INVESTIGATION OF EXON 12 MUTATIONS IN THE *JANUS KINASE 2* (*JAK 2*) GENE AMONG POLYCYTHEMIA VERA PATIENTS FROM HOSPITAL PAKAR UNIVERSITI SAINS MALAYSIA

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UNIVERSITI SAINS MALAYSIA

2025

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by

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Dissertation submitted in partial fulfillment of the requirements for the degree

of

Bachelor of Health Science (Honours)

(Biomedicine)

January 2025

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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(NUR HASYA ATHIRAH BINTI HANIZAM)

Date: 17/01/2025

ACKNOWLEDGEMENTS

Alhamdulillah, in the name of Allah, The Most Gracious and The Most Merciful, I am very grateful to God, for giving me the strength and spirit that I needed throughout this whole semester to complete my research project successfully at the School of Health Science, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan. I would like to grab this opportunity to acknowledge as well as express my utmost gratitude to everyone who has been giving me continuous support and lending a hand to accomplish this project. My deepest appreciation goes to my supervisor, Dr, Nik Norliza Nik Hassan, alongside my co-supervisor, Dr. Razan Hayati Zulkeflee for their guidance, advice, encouragement, and kindness that allows me to finish this research project. I would also like to thank Prof. Dr. Rosline Hassan, Dr. Marini Ramli, Dr. Zefarina Zulkafli, Mr. Mohamad Ros Sidek, and Ms. Nur Alia Najiehah Suhaimi as they play a very important role in the accomplishment of my research project. My appreciation also goes to all the lab staff who have helped me in any way especially the ones at the Biomedicine Laboratory, Central Research Laboratory (CRL), and Human Genome USM for allowing me to work in their establishments for the sake of finishing this research project. The funding of this project is from the Short-Term Grant USM (304/PPSP/6315778). I would like to thank my parents, Dr. Hanizam Hashim and Mrs. Nor Azleena Omar, and siblings for their continuous support and reassurance that gave me the strength to accomplish the project as well as thesis writing. Finally, I would also want to take my friends especially, Mr. Farres Nizam, Ms. Nurul Alya Mazri, Ms. Angelyn Lee Chui Yee, and Ms. Hanina Sofea Faizal for their unwavering moral support throughout this project.

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ABBREVIATIONS

AML	-	Acute Myeloid Leukemia
ARMS	-	Amplification Refractory Mutation System
ASO	-	Allele-Specific Oligonucleotide
AS-PCR	-	Allele-Specific PCR
BLAST	-	Basic Local Alignment Search Tool
BP	-	Blast Phase
Bp	-	Base Pair
CML	-	Chronic Myeloid Leukemia
ddNTPs	-	Dideoxynucleotides
DNA	-	Deoxyribonucleic Acid
EPO	-	Erythropoietin
EPOR	-	EPO Receptor
ET	-	Essential Thrombocythemia
F	-	Phenylalanine
FBC	-	Full Blood Count
G-CSFR	-	Granulocyte Colony-Stimulating Factor Receptor
GLVCO A	-	Glycophorin A
H&E	-	Hematoxylin And Eosin
Hb	-	Haemoglobin
Hct	-	Haematocrit
HPIS	-	Hospital Patient Information System
HPUSM	-	Hospital Pakar Universiti Sains Malaysia
HRM	-	High-Resolution Melt
IDT	-	Integrated Dna Technologies
IHC	-	Immunohistochemistry
iPSC	-	Induced Pluripotent Stem Cell
IQR	-	Interquartile Ranges
JAK 2	-	Janus Kinase 2
KPP	-	Klinik Pakar Perubatan
LDH	-	Lactate Dehydrogenase
M:E	-	Myeloid-To-Erythroid

MIPSS	-	Mutation-Enhanced International Prognostic Scoring System
MPN	-	Myeloproliferative Neoplasm
MPO	-	Myeloperoxidase
NCBI	-	National Center for Biotechnology Information
NGS	-	Next-Generation Sequencing
OSA	-	Obstructive Sleep Apnoea
PCR	-	Polymerase Chain Reaction
PMF	-	Myelofibrosis
Post PV-MF	-	Post-Polycythemia Vera Myelofibrosis
PV	-	Polycythemia Vera
RBC	-	Red Blood Cell
RETIC	-	Reticulin
SD	-	Standard Deviations
TAE	-	Tris-Acetate-EDTA
TBE	-	Tris-Borate-Edta
Tm	-	Melting Temperature
TWC	-	Total White Cell
USA	-	United States Of America
V	-	Valine
VUS	-	Variants Of Unknown Significance
WBC	-	White Blood Cell
WHO	-	World Health Organization

PENYIASATAN MUTASI EKSON 12 DALAM GEN *JANUS KINASE 2 (JAK2)* DALAM KALANGAN PESAKIT POLYCYTHEMIA VERA DI HOSPITAL PAKAR UNIVERSITI SAINS MALAYSIA

ABSTRAK

Polycythemia vera (PV) adalah gangguan myeloproliferatif yang dicirikan oleh pengeluaran sel darah merah yang berlebihan. Mutasi pada *JAK2* exon 12 kurang dikaji berbanding mutasi *JAK2* V617F. Kajian ini bertujuan untuk menentukan kekerapan mutasi *JAK2* exon 12 di kalangan pesakit PV di Hospital Pakar Universiti Sains Malaysia (HPUSM) dan meneroka hubungannya dengan parameter klinikal dan hematologi. Sebanyak 86 pesakit PV telah disaring untuk mutasi *JAK2* exon 12 menggunakan reaksi rantai polimerase (PCR) diikuti dengan penjujukan Sanger. Pesakit dibahagikan kepada kumpulan yang berbeza berdasarkan kewujudan mutasi seperti *JAK2* exon 12, mutasi *JAK2* V617F atau kedua-dua mutasi. Data klinikal, termasuk umur, jantina, splenomegali, dan trombosis, diperoleh daripada rekod perubatan. Parameter hematologi, seperti hemoglobin, hematokrit, kiraan sel darah putih (WBC), kiraan platelet, dan laktat dehidrogenase (LDH), dianalisis. Lima belas pesakit didapati positif untuk mutasi *JAK2* exon 12, yang menunjukkan dua jenis mutasi seperti mutasi sisipan dan mutasi titik. Menariknya, koeksistensi mutasi *JAK2* V617F dan exon 12 ditemui pada lapan pesakit (9.32%), menunjukkan potensi mekanisme genetik yang kompleks. Pesakit dengan kedua-dua mutasi *JAK2* V617F dan exon 12 mempunyai purata umur tertinggi serta peningkatan kiraan platelet dan WBC, menunjukkan perjalanan penyakit yang lebih agresif. Mutasi *JAK2* V617F dikaitkan dengan kadar trombosis dan splenomegali yang lebih tinggi berbanding mutasi exon 12 sahaja. Analisis morfologi mendedahkan keberadaan sel yang berlebihan dalam sumsum tulang pesakit positif *JAK2* exon 12, dengan pembahagian

sel eritroid dan sel megakaryosit yang ketara. Kajian ini menekankan kepentingan klinikal mutasi *JAK2* exon 12, menyerlahkan impak berbeza mereka terhadap perkembangan penyakit dan ciri-ciri klinikal berbanding mutasi *JAK2* V617F.

INVESTIGATION OF EXON 12 MUTATIONS IN THE *JANUS KINASE 2 (JAK 2)* GENE AMONG POLYCYTHEMIA VERA PATIENTS FROM HOSPITAL PAKAR UNIVERSITI SAINS MALAYSIA

ABSTRACT

Polycythemia vera (PV) is a myeloproliferative disorder characterized by excessive red blood cell production. While *JAK2* V617F mutations have been extensively studied, mutations in *JAK2* exon 12 remain under-explored. This study aimed to determine the prevalence of *JAK2* exon 12 mutations among PV patients at Hospital Pakar Universiti Sains Malaysia (HPUSM) and explore their associations with clinical and hematological parameters. A total of 86 PV patients were screened for *JAK2* exon 12 mutations using polymerase chain reaction (PCR) followed by Sanger sequencing. Patients were categorized into groups based on the presence of *JAK2* exon 12 mutations, *JAK2* V617F mutations, both mutations, or neither. Clinical data, including age, gender, splenomegaly, and thrombosis, were obtained from medical records. Hematological parameters, such as hemoglobin, hematocrit, white blood cell (WBC) count, platelet count, and lactate dehydrogenase (LDH), were analyzed. Fifteen patients tested positive for *JAK2* exon 12 mutations, revealing two mutation types such as insertion mutations and point mutations. Interestingly, co-occurrence of *JAK2* V617F and exon 12 mutations was found in eight patients (9.32%), indicating the potential for complex genetic mechanisms. Patients with both *JAK2* V617F and exon 12 mutations had the highest mean age and elevated platelet and WBC counts, indicating a more aggressive disease course. *JAK2* V617F mutations were associated with higher rates of thrombosis and splenomegaly compared to exon 12-only mutations. Morphological analysis revealed hypercellularity in the bone marrow of *JAK2* exon 12-positive patients, with significant erythroid and megakaryocytic proliferation. This study underscores the clinical significance of *JAK2* exon 12 mutations, highlighting their distinct impact on disease progression and clinical features compared to *JAK2* V617F mutations.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The World Health Organization (WHO) classifies polycythemia vera (PV) as a myeloproliferative neoplasm (MPN). It is defined by abnormal myeloid haematopoiesis proliferation, which results in excessive red cell formation (Lu & Chang, 2023). The overproduction of blood cells can result in complications such as, thrombosis, which affects roughly 29% of PV patients, where mostly suffered for arterial thrombosis. Furthermore, PV patients are at risk of progressing to post-polycythemia vera myelofibrosis (post PV-MF) and blast phase (BP), with reported progression in 9% and 3% of patients, respectively (Benevolo *et al.*, 2021). PV is also associated with a lower life expectancy because of these consequences, including the likelihood of acquiring secondary malignancies with a poor prognosis and limited response to therapy.

The diagnostic approach for PV has been standardized through the criteria provided by WHO, which was significantly refined in 2022. These criteria are divided into major and minor categories, and a confirmed diagnosis generally requires the presence of either all three major criteria or two major criteria along with the minor criterion. Those criteria are

a) Elevation of haemoglobin or haematocrit levels, specifically, haemoglobin levels above 16.5 g/dL in men and 16.0 g/dL in women, or haematocrit levels exceeding 49% in men and 48% in women.

Alternatively, a red cell mass greater than 25% above the predicted normal value also fulfills this criterion.

b) A bone marrow biopsy showing hypercellularity with trilineage proliferation (panmyelosis) provides morphological confirmation of PV, often highlighting increased erythroid, granulocytic, and megakaryocytic lines.

c) The detection of the *JAK2* V617F mutation or mutations in *JAK2* exon 12.

In addition to the major criteria, an abnormal or low serum erythropoietin (EPO) level constitutes the minor criterion, reflecting the suppression of natural erythropoiesis due to the autonomous production of red blood cells in PV. While the bone marrow biopsy is often recommended for more precise morphological analysis, it may not be required in certain cases where sustained high haemoglobin (Hb) (>18.5g/dL in men or >16.5g/dL in women) and haematocrit (Hct) levels (>55.5% in men or >49.5% in women) and a confirmed *JAK2* mutation exist (Tefferi & Barbui, 2023).

Patients with *JAK2* exon 12 mutations generally show higher haemoglobin levels but lower platelet and white blood cell counts compared to those with *JAK2* V617F mutations. However, despite these differences, both groups face a similar likelihood of complications such as thrombosis, progression to myelofibrosis, leukemia, and overall mortality rates (Guglielmelli et al., 2024). PV presents with a range of clinical features that differ from patient to patient. For instance, headaches are the most common symptom, reported by 96.7% of individuals. Itching, or pruritus, affects 63.3% of patients, making it another prominent symptom. Splenomegaly, the enlargement of the spleen, is observed in about 46.7% of cases and may indicate the advancement of the disease. Thrombosis is a notable complication, with 13.4% of patients experiencing blood clots at diagnosis and a total of 20% being affected overall (Husam & Almothafar, 2022). This demonstrates the significant risk of thrombotic events in PV patients, which remains a concern regardless of the mutation type.

Disease progression is another major issue, with 16.6% of patients developing myelofibrosis, a severe condition where the bone marrow becomes scarred, hindering its ability to produce blood cells properly. Additionally, PV can advance to acute leukemia, a more aggressive phase of the disease. Despite the variations in haemoglobin levels seen in *JAK2* exon 12 mutations, the risks of complications, including thrombosis and progression to myelofibrosis or leukemia, are comparable to those with *JAK2* V617F mutations. Therefore, ongoing monitoring of blood counts and potential disease progression is crucial in managing PV.

The *Janus Kinase 2* (JAK 2) mutation, particularly the V617F form, is a major driver in the pathogenesis of MPNs. This mutation activates the *JAK2* protein constitutively, resulting in uncontrolled blood cell proliferation. The *JAK2* V617F mutation, which is an exon 14 guanine-to-thymidine somatic mutation, is present in more than 90% of PV patients (Akram et al., 2018). This mutation is a critical diagnostic marker and plays an important role in PV pathogenesis by increasing abnormal haematopoiesis. Approximately 2% of PV patients do not have the *JAK2* V617F mutation. In these cases, insertions and deletions in exon 12 of the *JAK2* gene were identified as an alternate pathogenic pathway (Guglielmelli et al., 2024).

JAK2 exon 12 mutations are responsible for 2 to 5% of PV cases, especially in *JAK2 V617F*-negative patients. These mutations activate the JAK-STAT signaling system persistently, resulting in increased red blood cell synthesis and isolated erythrocytosis (Soubortseva *et al.*, 2023). Clinically, exon 12-mutated PV patients show at a younger age with high haemoglobin and haematocrit levels, which are frequently accompanied by low EPO. Mouse models and human studies show that the mutation enhances erythropoiesis, presumably through dysregulation of iron metabolism and altered cytokine receptor interactions. Despite changes in clinical presentation, the long-term prognosis for exon 12-mutated PV is similar to that of *V617F*-mutated patients(Tondeur *et al.*, 2021). These findings highlight the relevance of molecular diagnostics in the accurate categorization and management of PV.

Therefore, comprehensive genetic screening is essential for several reasons. It allows for the detection of less common genetic mutations that might not be identified through standard diagnostic tests. This is especially important for PV patients, where alternative mutations like those in *JAK2* exon 12 may be present. Identifying these mutations can lead to accurate diagnoses and precisely tailored treatment strategies, thereby enhancing patient outcomes. Moreover, understanding the full spectrum of *JAK2* mutations aids in assessing disease prognosis and response to therapy. Different mutations can impact the severity of the disease and progression in various ways. For example, patients with *JAK2* exon 12 mutations might exhibit distinct clinical features compared to those with the *JAK2* V617F mutation. By identifying these variations, clinicians can refine treatment approaches and better monitor disease progression. Furthermore, by employing Sanger Direct Sequencing, this research aims to address existing gaps in our understanding of *JAK2* exon 12 mutations in PV patients.

1.2 Problem Statement

PV is a myeloproliferative disorder characterized by the excessive production of red blood cells (Lu & Chang, 2023), which can lead to various complications, including thrombosis and cardiovascular issues. The *JAK2* V617F mutation has been identified as a key factor in the majority of PV cases, serving as a crucial diagnostic marker (Ibrahim et al., 2019). However, a subset of PV patients who are negative for the *JAK2* V617F mutation presents a significant diagnostic challenge (Guglielmelli et al., 2024). Among these patients, *JAK2* exon 12 mutations have been recognized as another genetic factor contributing to the disease, yet their prevalence and clinical significance are not well understood.

While the role of *JAK2* V617F in PV is well-documented, the lack of comprehensive data on *JAK2* exon 12 mutations in PV patients creates a diagnostic gap. This gap leads to challenges in accurately identifying and managing these patients, potentially resulting in delayed or inadequate treatment. The specific types of exons 12 mutations, their demographic associations, and their impact on the clinical and haematological presentation of PV remain inadequately studied, limiting the ability to develop effective diagnostic and therapeutic strategies.

Existing research predominantly focuses on the *JAK2* V617F mutation, with limited studies investigating the significance of exon 12 mutations in PV patients. This lack of data creates a knowledge gap, particularly in understanding how these mutations influence the presentation of the disease and progression in different demographic groups.

This study aims to identify and characterize the types of *JAK2* exon 12 mutations present in PV patients from HPUSM, explore associations between these mutation types and patient demographics, and investigate the potential impact of *JAK2* exon 12 mutations on the clinical presentation and haematological parameters of these patients.

1.3 Study Objective

1.3.1 General Objective

To investigate the prevalence, molecular characteristics, and clinical and haematological associations of *JAK2* exon 12 mutations in PV patients from HPUSM.

1.3.2 Specific Objectives

I. To identify and characterize the types of *JAK2* exon 12 mutations present in PV patients from HPUSM.

II. To explore associations between *JAK2* exon 12 mutation types and patients' demographics (age and gender), and clinical presentations (hepatomegaly and splenomegaly) in PV patients.

III. To investigate the associations of *JAK2* exon 12 mutations on haematological parameters (haemoglobin, red blood cells, total white cells, and platelet counts) and morphology of PV patients.

1.4 Hypothesis

1.4.1 Null Hypothesis

I. There are no distinct types of *JAK2* exon 12 mutations present in PV patients from HPUSM.

II. There are no significant associations between *JAK2* exon 12 mutation types and patients' demographics (age and gender) or clinical presentations (hepatomegaly and splenomegaly) in PV patients.

III. There are no significant impacts of *JAK2* exon 12 mutations on the haematological parameters (haemoglobin, haematocrit, red blood cells, total white cells, and platelet counts) and morphology of PV patients.

1.4.2 Alternative Hypothesis

I. There are distinct types of *JAK2* exon 12 mutations present in PV patients from HPUSM.

II. There are significant associations between *JAK2* exon 12 mutation types and patients' demographics (age and gender) or clinical presentations (hepatomegaly and splenomegaly) in PV patients.

III. There are significant impacts of *JAK2* exon 12 mutations on the haematological parameters (haemoglobin, haematocrit, red blood cells, total white cells, and platelet counts) and morphology of PV patients.

1.5 Significance of Study

PV is a myeloproliferative disorder that leads to excessive red blood cell production (Lu & Chang, 2023), increasing the risk of serious complications such as thrombosis and cardiovascular issues. The *JAK2* V617F mutation is a well-established diagnostic marker for PV, but diagnosing patients who are negative for this mutation remains challenging. Therefore, understanding the role of *JAK2* exon 12 mutations in these patients could provide crucial insights into their accurate diagnosis and treatment.

This study will provide valuable new information on *JAK2* exon 12 mutations in PV patients, a group that has not been extensively studied. By identifying and characterizing these mutations, the research will contribute to a deeper understanding of the genetic factors involved in PV. This will help bridge the gap in current knowledge and may lead to advancements in our understanding of the underlying mechanisms of the disease.

This research aligns with the mission of advancing medical knowledge and improving patient care at HPUSM. It shows a commitment to tackling important gaps in the field of haematology and highlights a personal dedication to providing new insights that could help us better understand and manage PV.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Polycythemia Vera

2.1.1 Definition and Classification

PV is a type of myeloproliferative neoplasm (MPN), a group of clonal hematologic disorders characterized by the abnormal proliferation of bone marrow stem cells. MPNs are primarily defined by the overproduction of one or more blood cell lines which include erythrocytes, leukocytes, or platelets that are due to mutations in hematopoietic stem cells. This group of disorders includes PV, primary myelofibrosis (PMF), and essential thrombocythemia (ET), chronic myeloid leukemia (CML) (Bhargava, 2021). PV is particularly characterized by clonal erythrocytosis, marked by an abnormal increase in red blood cells due to the mutation in the *Janus Kinase 2* (*JAK2)* gene, specifically the *V617F* mutation. Ramakrishnan (2023) reported approximately about 95% to 98% of PV cases possessed *JAK2* gene mutation specifically the *V617F* mutation while the remaining harbour *JAK2* exon 12 mutations. The *JAK2* mutation leads to the increased sensitivity of hematopoietic stem cells to erythropoietin (EPO), resulting in uncontrolled erythrocyte production.

PV is classified as primary polycythemia, distinguishing it from secondary polycythemia, which is driven by external factors such as chronic hypoxia or EPO-secreting tumours. PV is a myeloproliferative disorder originating from a mutation within the bone marrow, leading to an uncontrolled production of red blood cells. The *JAK2* V617F mutation plays a central role in this process by causing hypersensitivity to EPO or enabling erythroid progenitors to grow independently of EPO. PV can also present with additional features such as leucocytosis, thrombocytosis, and splenomegaly, and carries risks of progression to post-polycythemia vera myelofibrosis (post-PV MF) or acute myeloid leukemia (AML). Clinical manifestations include fatigue, itching, abdominal pain, weight loss, and increased risk of thrombotic events (Ismail et al., 2023).

In contrast, secondary polycythemia arises due to external factors that increase EPO levels, stimulating red blood cell production. Common causes include chronic hypoxia from lung diseases, obstructive sleep apnoea (OSA), or living at high altitudes. It can also be triggered by EPO-secreting tumours, such as renal cell carcinoma (Ismail et al., 2023). The diagnosis of secondary polycythemia is supported by elevated EPO levels and the absence of the *JAK2* mutation. The differentiation between primary and secondary polycythemia is crucial, has it differed between malignancy and reactive causes.

2.1.2 Epidemiology and Incidence of PV in Malaysia

PV makes up roughly 48% of MPN cases in the U.S., where it is most commonly reported among older Caucasian adults (Mehta et al., 2014). PV exhibits a rich variety of epidemiological patterns around the world. Its prevalence and presentation seem to be influenced by genetic background, demographic profiles, and environmental contexts unique to each region. Typically, PV incidence is higher among older populations, reflecting the progressive nature of clonal blood disorders like myeloproliferative neoplasms. In Sweden, one of the more comprehensively studied populations, PV has an age-standardized rate of 1.48 per 100,000 person-years, with significantly higher rates observed among those aged 60 and older

(Hultcrantz et al., 2020). In the United States, the incidence is slightly higher, with around 1.9 cases per 100,000 person-years.

This regional pattern persists throughout Asia, with China and Japan exhibiting comparable patterns. In China, PV accounts for approximately 46 to 48% of MPN cases. The *JAK2* V617F mutation is detected in 85 to 91% of PV cases, making it a useful diagnostic marker (Lin et al., 2015). While PV prevalence in China is generally lower than in Western countries, the difference may be due to both hereditary and environmental factors. Around 40% of MPN cases in Japan involve PV. Unlike many Western countries, the gender distribution of Japanese PV patients is well equal, with individuals frequently diagnosed beyond the age of 50. The *JAK2* mutation is seen in 80 to 85% of these patients (Ishitsuka et al., 2017).

Western countries like Canada and Germany exhibit PV trends similar to those in the U.S. and Sweden. Canadian studies place PV at around 40-50% of MPN cases, with an incidence rate of about 1.6 per 100,000 person-years. These cases are also more common among older adults, with Caucasians more frequently affected (Heppner et al., 2019). In Germany, PV represents about 45% of MPN cases, with incidence rates varying between 0.5 and 1.5 per 100,000 person-years. The disease predominantly affects older men, echoing patterns seen across much of Europe and North America (Kaifie et al., 2016).

These regional patterns align with global data, where PV prevalence varies but consistently shows higher rates among older populations and certain ethnic groups. For a comprehensive comparison of PV prevalence across different countries, refer to Table 2.1.

Table 2. 1 : Prevalence and Incidence of PV in Selected Countries.

Country	PV prevalence (% of MPN Cases)	Incidence Rate (per 100,000 person-years)	Key Demographics
Malaysia	47.8 %	Data limited	Older adults, male and higher among Chinese ethnicity (Zulkeflee et al., 2021)
Indonesia	Limited	Data limited	Likely similar pattern to Malaysia, older adults (Yassin et al., 2018)
Sweden	45 – 50 %	1.48	Higher in older adults (Hultcrantz et al., 2020)
United States	48 %	1.9	Older adults, Caucasians more affected (Mehta et al., 2014)
China	46 – 48 %	Data limited	Older adults, *JAK2* mutation in 85-91% (Lin et al., 2015)
Canada	40 – 50 %	1.6	Older adults, Caucasian ethnicity (Heppner et al., 2019)
Germany	45 %	0.5-1.5	Predominantly older adults, male (Kaifie et al., 2016)
Japan	40 %	Data limited	Equal gender, *JAK2* mutation in 80-85% (Ishitsuka et al., 2017)

In Malaysia, the epidemiology of PV and other MPN has been less extensively studied. A retrospective national registry from 2009 to 2015 documented a total of 385 PV cases, making up 38.1% of all MPN cases recorded during the study period. The average age of PV patients was 54 years, with a slight male predominance. Notably, the ethnic distribution showed a higher incidence of PV among the Chinese population, followed by Indian and Malay descent (Yap et al., 2018). Additionally, this study found that the *JAK2* V617F mutation in PV patients was significantly linked to a higher risk of arterial thrombosis, especially among older patients with hypertension (Yap et al., 2018). A more recent study, conducted from 2016 to 2020, also reported PV as representing about 47.8% of MPN cases. This recent data showed, PV patients had an average age of 58.7 years, with men accounting for 64.5% of cases. The study further affirmed that PV was more common among Malaysian Chinese, followed by Indian and Malay populations, suggesting that ethnic and genetic factors may contribute to PV prevalence (Zulkeflee et al., 2021). Although specific statistics on PV in neighbouring Indonesia are limited, the disease pattern likely mirrors that of Malaysia, commonly affecting older adults and involving the *JAK2* V617F mutation as a key diagnostic marker (Yassin et al., 2018).

However, PV exhibits common epidemiological trends globally, including higher prevalence among older adults and strong associations with the *JAK2* V617F mutation, regional differences influenced by genetics, ethnicity, and healthcare access highlight gaps in our understanding, particularly in Southeast Asia. Countries like Sweden and the United States benefit from extensive registries that provide clearer insights into the demographic and genetic patterns of patients, whereas in Malaysia and neighbouring countries, data remains limited, especially on rarer mutations like *JAK2* exon 12 mutation in PV patients. Existing Malaysian studies predominantly focus on the *JAK2* V617F mutation, leaving an important gap in understanding the prevalence, types, and clinical impact of *JAK2* exon 12 mutations.

2.1.3 Clinical Presentation

The clinical manifestations of PV are diverse and can significantly impact the quality of life of affected individuals. Common symptoms include fatigue, pruritus (itching), abdominal pain, and unexplained weight loss. These symptoms are often accompanied by more severe complications such as thrombotic events, including stroke and venous thromboembolism, which are major contributors to morbidity and mortality in PV patients. One of the hallmark features of PV is splenomegaly, which occurs in approximately 32.2% of patients in the Malaysian cohort (Yap et al., 2018). Splenomegaly can lead to symptoms such as abdominal discomfort. Additionally, microcirculatory disturbances, such as erythromelalgia which is a painful burning sensation in the hands and feet, are common in PV and can be particularly distressing for patients.

Hepatomegaly, enlargement of liver, is another known consequence of PV, a myeloproliferative condition characterized by excessive blood cell synthesis. This disorder occurs as a result of increased blood viscosity, which causes issues such as portal vein thrombosis and elevated pressure in the vascular system of the liver, as well as vascular abnormalities such as aberrant blood cell infiltration and engorged hepatic veins that are not malignant (Ahmed & Abid, 2019). Laboratory findings in PV typically include elevated haemoglobin levels, reduced or suppressed EPO levels, and the presence of the *JAK2* V617F mutation. In some cases, bone marrow biopsy may reveal hypercellularity with trilineage growth, further supporting the diagnosis (Ismail et al., 2023).

2.1.4 Diagnostic Criteria

The diagnosis of PV relies on a combination of clinical presentation, laboratory tests, and molecular analyses. According to the WHO 2022 criteria, a diagnosis of PV requires meeting either all three major criteria or the first two major criteria plus the minor criterion, as summarized in Table 2.2. The major diagnostic indicators include elevated haemoglobin levels, which are greater than 16.5 g/dL in men and greater than 16.0 g/dL in women, or haematocrit levels, which exceed 49 % in men and 48 % in women. Other indicators include hypercellular bone marrow with trilineage growth and the presence of *JAK2* mutations. The minor criterion is defined by subnormal serum EPO levels, as described by Almohmmad et al. in 2022.

Table 2. 2: World Health Organization Criteria for the Diagnosis of PV (Sources: The new WHO 2022 and ICC proposals for the classification of myelodysplastic neoplasms (2024).

Diagnosis of PV are based on the criteria below:	
Major Criteria	
Criterion No. 1 (clinical)	
Haemoglobin	>16.5 g/dL in men, >16.0 g/dL in women, or
Haematocrit	>49% in men, >48% in women, or
Red cell mass	25% increase above the mean normal predicted value
Criterion No. 2 (morphologic)	
Bone marrow morphology	Hypercellularity for age with trilineage growth (panmyelosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
Criterion No.3 (genetic)	
JAK2 V617F mutation or	Present
JAK2 exon 12 mutation	Present
Minor criterion	
Serum erythropoietin level	Subnormal

This structured diagnostic approach ensures the exclusion of secondary erythrocytosis and other myeloproliferative neoplasms. It emphasizes the importance of a comprehensive clinical, laboratory, and genetic assessment. Additionally, the inclusion of serum EPO levels as a minor criterion enhances diagnostic accuracy, particularly in borderline cases. These criteria guide clinicians in the early detection and management of PV and help reduce the risk of complications such as thrombosis and progression to myelofibrosis or acute leukemia.

Blood tests such as full blood count (FBC) play a critical role in identifying elevated red blood cell mass, leucocytosis, and thrombocytosis. Elevated haemoglobin or haematocrit levels combined with clinical features like splenomegaly or aquagenic pruritus strongly suggest PV. Bone marrow biopsy showing hypercellularity for age, with increased erythroid, granulocytic, and megakaryocytic proliferation (panmyelosis), further supports the diagnosis. However, diagnostic challenges remain, particularly in patients with early-stage disease where symptoms are nonspecific. Clinical features such as headaches, dizziness, and fatigue may overlap with other conditions. Molecular testing for *JAK2* mutations, especially *JAK2* V617F and *JAK2* Exon 12, is thus crucial for confirming PV in uncertain cases (Almohmmad et al., 2022).

2.2 Pathophysiology of PV

The pathophysiology of PV is complex interactions between genetic mutations and cellular mechanisms that result in a range of clinical manifestations and complications. At the core of PV is the dysregulation of hematopoietic stem cells, which are responsible for producing blood cells. In PV, these stem cells proliferate uncontrollably, particularly affecting the erythroid lineage. The primary driver of this dysregulation is a mutation in the *JAK2* gene, which is involved in the JAK-STAT signalling pathway. This pathway normally regulates cell growth and division. In PV, mutations such as *JAK2* V617F cause the erythroid progenitor cells to become hypersensitive to EPO a hormone that stimulates red blood cell production. Despite EPO levels being low or normal, these progenitor cells continue to produce excess red blood cells, leading to erythrocytosis, a key feature of PV (Bose & Verstovsek, 2019).

Moreover, certain PV cases are driven by *JAK2* exon 12 mutations, such as *JAK2p.N542_E543del*, are known to activate the JAK-STAT signalling pathway in a different way compared to *JAK2* V617F mutations. This activation initiates a highly erythroid-specific signalling cascade, resulting in enhanced erythropoiesis and an erythroid-dominant myeloproliferative phenotype in PV patients (Tefferi et al., 2017). Patients with *JAK2* exon 12 mutations frequently have isolated erythrocytosis and lower leukocyte and platelet counts than those with *JAK2* V617F. Exon 12 mutations increase phosphorylation of STAT1 and ERK while decreasing activation of STAT3 and AKT, a pattern that improves erythroid differentiation but has no significant effect on other blood lineages, explaining why erythrocytosis is more common than thrombocytosis. Additionally, *JAK2* exon 12 mutations have been shown to increase the number of mature erythroid cells, particularly orthochromatic erythroblasts, and to induce a higher expression of adult haemoglobin, which mirrors the clinical erythrocytosis in PV patients with this mutation (Nilsri et al., 2021).

Therapeutically, cells with *JAK2* exon 12 mutations respond well to interferon alpha and arsenic trioxide, which preferentially inhibit proliferation and promote apoptosis in mutated cells, positioning these agents as potential treatments for PV cases involving *JAK2* exon 12 mutations (Nilsri et al., 2021). This mutation-driven signalling difference also explains why PV patients with *JAK2* exon 12 mutations are often younger and present with erythroid-dominant symptoms rather than the trilineage hyperplasia seen with *JAK2* V617F (Tefferi et al., 2017). These findings highlight the importance of *JAK2* exon 12 mutations in the pathogenesis of PV, driven by the distinct and potent activation of STAT1 and ERK pathways, and support further exploration of specific therapies that target this mutation-driven phenotype.

Other than that, bone marrow hypercellularity is a hallmark of PV and reflects the overproduction of all three hematopoietic lineages which involved erythroid, granulocytic, and megakaryocytic cells. The increased cellularity contributes to the progression of the disease by overwhelming the capacity of marrow to produce healthy blood cells and leading to symptoms such as anemia and cytopenia as the disease progresses (Tefferi & Barbui, 2023).

One of the significant complications of PV is an increased risk of thrombosis. Elevated red blood cell mass increases blood viscosity, which can impair blood flow and promote clot formation. According to studies by Bose and Verstovsek (2019), there is a strong correlation between high white blood cell counts and increased thrombotic risk in PV patients. Leucocytosis contributes to this hypercoagulable state by promoting inflammation and endothelial damage. Another common feature is elevated platelet counts that increase the risk of thrombosis due to both the increased number and abnormal function of platelets. This hypercoagulability makes thrombotic events a critical concern in PV management.

PV patients frequently experience symptoms related to microvascular disturbances. These symptoms can include headaches, dizziness, visual disturbances, and erythromelalgia which is a condition characterized by redness, warmth, and pain in the extremities. These manifestations are attributed to abnormal interactions between platelets and the endothelium, as well as increased blood viscosity. Additionally, pruritus, particularly aquagenic pruritus, which is itching triggered by water exposure, is a common symptom in PV patients. This itching is thought to be linked to the release of cytokines from activated basophils and mast cells, contributing to the overall symptom burden in PV (Bose & Verstovsek, 2019).

Furthermore, PV can progress to more severe conditions, such as myelofibrosis and AML. Myelofibrosis, occurring in an estimated 15 to 25 % of PV patients, is characterized by the replacement of bone marrow with fibrous tissue, leading to pancytopenia, which is a reduction in all blood cell types, splenomegaly, and a shift towards extramedullary haematopoiesis, or blood cell production outside the marrow (Paz et al., 2020). The presence of bone marrow fibrosis at diagnosis is a significant indicator of potential rapid progression to myelofibrosis (Bose and Verstovsek, 2019). Several high-risk factors, such as elevated *JAK2* allele burden, additional mutations beyond *JAK2* such as SRSF2, and increased leukocyte counts, have been associated with higher chances of developing post PV-MF (Gerds et al., 2023). In comparison, progression to AML is less common but represents an aggressive and life-threatening stage of PV, occurring in approximately 5 to 10 % of cases (Paz et al., 2020). Factors such as older age, high leukocyte counts, and the presence of high-risk mutations, including TP53 and certain splicing factors, increase the likelihood of AML transformation. The development of prognostic models, such as the Mutation-Enhanced International Prognostic Scoring System (MIPSS), helps in assessing the risk of disease progression and guiding treatment strategies (Tefferi and Barbui, 2023).

2.3 *Janus Kinase 2 (JAK2)* Gene and Mutations

2.3.1 Overview of the *JAK2* Gene: Structure and Function

The *JAK2* gene, located on chromosome 9p24, encodes a non-receptor tyrosine kinase crucial in regulating haematopoiesis, or blood cell production. The *JAK2* protein is involved in signal transduction pathways initiated by cytokine and growth factor receptors such as the EPO receptor (EPOR), thrombopoietin receptor, and granulocyte colony-stimulating factor receptor (G-CSFR) (Ramakrishnan et al., 2023). Structurally as shown in Figure 2 below, the *JAK2* protein contains several domains which are an N-terminal FERM domain, a SH2-like domain, and two kinase domains (JH1 and JH2), with JH2 acting as a regulatory pseudo kinase that controls the catalytic activity of the JH1 domain (Skoda et al., 2015).

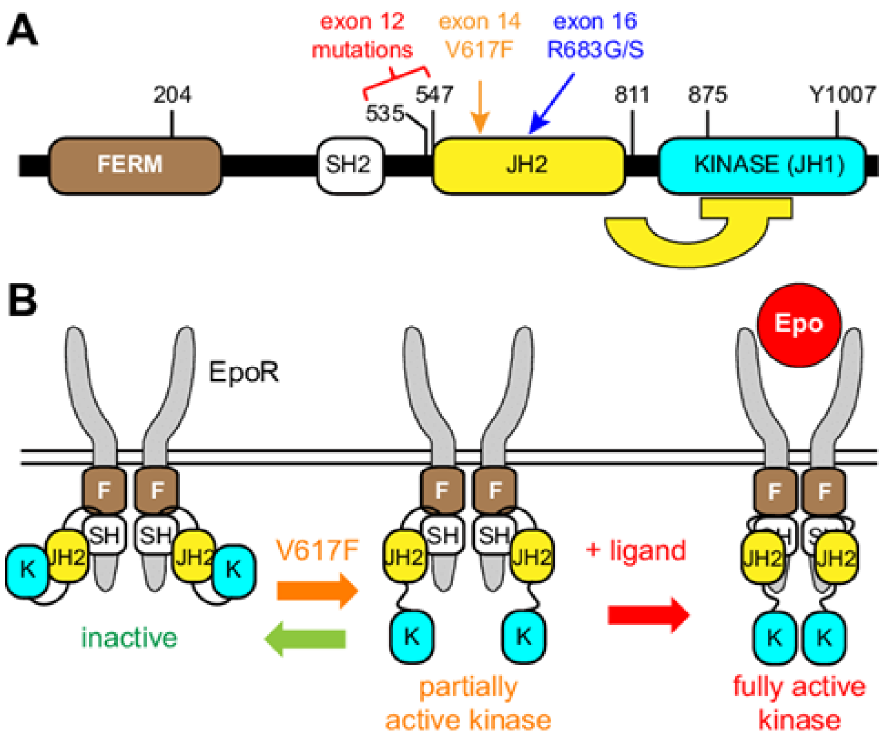

Figure 2. 1: Domain structure of the *JAK2* protein (Skoda et al., 2015).

Upon activation through ligand binding, *JAK2* triggers the JAK-STAT signalling pathway, leading to the transcription of genes that promote cell proliferation and differentiation. This pathway is particularly essential in erythropoiesis, the process by which red blood cells are produced.

2.3.2 *JAK2* V617F Mutation

The *JAK2* V617F mutation is the most frequent in PV, found in approximately 95% of PV patients. This mutation involves a substitution of valine (V) with phenylalanine (F) at position 617 in exon 14, which leads to constitutive activation of the JAK-STAT pathway. The mutation disrupts the regulatory function of the JH2 pseudo kinase domain, causing increased kinase activity and uncontrolled erythropoiesis (Khosravi et al., 2023).

This mutation enhances the kinase activity of *JAK2,* leading to excessive blood cell production, and is a primary driver of PV (Ramakrishnan et al. 2023). Higher allele burdens of the *V617F* mutation are associated with more severe clinical manifestations, including increased thrombotic risk. Studies also emphasize that patients with lower allelic burden of *JAK2* mutations tend to have better clinical outcomes, underlining the importance of monitoring allelic burden in treatment (Guglielmelli et al. 2024).

2.3.3 *JAK2* exon 12 mutations

In about 2 to 5% of PV cases exon 12 mutations represent an alternative molecular mechanism. These mutations include insertions, deletions, or duplications in the *JAK2* exon 12 regions, affecting amino acids 536 to 547. Like the *V617F* mutation, these exon 12 variants result in constitutive activation of *JAK2* and promote erythropoiesis, but their functional impacts and clinical presentations can vary (Hao et al. 2024; Dvoracek et al. 2023).

2.3.3.1 Types of Mutations/Deletions Identified in JAK2 Exon 12

JAK2 exon 12 mutations are known to play a significant role in PV, especially in cases that test negative for the more common *JAK2* V617F mutation. These exon 12 mutations are typically associated with isolated erythrocytosis without concurrent leucocytosis or thrombocytosis, making them distinct from V617F-positive PV cases. In various studies, specific mutations have been identified within exon 12, providing insight into the molecular underpinnings of PV in these patients.

For instance, a study conducted on Taiwanese PV patients revealed exon 12 mutations such as *N542-E543del, F537-K539delinsL, and I540-E543delinsKK* in patients who were V617F-negative, suggesting that these alterations contribute to the disease in a similar manner (Bose & Verstovsek, 2019). Similarly, research from Poland found mutations including *H538-K539delinsL, E543-D544del, and N542-E543del* among V617F-negative PV patients, reinforcing the relevance of these exon 12 mutations in a subset of PV cases (Tefferi, 2014).

Meanwhile, a recent analysis of Vietnamese PV patients detected two unique mutations which are, c.1592A>*G (p.H531R)* and *c.1616A>G (p.K539R),* though in silico analysis suggested that these particular variants might not significantly impact the kinase activity of *JAK2*, thereby possibly exhibiting a milder clinical presentation (Nguyen et al., 2022). Such findings underscore the diversity of mutations within exon 12 and highlight the importance of genetic testing to differentiate between *JAK2* mutation types for accurate diagnosis and management of PV.

Despite the relatively benign clinical course of exon 12 mutations compared to *V617F*, their identification is crucial as they define a unique clinical subset of PV, which can respond well to phlebotomy but still requires monitoring due to a risk of progression to myelofibrosis or other complications (Kurochkin et al., 2022).

2.3.4 Significance of Differences Between *JAK2* V617F and Exon 12 Mutations

The *JAK2* V617F and exon 12 mutations represent two distinct molecular pathways leading to PV. While both mutations enhance *JAK2* kinase activity and contribute to the disease's pathogenesis, there are significant differences between the two. The *JAK2* V617F mutation is much more prevalent, being present in approximately 95% of PV cases, whereas exon 12 mutations are observed in only 2 to 5% of cases. This disparity in prevalence underscores the dominant role of *JAK2* V617F in the molecular etiology of PV (Ramakrishnan et al. 2023).

From a clinical perspective, *JAK2* V617F mutations are often associated with more aggressive disease phenotypes. Patients with this mutation tend to exhibit increased leucocytosis, thrombocytosis, and a heightened risk of thrombotic events. In contrast, exon 12 mutations are generally linked to isolated erythrocytosis, with less significant leucocytosis and thrombocytosis, indicating a milder disease course (Hao et al. 2024). These differences in clinical manifestations highlight the need for a differentiated approach when managing patients with these mutations.

Diagnosis and treatment strategies further differentiate these mutations. *JAK2* V617F mutations are widely screened for and are easier to detect through routine molecular testing such as Allele-Specific Oligonucleotide (ASO) PCR or Amplification Refractory Mutation System (ARMS) PCR. On the other hand, detecting exon 12 mutations requires more specific molecular techniques, such as

Sanger sequencing, due to the heterogeneity of the mutations involved. These diagnostic challenges emphasize the need for tailored approaches when treating PV patients with different *JAK2* mutations.

Table 2.3 summarizes the significant differences between *JAK2* V617F and exon 12 mutations (Maddali et al., 2020), providing a clear view of how these mutations influence disease course and management strategies.

Table 2. 3: Differences in Significance Between *JAK2* V617F and Exon 12 Mutations in PV. (Source: Annals of Haematology (2020)).

Criteria	*JAK2* V617F Mutation	*JAK2* exon 12 mutation
Prevalence	Found in ~95% of PV cases	Found in ~2–5% of PV cases
Disease Phenotype	More aggressive, with leucocytosis and thrombocytosis	Milder, often with isolated erythrocytosis
Diagnostic Approach	Routine molecular testing, e.g., ASO and ARMS PCR	Requires specific techniques, e.g., Sanger sequencing
Bone Marrow Findings	Panmyelosis with megakaryocyte clustering	Often isolated erythroid hyperplasia
Epo	Typically, low	May not always be suppressed

2.4 Clinical Significance of *JAK2* exon 12 Mutations

The *JAK2* V617F and exon 12 mutations are pivotal in understanding the molecular mechanisms underlying PV. These mutations activate the *JAK2* kinase pathway, leading to increased red blood cell production and contributing to the pathogenesis of the disease. However, the clinical implications, prevalence, and prognostic outcomes associated with these mutations differ significantly. An organized comparison of these differences is essential for guiding clinical management and therapeutic approaches. Table 2.4 summarizes the key distinctions