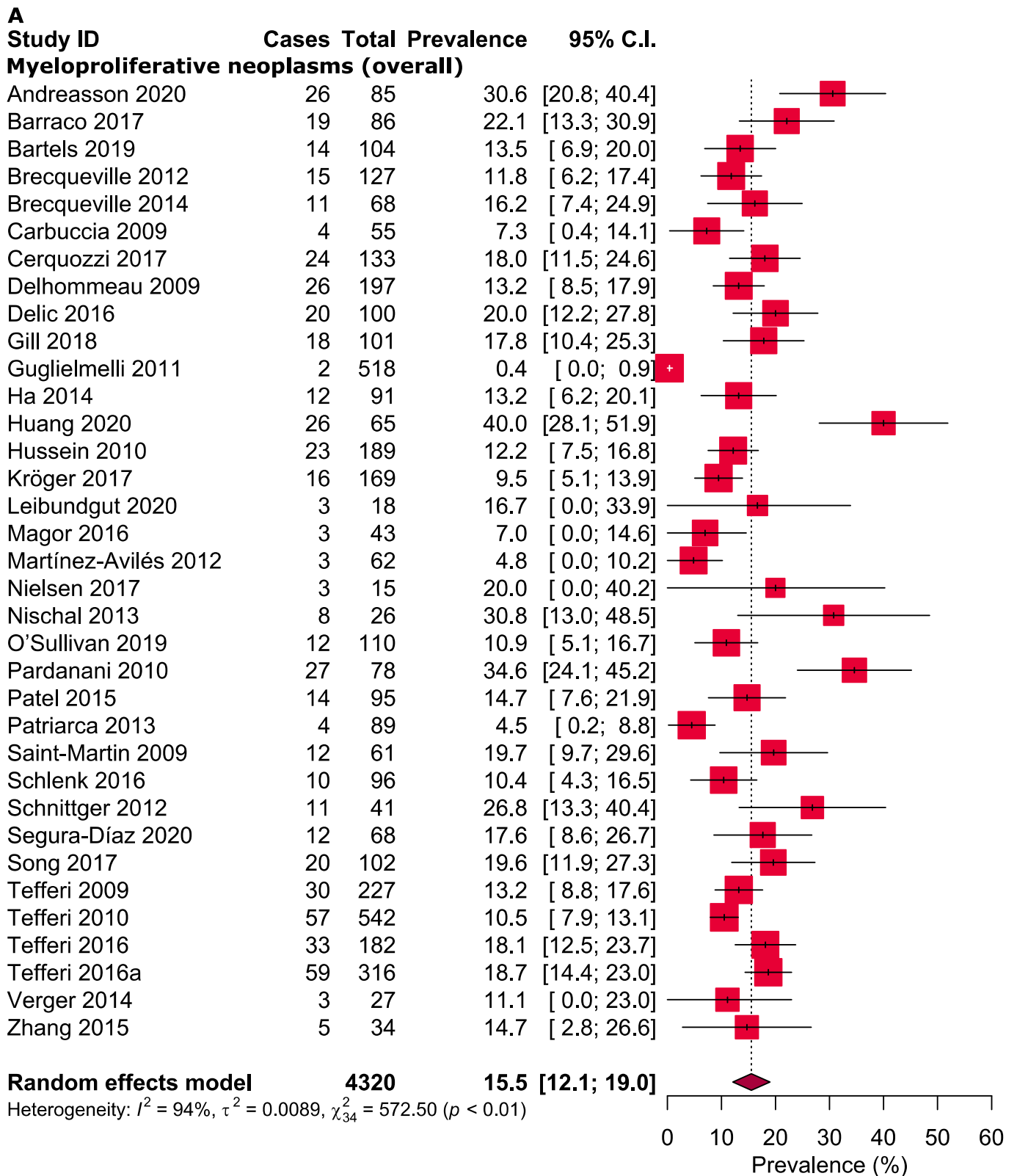


Figure 2. Cont.

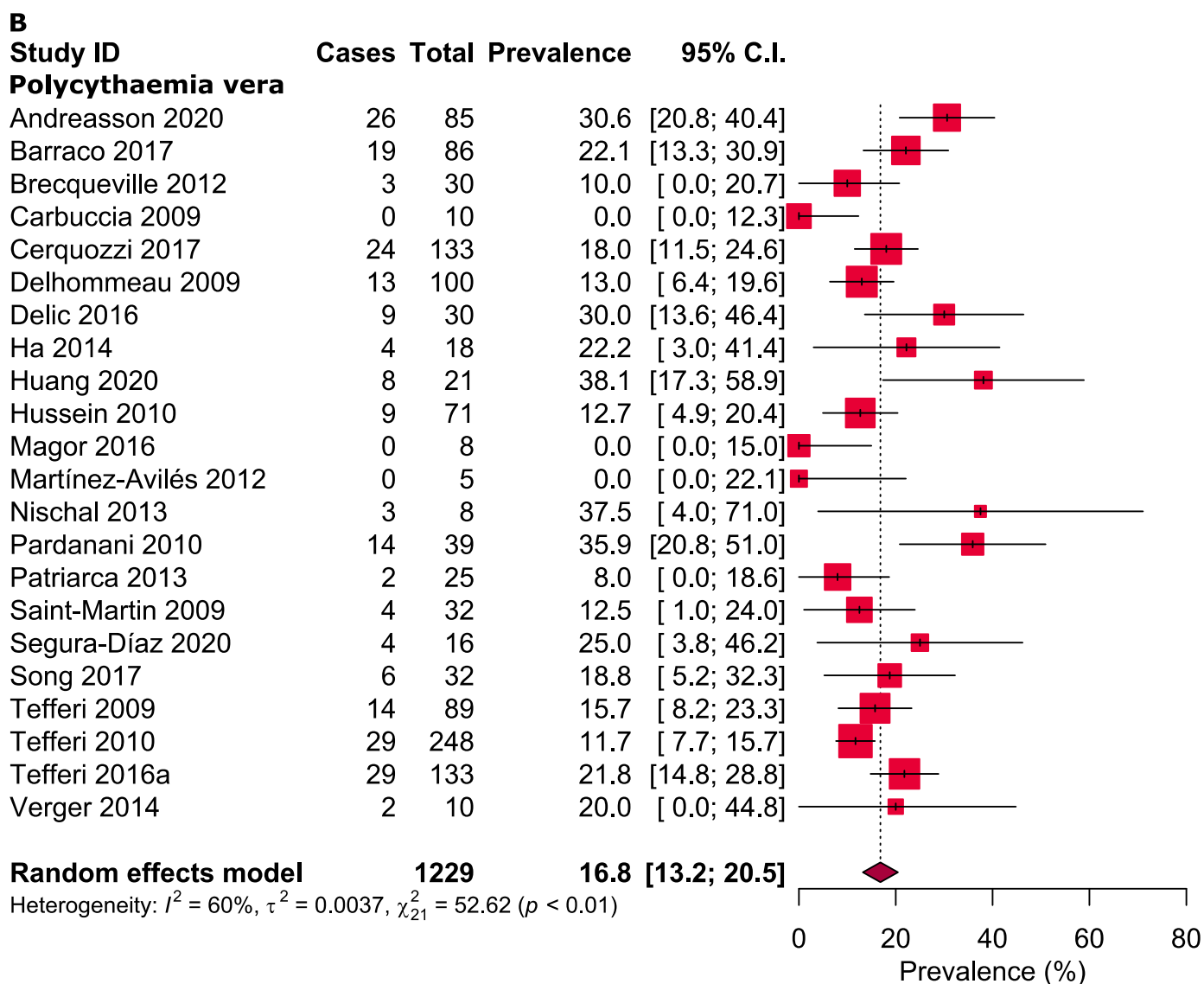


Figure 2. Cont.

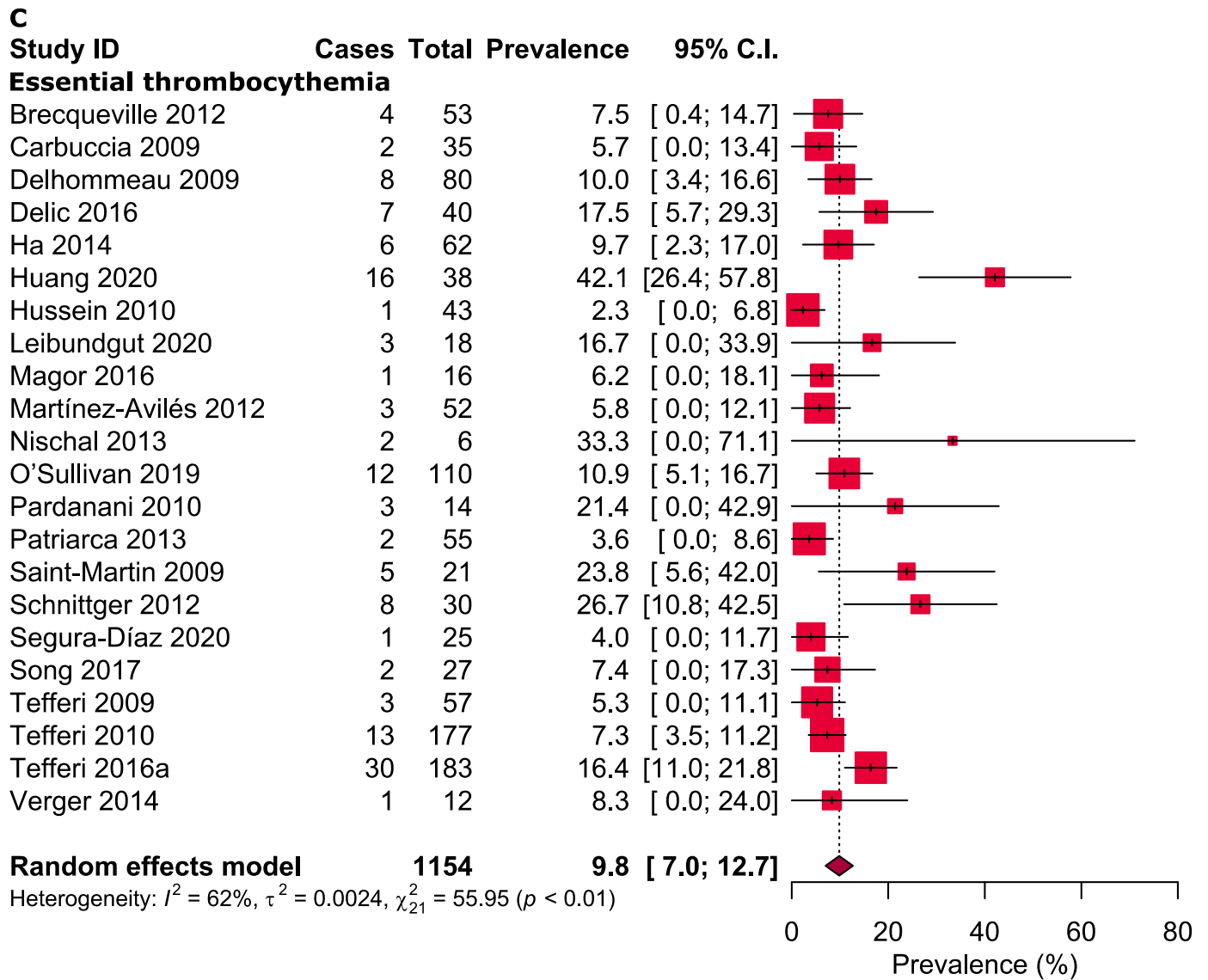
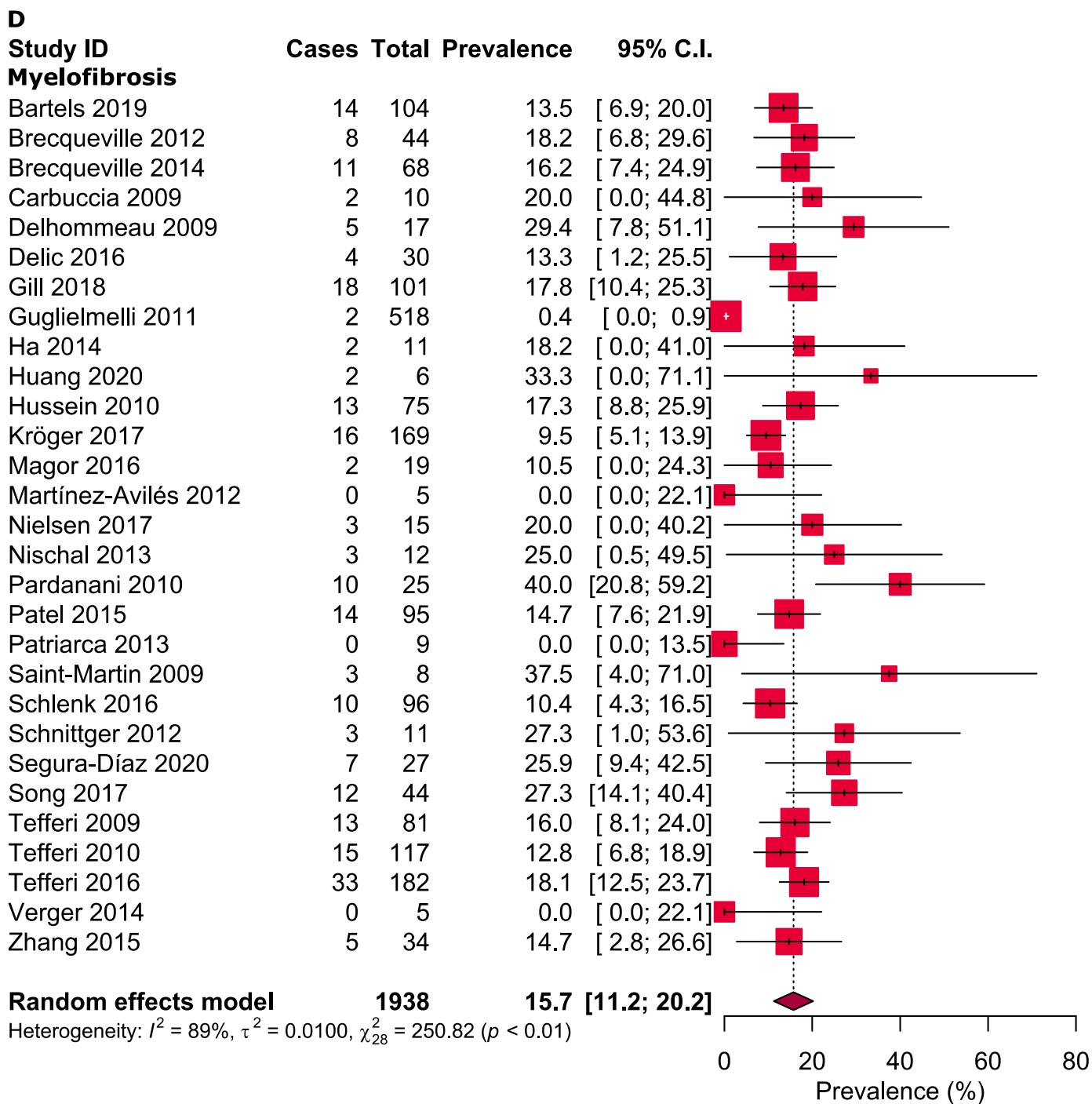


Figure 2. Cont.



**Figure 2.** (A) Prevalence of *TET2* gene mutations in patients with MPN (overall). (B) Prevalence of *TET2* gene mutations in patients with PV. (C) Prevalence of *TET2* gene mutations in patients with ET. (D) Prevalence of *TET2* gene mutations in patients with MF.

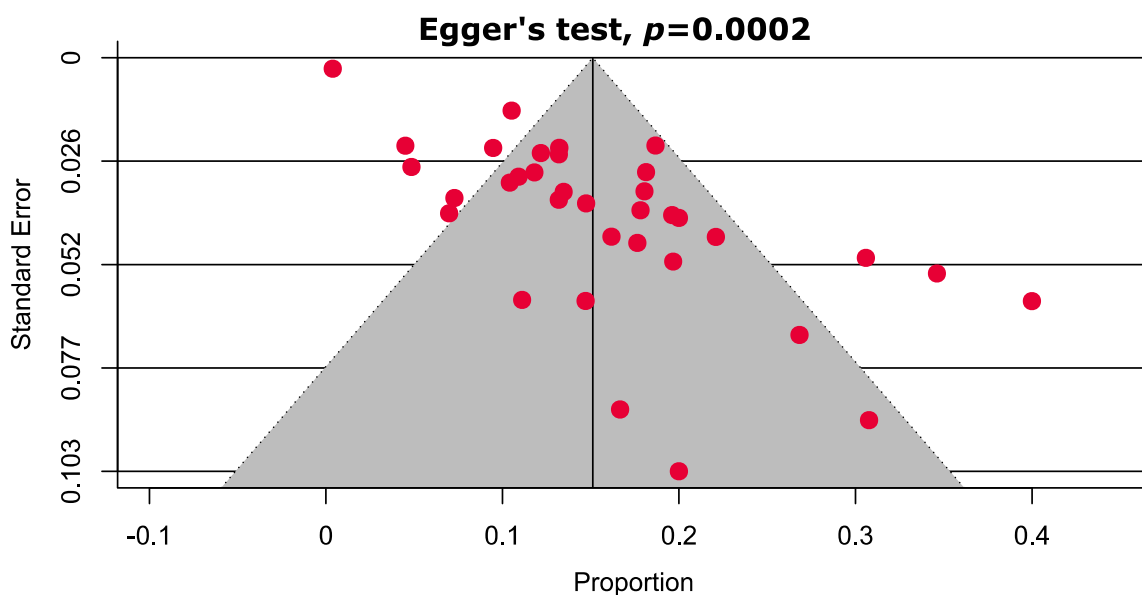


Figure 3. Funnel plot estimating the prevalence of *TET2* gene mutations in patients with MPN (overall).

Table 3. Sensitivity analyses.

Strategies of Sensitivity Analyses	Prevalence [95% CIs] (%)	Difference of Pooled Prevalence Compared to the Main Result	Number of Studies Analysed	Total Number of Subjects	Heterogeneity	
					<i>I</i> <sup>2</sup>	<i>p</i> -Value
<b>Myeloproliferative neoplasms (overall)</b>						
Excluding small studies	13.6 [8.8–18.4]	1.9% lower	15	3117	96%	<0.0001
Excluding low- and moderate-quality studies	15.4 [11.3–19.6]	0.1% lower	24	3485	95%	<0.0001
Excluding outlier studies	13.9 [12.0–15.9]	1.6% lower	31	3633	63%	<0.0001
<b>Polycythaemia vera</b>						
Excluding small studies	15.6 [11.0–20.3]	1.2% lower	4	614	59%	0.06
Excluding low- and moderate-quality studies	18.6 [14.4–22.7]	1.8% higher	13	946	59%	0.003
Excluding outlier studies	15.4 [12.0–18.7]	1.4% lower	19	1161	54%	0.01
<b>Essential thrombocythaemia</b>						
Excluding small studies	11.3 [5.9–16.8]	1.5% higher	3	470	72%	0.02
Excluding low- and moderate-quality studies	11.1 [7.1–15.0]	1.3% higher	12	839	72%	<0.0001
Excluding outlier studies	8.4 [6.0–10.8]	4.4% lower	19	1096	48%	0.01
<b>Myelofibrosis</b>						
Excluding small studies	11.7 [4.0–19.5]	4.0% lower	6	1191	95%	<0.0001
Excluding low- and moderate-quality studies	14.0 [8.9–19.1]	1.7% lower	18	1700	91%	<0.0001
Excluding outlier studies	14.5 [12.4–16.7]	1.2% lower	25	1377	18%	0.46

CIs: Confidence intervals.

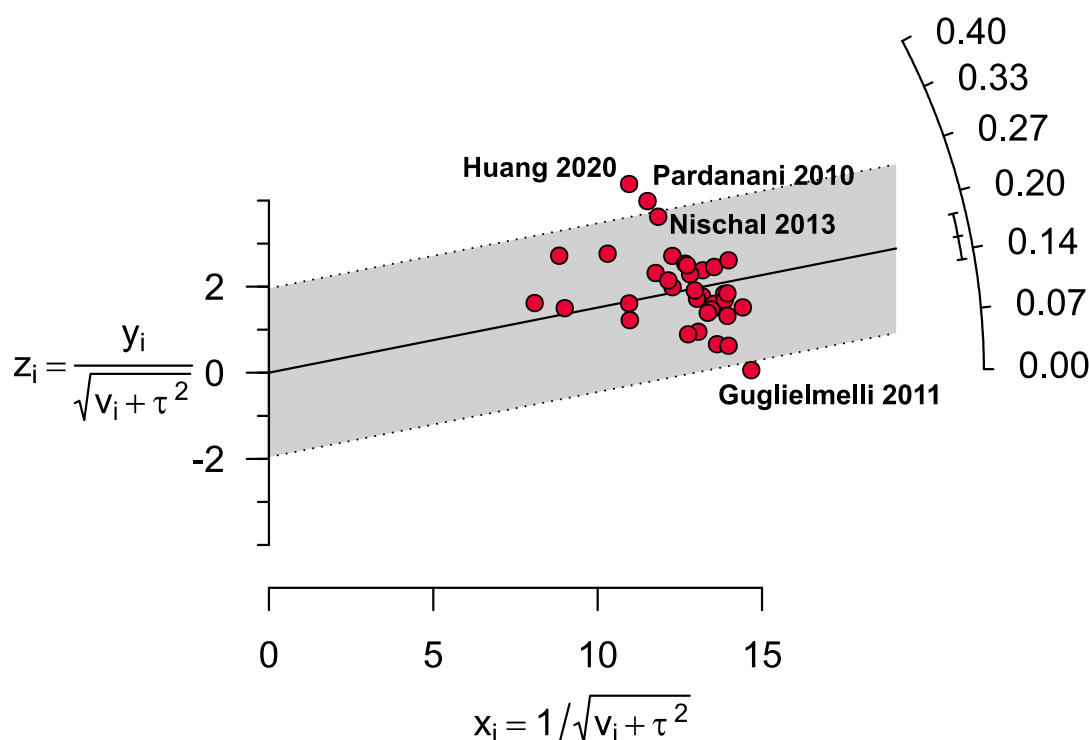


Figure 4. Galbraith plot analysing MPN (overall) identified four outlier studies.

#### 4. Discussion

The overall prevalence of *TET2* gene mutations among *BCR-ABL*-negative MPN patients was estimated to be 15.5%. This estimate is similar to the occurrence of *TET2* somatic mutations in patients with various myeloid cancers [11]. Compared with other myeloid malignancies, the prevalence of *TET2* gene mutations among patients with *BCR-ABL*-negative MPN appears to be lower. Among patients with myelodysplastic syndromes (MDS), the prevalence of these mutations has been estimated to be 18–35% [11,53–58], 36–60% for those with chronic myelomonocytic leukaemia (CMML) [54,59–61], 24% in cases with AML, 22% in chronic myelogenous leukaemia (CML) [11], 20% in mastocytosis [62,63] and about 30% in patients with blastic plasmacytoid dendritic cell neoplasm [64,65].

The results appear to confirm the observation that epigenetic regulators like the *TET2* gene have mutated more frequently among those patients with PV ( $p = 0.05$ ), compared with those with either MF ( $p = 0.02$ ) or ET ( $p = 0.023$ ) [27]. According to a meta-analysis that analysed the frequency of three main genes (*JAK2*, *MPL* and *CALR*) in MPN from 2000 to 2018 [66], for the most common gene mutation *JAK2* V617F, *TET2* showed a lower prevalence as compared to *JAK2* V617F in PV (46.7–100.0%), ET (31.3–72.1%) and MF (25.0–85.7%). For the *MPL* gene, our results displayed a higher proportion in PV (16.8% vs. 0%) but similar percentages in ET (9.8% vs. 0.9–12.5%) and MF (15.7% vs. 0–17.7%). Finally, in relation to the last common driver gene, *CALR*, *TET2* manifested a higher prevalence in PV (16.8% vs. 0%), a lower prevalence in ET (9.8% vs. 12.6–50%) and a similar proportion in MF (15.7% vs. 10–100%). From these results, it appears that the *TET2* gene mutations have distributed more evenly across MPN subcategories in contrast to the three main driver mutation genes [47].

This study has several notable strengths. To our knowledge, no meta-analysis has previously investigated the prevalence of *TET2* gene mutations in patients with MPN. This meta-analysis included studies from six databases using robust search strategies without any language restrictions. All the sensitivity analyses produced similar results to the overall findings, suggesting that the main result is likely to be robust and credible.



Nevertheless, there are a few limitations. Several meta-analyses exhibited high heterogeneity, indicating considerable variability among the results from the included studies. After excluding the four outlier studies identified by the Galbraith plot, heterogeneity was reduced from 94% to 63% across all MPN studies, 60% to 54% for PV, 62% to 48% for ET and 89% to 18% for MF, suggesting that these four studies were an important source of heterogeneity. Several factors may further explain this heterogeneity. One of the outlier studies [29] recorded a very low prevalence of *TET2* gene mutations (0.4%). This may be due to the use of a different method (HRM analysis) that may be less sensitive to identifying the mutations compared with most other studies. Notably, a similar result was also observed in one of the two other studies [35,44] that also employed the same analytical technique. Different etiological exposures might occur in different regions, resulting in differences in the prevalence estimates across the different studies [67]. In support of this hypothesis, a lower prevalence was recorded in Australia and Italy, whereas a higher result was identified in China and the United States. Variations in the use of different diagnostic guidelines may have also affected the estimates of prevalence and further contributed to the heterogeneity of results between studies. The discovery of the *JAK2* gene mutations in 2005 and their subsequent inclusion in the diagnostic criteria [68] for MPN, PV and ET but not MF [2,69] may account for some of the differences observed among the studies. A stepwise increase in *TET2* gene mutations in MPN was observed with subsequent versions of the WHO classification and diagnostic criteria among all cases of MPN (12.9% for WHO criteria 2001, 14.5% for WHO criteria 2008 and 20.1% for WHO criteria 2016), PV (13.7% for WHO criteria 2001, 16.9% for WHO criteria 2008 and 21.4% for WHO criteria 2016) and ET (5.3% for WHO criteria 2001, 9.4% for WHO criteria 2008 and 20.3% for WHO criteria 2016) but not in MF (17.5% for WHO criteria 2001, 14.4% for WHO criteria 2008 and 16.5% for WHO criteria 2016).

Another limitation of this meta-analysis is that the prevalence of MPN may be underestimated in some studies. Patients with MPN can be relatively symptom-free for many years so people, with little contact with health services, can remain undiagnosed for long periods [70]. Estimates of the prevalence of *TET2* mutations in MPN may be underestimated, particularly in less-developed countries or among disadvantaged groups in well-developed countries.

Finally, the included studies largely focused on the allele frequencies of the main driver mutations (*JAK2*, *MPL* and *CALR*) and did not permit any analysis of the allelic frequencies of the *TET2* mutant allele in MPN.

## 5. Conclusions

This meta-analysis provides the most comprehensive currently available estimate of the overall prevalence of *TET2* gene mutations (15.5%) among patients with MPN. However, substantial heterogeneity was evident among the results included in this meta-analysis, likely related to factors such as regional differences in patients included in studies and variations in the diagnostic criteria employed by the studies. This heterogeneity suggests that caution should be employed with using the estimates of prevalence.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/cancers13123078/s1>, Figure S1: prevalence of *TET2* gene mutations based on continents, Figure S2: Prevalence of *TET2* gene mutations based on countries, Figure S3: Prevalence of *TET2* gene mutations based on the WHO classification and diagnostic criteria of MPN, Figure S4: Prevalence of *TET2* gene mutations based on different methods used to detect *TET2* gene mutations in MPN, Figure S5: Subgroup analysis. Prevalence of *TET2* gene mutations in patients with (A) PMF and (B) SMF, Figure S6: Funnel plots estimating the prevalence of *TET2* gene mutations in patients with (A) polycythaemia vera, (B) essential thrombocythaemia and (C) myelofibrosis, Figure S7: Sensitivity analyses, Table S1: Search strategies, Table S2: Quality assessment of the included cross-sectional studies, Table S3: Quality assessment of the included case-control studies.

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## References

1. Campregher, P.V.; de Souza Santos, F.P.; Perini, G.F.; Hamerschlag, N. Molecular biology of Philadelphia-negative myeloproliferative neoplasms. *Rev. Bras. Hematol. Hemoter.* **2012**, *34*, 150–155. [CrossRef]
2. Tefferi, A.; Thiele, J.; Vardiman, J.W. The 2008 World Health Organization classification system for myeloproliferative neoplasms: Order out of chaos. *Cancer* **2009**, *115*, 3842–3847. [CrossRef]
3. Tefferi, A.; Elliott, M. Thrombosis in myeloproliferative disorders: Prevalence, prognostic factors, and the role of leukocytes and JAK2V617F. In *Seminars in Thrombosis and Hemostasis*; Thieme Medical Publishers, Inc.: New York, NY, USA, 2007; pp. 313–320.
4. Nangalia, J.; Massie, C.E.; Baxter, E.J.; Nice, F.L.; Gundem, G.; Wedge, D.C.; Avezov, E.; Li, J.; Kollmann, K.; Kent, D.G. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N. Engl. J. Med.* **2013**, *369*, 2391–2405. [CrossRef] [PubMed]
5. Tenedini, E.; Bernardis, I.; Artusi, V.; Artuso, L.; Roncaglia, E.; Guglielmelli, P.; Pieri, L.; Bogani, C.; Biamonte, F.; Rotunno, G. Targeted cancer exome sequencing reveals recurrent mutations in myeloproliferative neoplasms. *Leukemia* **2014**, *28*, 1052–1059. [CrossRef]
6. Reinig, E.; Yang, F.; Traer, E.; Arora, R.; Brown, S.; Rattray, R.; Braziel, R.; Fan, G.; Press, R.; Dunlap, J. Targeted next-generation sequencing in myelodysplastic syndrome and chronic myelomonocytic leukemia aids diagnosis in challenging cases and identifies frequent spliceosome mutations in transformed acute myeloid leukemia. *Am. J. Clin. Pathol.* **2016**, *145*, 497–506. [CrossRef]
7. Vainchenker, W.; Kralovics, R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood* **2017**, *129*, 667–679. [CrossRef] [PubMed]
8. Chia, Y.C.; Islam, M.A.; Woon, P.Y.; Johan, M.F.; Hassan, R.; Ramli, M. Molecular genetics of thrombotic myeloproliferative neoplasms: Implications in precision oncology. *Genes Dis.* **2021**. [CrossRef]
9. Scott, L.M.; Rebel, V.I. JAK2 and genomic instability in the myeloproliferative neoplasms: A case of the chicken or the egg? *Am. J. Hematol.* **2012**, *87*, 1028–1036. [CrossRef]
10. Kameda, T.; Shide, K.; Yamaji, T.; Kamiunten, A.; Sekine, M.; Taniguchi, Y.; Hidaka, T.; Kubuki, Y.; Shimoda, H.; Marutsuka, K.; et al. Loss of TET2 has dual roles in murine myeloproliferative neoplasms: Disease sustainer and disease accelerator. *Blood* **2015**, *125*, 304–315. [CrossRef]
11. Delhommeau, F.; Dupont, S.; Della Valle, V.; James, C.; Trannoy, S.; Massé, A.; Kosmider, O.; Le Couedic, J.P.; Robert, F.; Alberdi, A.; et al. Mutation in TET2 in myeloid cancers. *N. Engl. J. Med.* **2009**, *360*, 2289–2301. [CrossRef]
12. Swierczek, S.I.; Yoon, D.; Bellanné-Chantelot, C.; Kim, S.J.; Saint-Martin, C.; Delhommeau, F.; Najman, A.; Prchal, J.T. Extent of hematopoietic involvement by TET2 mutations in JAK2V617F polycythemia vera. *Haematologica* **2011**, *96*, 775–778. [CrossRef]
13. Moola, S.; Munn, Z.; Tufanaru, C.; Aromataris, E.; Sears, K.; Sfetcu, R.; Currie, M.; Lisy, K.; Qureshi, R.; Mattis, P.; et al. Chapter 7: Systematic reviews of etiology and risk. In *JBI Manual for Evidence Synthesis*; Aromataris, E., Munn, Z., Eds.; JBI: Adelaide, Australia, 2020. Available online: <https://synthesismanual.jbi.global> (accessed on 12 December 2020).
14. Ha, J.S.; Jeon, D.S.; Kim, J.R.; Ryoo, N.H.; Suh, J.S. Analysis of the ten-eleven translocation 2 (TET2) gene mutation in myeloproliferative neoplasms. *Ann. Clin. Lab. Sci.* **2014**, *44*, 173–179. [PubMed]
15. Abdel-Wahab, O.; Levine, R.L. EZH2 mutations: Mutating the epigenetic machinery in myeloid malignancies. *Cancer Cell* **2010**, *18*, 105–107. [CrossRef]
16. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Group, P. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* **2009**, *6*, e1000097. [CrossRef]
17. Islam, M.A.; Alam, S.S.; Kundu, S.; Hossan, T.; Kamal, M.A.; Cavestro, C. Prevalence of Headache in Patients with Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis of 14,275 Patients. *Front. Neurol.* **2020**, *11*, 562634. [CrossRef]
18. Viechtbauer, W. Conducting meta-analyses in R with the metafor package. *J. Stat. Softw.* **2010**, *36*, 1–48. [CrossRef]

19. Ihle, M.A.; Fassunke, J.; König, K.; Grünewald, I.; Schlaak, M.; Kreuzberg, N.; Tietze, L.; Schildhaus, H.-U.; Büttner, R.; Merkelbach-Bruse, S. Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p. V600E and non-p. V600E BRAF mutations. *BMC Cancer* **2014**, *14*, 13. [[CrossRef](#)] [[PubMed](#)]
20. Andréasson, B.; Pettersson, H.; Wasslavik, C.; Johansson, P.; Palmqvist, L.; Asp, J. ASXL1 mutations, Previous vascular complications and age at diagnosis predict survival in 85 WHO-defined polycythaemia vera patients. *Br. J. Haematol.* **2020**, *189*, 913–919. [[CrossRef](#)]
21. Barraco, D.; Cerquozzi, S.; Gangat, N.; Patnaik, M.M.; Lasho, T.; Finke, C.; Hanson, C.A.; Ketterling, R.P.; Pardanani, A.; Tefferi, A. Monocytosis in polycythemia vera: Clinical and molecular correlates. *Am. J. Hematol.* **2017**, *92*, 640–645. [[CrossRef](#)] [[PubMed](#)]
22. Bartels, S.; Faisal, M.; Büsche, G.; Schlue, J.; Hasemeier, B.; Schipper, E.; Vogtmann, J.; Westphal, L.; Lehmann, U.; Kreipe, H. Mutations associated with age-related clonal hematopoiesis in PMF patients with rapid progression to myelofibrosis. *Leukemia* **2019**, *34*, 1364–1372. [[CrossRef](#)]
23. Brecqueville, M.; Rey, J.; Bertucci, F.; Coppin, E.; Finetti, P.; Carbuccia, N.; Cervera, N.; Gelsi-Boyer, V.; Arnoulet, C.; Gisserot, O.; et al. Mutation analysis of ASXL1, CBL, DNMT3A, IDH1, IDH2, JAK2, MPL, NF1, SF3B1, SUZ12, and TET2 in myeloproliferative neoplasms. *Genes Chromosomes Cancer* **2012**, *51*, 743–755. [[CrossRef](#)]
24. Brecqueville, M.; Rey, J.; Devillier, R.; Guille, A.; Gillet, R.; Adélaide, J.; Gelsi-Boyer, V.; Arnoulet, C.; Chaffanet, M.; Mozziconacci, M.J.; et al. Array comparative genomic hybridization and sequencing of 23 genes in 80 patients with myelofibrosis at chronic or acute phase. *Haematologica* **2014**, *99*, 37–45. [[CrossRef](#)]
25. Carbuccia, N.; Murati, A.; Trouplin, V.; Brecqueville, M.; Adelaide, J.; Rey, J.; Vainchenker, W.; Bernard, O.; Chaffanet, M.; Vey, N. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia* **2009**, *23*, 2183–2186. [[CrossRef](#)]
26. Cerquozzi, S.; Barraco, D.; Lasho, T.; Finke, C.; Hanson, C.A.; Ketterling, R.P.; Pardanani, A.; Gangat, N.; Tefferi, A. Risk factors for arterial versus venous thrombosis in polycythemia vera: A single center experience in 587 patients. *Blood Cancer J.* **2017**, *7*, 662. [[CrossRef](#)]
27. Delic, S.; Rose, D.; Kern, W.; Nadarajah, N.; Haferlach, C.; Haferlach, T.; Meggendorfer, M. Application of an NGS-based 28-gene panel in myeloproliferative neoplasms reveals distinct mutation patterns in essential thrombocythaemia, primary myelofibrosis and polycythaemia vera. *Br. J. Haematol.* **2016**, *175*, 419–426. [[CrossRef](#)] [[PubMed](#)]
28. Gill, H.; Ip, H.W.; Yim, R.; Tang, W.F.; Pang, H.H.; Lee, P.; Leung, G.M.K.; Li, J.; Tang, K.; So, J.C.C.; et al. Next-generation sequencing with a 54-gene panel identified unique mutational profile and prognostic markers in Chinese patients with myelofibrosis. *Ann. Hematol.* **2019**, *98*, 869–879. [[CrossRef](#)]
29. Guglielmelli, P.; Biamonte, F.; Score, J.; Hidalgo-Curtis, C.; Cervantes, F.; Maffioli, M.; Fanelli, T.; Ernst, T.; Winkelman, N.; Jones, A.V.; et al. EZH2 mutational status predicts poor survival in myelofibrosis. *Blood* **2011**, *118*, 5227–5234. [[CrossRef](#)] [[PubMed](#)]
30. Huang, X.; Wu, J.; Deng, X.; Xu, X.; Zhang, X.; Ma, W.; Hu, T.; Yang, J.; Guan, M.; Tang, G. Mutation profiles of classic myeloproliferative neoplasms detected by a customized next-generation sequencing-based 50-gene panel. *J. Bio-X Res.* **2020**, *3*, 13–20. [[CrossRef](#)]
31. Hussein, K.; Abdel-Wahab, O.; Lasho, T.L.; Van Dyke, D.L.; Levine, R.L.; Hanson, C.A.; Pardanani, A.; Tefferi, A. Cytogenetic correlates of TET2 mutations in 199 patients with myeloproliferative neoplasms. *Am. J. Hematol.* **2010**, *85*, 81. [[CrossRef](#)]
32. Kröger, N.; Panagiota, V.; Badbaran, A.; Zabelina, T.; Triviai, I.; Araujo Cruz, M.M.; Shahswar, R.; Ayuk, F.; Gehlhaar, M.; Wolschke, C.; et al. Impact of Molecular Genetics on Outcome in Myelofibrosis Patients after Allogeneic Stem Cell Transplantation. *Biol. Blood Marrow Transplant.* **2017**, *23*, 1095–1101. [[CrossRef](#)] [[PubMed](#)]
33. Oppliger Leibundgut, E.; Haubitz, M.; Burington, B.; Ottmann, O.G.; Spitzer, G.; Odenike, O.; McDevitt, M.A.; Röth, A.; Snyder, D.S.; Baerlocher, G.M. Dynamics of mutations in patients with essential thrombocythemia treated with imetelstat. *Haematologica* **2020**. [[CrossRef](#)] [[PubMed](#)]
34. Magor, G.W.; Tallack, M.R.; Klose, N.M.; Taylor, D.; Korbie, D.; Mollie, P.; Trau, M.; Perkins, A.C. Rapid Molecular Profiling of Myeloproliferative Neoplasms Using Targeted Exon Resequencing of 86 Genes Involved in JAK-STAT Signaling and Epigenetic Regulation. *J. Mol. Diagn.* **2016**, *18*, 707–718. [[CrossRef](#)]
35. Martinez-Aviles, L.; Besses, C.; Alvarez-Larran, A.; Torres, E.; Serrano, S.; Bellosillo, B. TET2, ASXL1, IDH1, IDH2, And c-CBL genes in JAK2-and MPL-negative myeloproliferative neoplasms. *Ann. Hematol.* **2012**, *91*, 533–541. [[CrossRef](#)] [[PubMed](#)]
36. Myrtue Nielsen, H.; Lykkegaard Andersen, C.; Westman, M.; Sommer Kristensen, L.; Asmar, F.; Arvid Kruse, T.; Thomassen, M.; Stauffer Larsen, T.; Skov, V.; Lotte Hansen, L.; et al. Epigenetic changes in myelofibrosis: Distinct methylation changes in the myeloid compartments and in cases with ASXL1 mutations. *Sci. Rep.* **2017**, *7*, 6774. [[CrossRef](#)]
37. Nischal, S.; Bhattacharyya, S.; Christopheit, M.; Yu, Y.; Zhou, L.; Bhagat, T.D.; Sohal, D.; Will, B.; Mo, Y.; Suzuki, M.; et al. Methylome profiling reveals distinct alterations in phenotypic and mutational subgroups of myeloproliferative neoplasms. *Cancer Res.* **2013**, *73*, 1076–1085. [[CrossRef](#)]
38. O’Sullivan, J.M.; Hamblin, A.; Yap, C.; Fox, S.; Boucher, R.; Panchal, A.; Alimam, S.; Dreau, H.; Howard, K.; Ware, P.; et al. The poor outcome in high molecular risk, hydroxycarbamide-resistant/intolerant ET is not ameliorated by ruxolitinib. *Blood* **2019**, *134*, 2107–2111. [[CrossRef](#)] [[PubMed](#)]
39. Pardanani, A.; Lasho, T.; Finke, C.; Oh, S.T.; Gotlib, J.; Tefferi, A. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. *Leukemia* **2010**, *24*, 1713–1718. [[CrossRef](#)] [[PubMed](#)]

40. Patel, K.P.; Newberry, K.J.; Luthra, R.; Jabbour, E.; Pierce, S.; Cortes, J.; Singh, R.; Mehrotra, M.; Routbort, M.J.; Luthra, M.; et al. Correlation of mutation profile and response in patients with myelofibrosis treated with ruxolitinib. *Blood* **2015**, *126*, 790–797. [[CrossRef](#)]
41. Patriarca, A.; Colaizzo, D.; Tiscia, G.; Spadano, R.; Di Zaccaro, S.; Spadano, A.; Villanova, I.; Margaglione, M.; Grandone, E.; Dragani, A. TET2 mutations in Ph-negative myeloproliferative neoplasms: Identification of three novel mutations and relationship with clinical and laboratory findings. *BioMed Res. Int.* **2013**, *2013*, 929840. [[CrossRef](#)]
42. Saint-Martin, C.; Leroy, G.; Delhommeau, F.; Panelatti, G.; Dupont, S.; James, C.; Plo, I.; Bordessoule, D.; Chomienne, C.; Delannoy, A.; et al. Analysis of the Ten-Eleven Translocation 2 (TET2) gene in familial myeloproliferative neoplasms. *Blood* **2009**, *114*, 1628–1632. [[CrossRef](#)]
43. Schlenk, R.F.; Stegelmann, F.; Reiter, A.; Jost, E.; Gattermann, N.; Hebart, H.; Waller, C.; Hochhaus, A.; Platzbecker, U.; Schafhausen, P.; et al. Pomalidomide in myeloproliferative neoplasm-associated myelofibrosis. *Leukemia* **2017**, *31*, 889–895. [[CrossRef](#)] [[PubMed](#)]
44. Schnittger, S.; Bacher, U.; Eder, C.; Dicker, F.; Alpermann, T.; Grossmann, V.; Kohlmann, A.; Kern, W.; Haferlach, C.; Haferlach, T. Molecular analyses of 15,542 patients with suspected BCR-ABL1-negative myeloproliferative disorders allow to develop a stepwise diagnostic workflow. *Haematologica* **2012**, *97*, 1582–1585. [[CrossRef](#)] [[PubMed](#)]
45. Segura-Díaz, A.; Stuckey, R.; Florido, Y.; González-Martín, J.M.; López-Rodríguez, J.F.; Sánchez-Sosa, S.; González-Pérez, E.; Perdomo, M.N.S.; del Mar Perera, M.; de la Iglesia, S.; et al. Thrombotic risk detection in patients with polycythemia vera: The predictive role of DNMT3A/TET2/ASXL1 mutations. *Cancers* **2020**, *12*, 934. [[CrossRef](#)]
46. Song, J.; Hussaini, M.; Zhang, H.; Shao, H.; Qin, D.; Zhang, X.; Ma, Z.; Hussain Naqvi, S.M.; Zhang, L.; Moscinski, L.C. Comparison of the Mutational Profiles of Primary Myelofibrosis, Polycythemia Vera, and Essential Thrombocytosis. *Am. J. Clin. Pathol.* **2017**, *147*, 444–452. [[CrossRef](#)]
47. Tefferi, A.; Pardanani, A.; Lim, K.H.; Abdel-Wahab, O.; Lasho, T.L.; Patel, J.; Gangat, N.; Finke, C.M.; Schwager, S.; Mullally, A.; et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. *Leukemia* **2009**, *23*, 905–911. [[CrossRef](#)]
48. Tefferi, A.; Lasho, T.L.; Abdel-Wahab, O.; Guglielmelli, P.; Patel, J.; Caramazza, D.; Pieri, L.; Finke, C.M.; Kilpivaara, O.; Wadleigh, M.; et al. IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. *Leukemia* **2010**, *24*, 1302–1309. [[CrossRef](#)] [[PubMed](#)]
49. Tefferi, A.; Lasho, T.L.; Finke, C.M.; Elala, Y.; Hanson, C.A.; Ketterling, R.P.; Gangat, N.; Pardanani, A. Targeted deep sequencing in primary myelofibrosis. *Blood Adv.* **2016**, *1*, 105–111. [[CrossRef](#)]
50. Tefferi, A.; Lasho, T.L.; Guglielmelli, P.; Finke, C.M.; Rotunno, G.; Elala, Y.; Pacilli, A.; Hanson, C.A.; Pancrazzi, A.; Ketterling, R.P.; et al. Targeted deep sequencing in polycythemia vera and essential thrombocythemia. *Blood Adv.* **2016**, *1*, 21–30. [[CrossRef](#)] [[PubMed](#)]
51. Verger, E.; Andreoli, A.; Chomienne, C.; Kiladjian, J.J.; Cassinat, B. TET2 gene sequencing may be helpful for myeloproliferative neoplasm diagnosis. *Br. J. Haematol.* **2014**, *165*, 416–419. [[CrossRef](#)]
52. Chunxia, Z.; Li, G.; Qianqian, J.; Zhenling, L.; Fanzhou, H.; Yayue, G.; Ming, G.; Shaohua, X.; Yin, T.; Yanrong, C.; et al. Symptom burden and its relationships with risk assessment and gene mutations in patients with primary myelofibrosis. *J. Leuk. Lymphoma* **2015**, *24*, 453–456.
53. Tefferi, A.; Lim, K.H.; Abdel-Wahab, O.; Lasho, T.L.; Patel, J.; Patnaik, M.M.; Hanson, C.A.; Pardanani, A.; Gilliland, D.G.; Levine, R.L. Detection of mutant TET2 in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML. *Leukemia* **2009**, *23*, 1343–1345. [[CrossRef](#)] [[PubMed](#)]
54. Shih, A.H.; Abdel-Wahab, O.; Patel, J.P.; Levine, R.L. The role of mutations in epigenetic regulators in myeloid malignancies. *Nat. Rev. Cancer* **2012**, *12*, 599–612. [[CrossRef](#)]
55. Guo, Z.; Zhang, S.-K.; Zou, Z.; Fan, R.-H.; Lyu, X.-D. Prognostic significance of TET2 mutations in myelodysplastic syndromes: A meta-analysis. *Leukemia Res.* **2017**, *58*, 102–107. [[CrossRef](#)] [[PubMed](#)]
56. Bejar, R.; Stevenson, K.; Abdel-Wahab, O.; Galili, N.; Nilsson, B.; Garcia-Manero, G.; Kantarjian, H.; Raza, A.; Levine, R.L.; Neuberg, D. Clinical effect of point mutations in myelodysplastic syndromes. *N. Engl. J. Med.* **2011**, *364*, 2496–2506. [[CrossRef](#)] [[PubMed](#)]
57. Kosmider, O.; Gelsi-Boyer, V.; Cheok, M.; Grabar, S.; Della-Valle, V.; Picard, F.; Viguié, F.; Quesnel, B.; Beyne-Rauzy, O.; Solary, E. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). *Blood* **2009**, *114*, 3285–3291. [[CrossRef](#)]
58. Itzykson, R.; Kosmider, O.; Cluzeau, T.; Mansat-De Mas, V.; Dreyfus, F.; Beyne-Rauzy, O.; Quesnel, B.; Vey, N.; Gelsi-Boyer, V.; Raynaud, S. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. *Leukemia* **2011**, *25*, 1147–1152. [[CrossRef](#)] [[PubMed](#)]
59. Kohlmann, A.; Grossmann, V.; Klein, H.U.; Schindela, S.; Weiss, T.; Kazak, B.; Dicker, F.; Schnittger, S.; Dugas, M.; Kern, W.; et al. Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. *J. Clin. Oncol.* **2010**, *28*, 3858–3865. [[CrossRef](#)]
60. Itzykson, R.; Kosmider, O.; Renneville, A.; Gelsi-Boyer, V.; Meggendorfer, M.; Morabito, M.; Berthon, C.; Adès, L.; Fenaux, P.; Beyne-Rauzy, O. Prognostic score including gene mutations in chronic myelomonocytic leukemia. *J. Clin. Oncol.* **2013**, *31*, 2428–2436. [[CrossRef](#)]



61. Grossmann, V.; Kohlmann, A.; Eder, C.; Haferlach, C.; Kern, W.; Cross, N.; Haferlach, T.; Schnittger, S. Molecular profiling of chronic myelomonocytic leukemia reveals diverse mutations in >80% of patients with TET2 and EZH2 being of high prognostic relevance. *Leukemia* **2011**, *25*, 877–879. [[CrossRef](#)]
62. Tefferi, A.; Levine, R.L.; Lim, K.H.; Abdel-Wahab, O.; Lasho, T.L.; Patel, J.; Finke, C.M.; Mullally, A.; Li, C.Y.; Pardanani, A.; et al. Frequent TET2 mutations in systemic mastocytosis: Clinical, KITD816V and FIP1L1-PDGFR $\alpha$  correlates. *Leukemia* **2009**, *23*, 900–904. [[CrossRef](#)] [[PubMed](#)]
63. Soucie, E.; Hanssens, K.; Mercher, T.; Georgin-Lavialle, S.; Damaj, G.; Livideanu, C.; Chandesris, M.O.; Acin, Y.; Létard, S.; de Sepulveda, P. In aggressive forms of mastocytosis, TET2 loss cooperates with c-KITD816V to transform mast cells. *Blood* **2012**, *120*, 4846–4849. [[CrossRef](#)] [[PubMed](#)]
64. Jardin, F.; Ruminy, P.; Parmentier, F.; Troussard, X.; Vaida, I.; Stamatoullas, A.; Leprêtre, S.; Penther, D.; Duval, A.B.; Picquenot, J.M. TET2 and TP53 mutations are frequently observed in blastic plasmacytoid dendritic cell neoplasm. *Br. J. Haematol.* **2011**, *153*, 413–416. [[CrossRef](#)] [[PubMed](#)]
65. Menezes, J.; Acquadro, F.; Wiseman, M.; Gomez-Lopez, G.; Salgado, R.; Talavera-Casanas, J.; Buno, I.; Cervera, J.; Montes-Moreno, S.; Hernandez-Rivas, J. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. *Leukemia* **2014**, *28*, 823–829. [[CrossRef](#)] [[PubMed](#)]
66. Mejía-Ochoa, M.; Toro, P.A.A.; Cardona-Arias, J.A. Systematization of analytical studies of polycythemia vera, essential thrombocythemia and primary myelofibrosis, and a meta-analysis of the frequency of JAK2, CALR and MPL mutations: 2000–2018. *BMC Cancer* **2019**, *19*, 590. [[CrossRef](#)] [[PubMed](#)]
67. Anderson, L.A.; Duncombe, A.S.; Hughes, M.; Mills, M.E.; Wilson, J.C.; McMullin, M.F. Environmental, Lifestyle, And familial/ethnic factors associated with myeloproliferative neoplasms. *Am. J. Hematol.* **2012**, *87*, 175–182. [[CrossRef](#)] [[PubMed](#)]
68. Levine, R.L.; Wadleigh, M.; Cools, J.; Ebert, B.L.; Wernig, G.; Huntly, B.J.; Boggon, T.J.; Wlodarska, I.; Clark, J.J.; Moore, S. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* **2005**, *7*, 387–397. [[CrossRef](#)] [[PubMed](#)]
69. Passamonti, F.; Maffioli, M. Update from the latest WHO classification of MPNs: A user’s manual. *Hematology* **2016**, *2016*, 534–542. [[CrossRef](#)]
70. Tefferi, A.; Vardiman, J.W. Classification and diagnosis of myeloproliferative neoplasms: The 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia* **2008**, *22*, 14–22. [[CrossRef](#)]