The Genetic Makeup of Myeloproliferative Neoplasms: Role of Germline Variants in Defining Disease Risk, Phenotypic Diversity and Outcome

Elena Masselli 1,2, Giulia Pozzi 1, Cecilia Carubbi 1,* and Marco Vitale 1,2,*

1 Department of Medicine and Surgery, Anatomy Unit, University of Parma, 43126 Parma, Italy; elena.masselli@unipr.it (E.M.); giulia.pozzi@unipr.it (G.P.)
2 University Hospital of Parma, AOU-PR, 43126 Parma, Italy
* Correspondence: cecilia.carubbi@unipr.it (C.C.); marco.vitale@unipr.it (M.V.)

Abstract: Myeloproliferative neoplasms are hematologic malignancies typified by a substantial heritable component. Germline variants may affect the risk of developing a MPN, as documented by GWAS studies on large patient cohorts. In addition, once the MPN occurred, inherited host genetic factors can be responsible for tuning the disease phenotypic presentation, outcome, and response to therapy. This review covered the polymorphisms that have been variably associated to MPNs, discussing them in the functional perspective of the biological pathways involved. Finally, we reviewed host genetic determinants of clonal hematopoiesis, a pre-malignant state that may anticipate overt hematologic neoplasms including MPNs.

Keywords: myeloproliferative neoplasms; polymorphism; GWAS; germline predisposition; inflammation; clonal hematopoiesis.

1. Introduction

Classical Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) are a group of closely related stem cell disorders, namely, polycythemia vera (PV), essential thrombocythemia (ET), and pre-fibrotic and overt primary myelofibrosis (prePMF and overtPMF, respectively). Myelofibrosis may also evolve from an antecedent PV or ET, therefore termed as post-PV or post-ET MF. Bone marrow myeloid lineage(s) expansion, coupled with a variable degree of reticulin/collagen fiber deposition, abnormal peripheral blood cell count, extramedullary hematopoiesis, organomegaly, and increased inflammatory burden are hallmarks of MPNs [1].

Clonal proliferation is triggered by the acquisition of somatic mutations in specific myeloid genes, operationally classified in driver mutations, e.g., JAK2V617F and JAK2 exon 12 mutations, MPLW515 mutations, and CALR indels, covering virtually all PV cases (~99%) and the vast majority of ET and PMF cases (85–90%), and less common non-driver mutations, including TET2, ASXL1, IDH1/2, EZH2, SRSF2, LNK, CBL, TP53, etc., occurring in fewer than 30% of patients during chronic phase but typically increasing with disease progression (accelerated/blastic phase) [2].

These mutations represent crucial molecular events in the pathogenesis of MPNs, affecting disease phenotype, course, and outcome [3], and have, therefore, been included into WHO diagnostic criteria as well as in the “genetically inspired” prognostic scoring systems for disease risk stratification (i.e., the Mutation-enhanced International Prognostic Scoring System for transplant-age patients (MIPSS70), the karyotype-enhanced MIPSS70 (MIPSS70 ver2.0), and the Genetically inspired Prognostic Scoring System) (GIPSS) [4–6].
However, data from epidemiological and familial studies clearly point out a heritable component affecting the risk of developing MPN and potentially contributing to the phenotypic pleiotropy observed despite shared driver mutations [7].

Hereditary predisposition to MPNs usually refers to common, low-penetrance, germline host genetic factors, detected in the general population, whose presence facilitates the acquisition of a somatic driver mutation in a pluripotent hematopoietic stem cell, giving rise to the malignant clone [8].

Genome-wide association studies have identified a number of germline genetic patterns associated to an increased propensity for developing sporadic MPNs [9–13]. In addition, a small number of genetic variants are associated with familial predisposition. In these cases, a germline variant has been recurrently identified in family clusters of MPNs [7].

In this review we covered the main host genetic variants that have been associated to familial and sporadic MPN in terms of (1) increased disease risk, (2) impact on disease phenotype and outcome, and (3) influence on therapy response, discussing them in the functional perspective of the biological pathways involved.

2. Host Genetic Variants Associated to Familial MPNs

Host genetic factors in familial MPNs include germline duplication of ATG2B and GSKIP [14], germline RBBP6 mutations [15], and germline variants in LRRC3 and BCORL1 [16]. Of note, these MPNs do not phenotypically differ from sporadic cases and, interestingly, the affected members may carry different somatic driver mutations (JAK2V617F, MPL, and CALR) [7,8]. MPN phenotype encompasses PV, ET, and PMF in the first two studies [14,15] and only PV in the paper by Hirvonen et al. [16]. In this latter case, all patients displayed JAK2V617F mutation.

It has been hypothesized that overexpression of ATG2B and GSKI, resulting from germline duplication, may account for increased fitness for cells subsequently acquiring somatic mutation(s), hinting, therefore, that germline variants and acquired somatic driver mutations are cooperative events for MPN development [14].

RBBP6 gene encodes for a ring finger E3 ubiquitin ligase involved in p53 degradation. RBBPR1569H germline variant alters the p53 binding site, thus conferring an increased risk of mutagenesis. In this case, therefore, the correlation with all three hallmark driver mutations is explained by genetic instability [15].

3. Host Genetic Variants Associated to Increased MPN Risk in General Population

Germline variants reaching the conventional threshold ($p < 5 \times 10^{-8}$) for genome-wide significant association with an increased MPN risk are summarized in Table 1.

| SNPs               | Gene Function                      | Associated Driver Mutations | Associated MPN Phenotype | Ref. |
|--------------------|------------------------------------|-----------------------------|--------------------------|------|
| JAK2 46/1 haplotype| Hematopoiesis, cytokine receptor signaling | All                         | All                      | [9–13] |
| TERT               | Telomere length                    | All                         | All                      | [10,12] [11,13] |
|                    |                                    | (>JK2V617F)                 | (>PV and PMF)            |      |
| MECOM              | HSC maintenance, differentiation    | JAK2V617F and CALR type 1/type 1-like | PV MF and ET (only in presence of CALR) | [12] [11] [13] |
|                    |                                    | none                        | ET (only in presence of JAK2V617F) | [11,12] |
| HBS1L-MYB          | Peripheral blood cell counts, fetal hemoglobin levels | none                        | n/a                      |      |
|                    |                                    | n/a                         | n/a                      |      |
| GFI1B              |                                    | n/a                         | n/a                      |      |
| Polymorphism | Function | Ref. | Haplotype |
|--------------|----------|------|----------|
| rs621940     | unknown  |      |          |
| rs1633768    | unknown  |      |          |
| rs524137     | unknown  |      |          |
| CHEK2        | DNA damage response | n/a | n/a |
| rs555607708  | DNA damage response | n/a | n/a |
| rs17879961   | DNA damage response | n/a | n/a |
| SH2B3        | HSC self-renewal, commitment, terminal differentiation of monocytes | n/a | n/a |
| rs7310615    | HSC self-renewal, commitment, terminal differentiation of monocytes | n/a | n/a |
| ATM          | HSC self-renewal, commitment, terminal differentiation of monocytes | n/a | n/a |
| rs1800057    | HSC self-renewal, commitment, terminal differentiation of monocytes | n/a | n/a |
| TET2         | HSC self-renewal, commitment, terminal differentiation of monocytes | n/a | n/a |
| rs1548483    | HSC self-renewal, commitment, terminal differentiation of monocytes | n/a | n/a |
| rs62329718   | HSC self-renewal, commitment, terminal differentiation of monocytes | n/a | n/a |
| PINT         | DNA damage response, hematopoietic stem cell maintenance, and differentiation (via PRC2) | n/a | n/a |
| rs58270997   | DNA damage response, hematopoietic stem cell maintenance, and differentiation (via PRC2) | n/a | n/a |
| THRB-RARB    | unknown | none | PMF |
| rs4858647    | unknown | none | PMF |
| GATA2        | HSC activity and self-renewal, myeloid and myelo-erythroid differentiation, erythroid precursors maintenance | n/a | n/a |
| rs9664772    | HSC activity and self-renewal, myeloid and myelo-erythroid differentiation, erythroid precursors maintenance | n/a | n/a |
| SCHIP1       | unknown | n/a | n/a |
| rs77249081   | unknown | n/a | n/a |
| KIF4A        | unknown | n/a | n/a |
| rs74676712   | unknown | n/a | n/a |
| NUDT3        | unknown | n/a | n/a |
| rs16466979   | unknown | n/a | n/a |
| MKL1         | unknown | n/a | n/a |
| rs61471615   | unknown | n/a | n/a |
| MRPS31       | unknown | n/a | n/a |
| rs8002412    | unknown | n/a | n/a |
| ZNF21        | HSC differentiation and B-lymphoid cell development | n/a | n/a |
| rs9946154    | HSC differentiation and B-lymphoid cell development | n/a | n/a |
| RUNX1        | Differentiation of megakaryocytes and lymphocytes | n/a | n/a |
| rs55857134   | Differentiation of megakaryocytes and lymphocytes | n/a | n/a |

Abbreviations: HSC: Hematopoietic Stem Cell; n/a: not assessed; PRC2: polycomb repressive complex 2; > indicates a stronger association.

Germline polymorphisms at JAK2 and TERT endow individuals with a predisposition to developing a MPN, which emerged from all the four main studies interrogating large patient and control data sets by a genome-wide association approach [10–13]. Polymorphisms at 3q26.2 and 3q26.3, involving MECOM and the intergenic region between HBS1L and MYB, have been associated to increased MPN risk in three out of the four above referenced studies [11–13]; the association did not emerge in the Icelandic population, described by Oddsson et al. [10].

Other recurrent variants include TET2, ATM, SH2B3, PINT, CHECK2, and GFI1B loci, first identified by Hind et al.[11] and very recently confirmed by Bao and colleagues[13].

### 3.1. The JAK2 46/1 Haplotype

The JAK2 “46/1” or “GGCC” haplotype has been the first germline risk variant described in MPNs, and simultaneously reported by three independent groups of investigators in 2009 [9,17,18]. The JAK2 46/1 haplotype maps on a region of about 250–280 Kb on the short arm of chromosome 9 including, in addition to JAK2, also Insulin-like 6 (INSL6) and INSL4 genes. The haplotype is tagged by a combination of four single nucleotide polymorphisms (SNPs)—rs3780367, rs10974944, rs12343867, and rs1159782—mapping on JAK2 introns 10, 12, 14, and 15 and generating the so-called “GGCC” sequence.

These SNPs are in complete linkage disequilibrium and, therefore, inherited en bloc. The frequency of this haplotype is around 45% in the general population and its presence
has been associated to an increased risk of MPN onset, preferentially — but not exclusively — carrying JAK2 driver mutations (including V617F exon 14 mutation and exon 12 mutations). This is likely due to an increased susceptibility to DNA damage and replication errors conferred by the haplotype, which may predispose to the acquisition of the somatic JAK2 mutations (“hypermutability hypothesis”) or, at the opposite, may confer a selective advantage to randomly JAK2-mutated clone (“fertile ground hypothesis”) [17].

Although the association is stronger with JAK2V617F-positive MPNs, recent GWAS studies demonstrate that the JAK2 46/1 haplotype reaches the genome-wide level of significance (p < 5 × 10⁸) also in JAK2V617F-negative cases [12] including those carrying CALR mutations [18]. These data, of course, should lead us to search for a broader explanation of the mechanisms by which the JAK2 46/1 haplotype predisposes to MPN, which likely goes beyond the “hypermutability” or “fertile ground” hypotheses, which can explain the association with JAK2V617F-positive but not with JAK2V617F-negative cases (i.e., increased mutation rate of the JAK2 locus and selective advantage of the JAK2V617F mutated clone) [18].

The demonstration of a broader, pleiotropic effects of these germline variants derives from a study conducted in patients with normal karyotype acute myeloid leukemia, showing that homozygosity or heterozygosity for the JAK2 46/1 haplotype was associated with myelomonocytic-biases malignant hematopoiesis and increased risk of death from infection due to an aberrant immune response [19].

In this regard, an intriguing perspective was provided by Hermouet and coll. [20], who hypothesized that the 46/1 haplotype may facilitate the overexpression of the JAK2 gene (by recombination or abnormal promoter binding/methylation) and the consequent activation of pro-proliferative and pro-survival pathways in myeloid cells, which, in turn, predisposes to an increased risk of DNA replication errors and mutations in pathogenetically relevant myeloid genes (including CALR, MPL, TET2, ASXL1, etc.). This would explain the association with JAK2-unmutated MPNs. Moreover, the overexpression of INSL4 and INSL6 (also part of the haplotype) in bone marrow stroma cells may lead to an increased production of pro-inflammatory cytokines, generating a permissive milieu for the mutated clone [20]. Overall, the JAK2 46/1 haplotype could be envisioned as a host genetic susceptibility factor for inappropriate myeloid response to cytokines, leading to an enhanced inflammatory state and increased risk of myeloid neoplasms.

3.2. Telomere Reverse Transcriptase Gene (TERT) Polymorphisms

The telomere reverse transcriptase gene (TERT) encodes for the catalytic component of telomerase, a ribonucleoprotein enzyme that stabilizes telomere length, preventing the activation of the cellular senescence program [21]. TERT biological function makes it a strong candidate for factors that influence cancer risk. Indeed, enhanced telomerase activity/expression has been extensively associated to several types of cancers [22]. The impact of short telomeress in disease was first appreciated in dyskeratosis congenita (DC), a disorder typified by the mucocutaneous triad (oral leukoplakia, abnormal skin pigmentation, and nail dystrophy), bone marrow failure, cancer predisposition, pulmonary fibrosis, and liver dysfunction [23]. The high penetrance of bone marrow failure among patients with classic DC highlights the importance of telomere length maintenance in hematopoiesis. Indeed, the mechanism underlying DC-related bone marrow failure appears to be related to a progressive depletion of functional hematopoietic progenitor and stem cells [24,25].

The rs2736100 SNP located in the second intron of the TERT gene at 5p15 has been associated to an increased risk of cancer [26], including MPNs. According to Tapper et al. [12], the association between the rs2736100 SNP and MPN risk reaches, however, genome-wide significance only when including JAK2V617F-positive cases. Hence, similarly to the JAK2 46/1 haplotype, the effects are stronger in JAK2V617F-mutated MPNs. According to Trifa et al. [18], the SNP was significantly associated with each single MPN, regardless of driver-mutation status.

This SNP was first described by Oddsson et al. [10] in 237 Icelanders diagnosed with MPN and, although the underlying functional mechanism is still debated, it is thought to
(1) directly increase the transcription of TERT, enhancing its expression, and (2) be in linkage disequilibrium with biologically plausible disease-causing mutations.

In addition to the rs2736100, other variants are enriched in MPN populations, specifically the rs7705526 and the rs2853677 [11,13], which, similarly to the rs2736100, emerged from previous GWAS cancer studies [27]. Hind et al. showed that the lead SNP rs2853677 is in moderate linkage disequilibrium with the rs2736100 [11].

Interestingly, a growing body of evidence has been accumulated on non-canonical functions of telomerase reverse transcriptase, including cell cycle regulation, promotion of cell growth and proliferation, and control of mitochondrial integrity following oxidative stress [28]. Wang et al. observed a dose-dependent effect of the rs2736100 G-allele on IL-6 levels in non-small cell lung cancer, suggesting a role for this SNP in IL6 gene expression modulation and cytokine production [29]. This observation is of utmost interest in the context of MPNs, in which the inflammatory background is the main trigger and driver for clonal evolution [30].

Besides TERT rs2736100, telomere length is genetically determined by, at least, 10 other SNPs (ZNF676 rs412658, CTC1 rs3027234, DHX35 rs6028466, PXX rs6772228, NAF1 rs7675998, ZNF208 rs8105767, OBFC1 rs9420907, ACYP2 rs11125529, TERC rs10936599, and ZBTB46 rs755017) [31–34]. This observation derives from studies on telomere length measured in leukocytes (LTL), which is considered a reliable surrogate for telomere length in other tissues, relatively stable over time. A polygenic risk score (named “teloscore”) based on the combination of these 11 SNPs—detected by GWAS—has been successfully utilized to investigate the association between genetic determinants of telomere length and increased cancer risk.

Giaccherini et al. [35] tested the association between teloscore (built by weighting the effects of each of the 11 SNPs on LTL) and MPN risk, surprisingly finding that genetically determined longer telomeres predict higher MPN risk. Individual SNP association studies (by allelic discrimination assays) confirmed the TERT rs2736100 C-allele as a high-risk variant for increased MPN susceptibility and additionally reported a novel association of the OBFC1 rs9420907 C allele and increased MPN risk.

3.3. Polymorphisms in 3q26 (MECOM and HBS1L-MYB)

Polymorphisms at 3q26.2 and 3q26.3, involving MECOM and the intergenic region between HBS1L and MYB (the so-called HMIP region), account for increased MPN risk.

MECOM (MDS1 and EVII Complex Locus) gene encodes for a transcription factor involved in hematopoietic stem cell maintenance, differentiation, and leukemogenesis [36]. MECOM has a well-established role in hematologic neoplasms. Germline mutations have been reported to be causative of a rare, inherited bone marrow failure syndrome with a megakaryocytic thrombocytopenia associated to various organ malformations with variable penetrance [37]. In addition, chromosomal rearrangements between 3q21 and 3q26 elicit high-risk acute myeloid leukemia, via the GATA2 enhancer reposition near the MECOM locus, which results in both EVII overexpression and GATA2 haploinsufficiency [38,39].

Three different MECOM SNPs have been identified as genome-wide significant loci in MPNs: the rs22018862 [12], the rs3861397 [11], and the rs9847631 [13]. In the study authored by Tapper et al. [12], the rs22018862 SNP, located in a non-coding region 153 Kb downstream of MECOM, was the only germline variant that, along with the JA2 46/1 haplotype, maintained genome-wide significance when analyses were restricted to JA2V617F-negative MPNs. Functional implications of this SNP are not established yet; however, based on publicly available databases, the authors hypothesized that this region may correspond to a regulatory element with enhancer-like activity. The rs22018862 SNP appears to correlate with PV and CALR-mutated ET and PMF [18].

HMIP SNPs, located in the intergenic region between HBS1L and MYB, account for interindividual variability of hematologic parameters in healthy subjects, particularly of platelet, monocyte, and erythrocyte counts [40–44]. Additionally, polymorphisms of the HBS1L-MYB intergenic region have been reported to influence fetal hemoglobin levels in
adults [45], with relevant functional and clinical implications in β hemoglobinopathies [46,47]. The mechanism by which these noncoding sequence variants affect multiple erythrocyte characteristics has been elucidated in subsequent, follow-up studies, demonstrating that *HMIP* polymorphisms affect regulatory elements bound by erythroid transcription factors. These elements interact with *MYB*, a critical regulator of erythroid development and HbF levels [48]. In fact, it has been shown that *MYB* plays crucial role in silencing the fetal and embryonic hemoglobin genes[49].

Two GWAS studies identified the rs9376092 SNP as a susceptibility germline variant for increased MPN risk [11,12]. The rs9376092 is in strong linkage disequilibrium with the other SNPs tagging the *HMIP* region. The polymorphic allele variant modulates the expression of both flanking genes, especially of *MYB*, which is down-modulated. Interestingly, *MYB* down-regulation is responsible for enhanced normal and clonal megakaryopoiesis, as demonstrated by the fact that *MYB* knockdown in normal human hematopoietic progenitors promotes megakaryocyte development [50], and that *MYB* knockdown in the murine Kit+Sca1+Lin- hematopoietic stem cell population generates a transplantable myeloproliferative phenotype that mimics ET [51].

Consistently, genotype–phenotype correlations by Trifa and Colleagues indicated that the rs9376092 SNP preferentially associates with *JAK2V617F*-positive ET [18].

Of note, *MYB* deregulation from a novel *EWSR1-MYB* fusion was detected at the time of leukemic evolution of a *JAK2V617F*-positive PMF case[52].

3.4. *GFI1B* and *CHEK2* Polymorphisms

*GFI1B* and *CHEK2* SNPs were first reported as genome-wide significant loci for increased MPN risk by Hind et al. [11]. Very recently, Bao and Colleagues provided a complete functional characterization of the mechanisms by which *GFI1B* and *CHEK2* genetic variations affect the MPN risk through the modulation of hematopoietic stem cell function[13].

*GFI1B* mutations have been associated to a rare, dominant, congenital platelet disorder known as *GFI1B*-related thrombocytopenia (*GFI1B*-RT), caused by the presence of truncated *GFI1B* proteins with dominant-negative properties on megakaryocytopenia and thrombopoiesis [53]. The SNPs accounting for MPN predisposition are the rs621940 [11], the rs1633768, and rs524137 [13], of which the last two are located in a region of hematopoietic-accessible chromatin located around 12 kb downstream of *GFI1B*.

The checkpoint kinase *CHEK2* is a critical component of the DNA damage response pathway. Germline variants reaching GWAS-significant association with increased MPN risk include the rs555607708 [11] and the I157T missense variant (rs17879961) [13], which have been previously linked to an increased risk for several types of cancer, including chronic lymphocytic leukemia [54].

Bao and coll. provided the first demonstration that the rs524137 SNP of *GFI1B* and the rs17879961 SNP of *CHEK2* are functionally relevant, by reducing, respectively, *GFI1B* expression and *CHEK2* function in hematopoietic stem cell and thereby increasing their self-renewal [13].

4. Host Genetic Variants Modulating Disease Phenotype and/or Outcome

Although not implicated in conferring an increased MPN risk, several SNPs have been described to harbor disease-modifying effects, in terms of phenotype, hematologic parameters at the time of MPN onset, disease course, and outcome. Moreover, the *JAK2* haplotype, repeatedly confirmed as a strong host genetic predisposition factor for MPN in GWAS, has been identified as a biomarker of disease outcome in PMF by Tefferi’s group. In a first study published in 2010, nullizygosity for the *JAK2* haplotype was associated to shortened survival in a cohort of 130 PMF patients [55]. Subsequently, in a follow-up study on a cohort of 414 molecularly annotated PMF, the authors confirmed that wild-type patients displayed inferior overall survival as compared to the other genotypes, independently from well-
established genetic and cytogenetic markers of poor outcome (karyotype, driver mutational status, presence of high-molecular-risk mutations) [56].

Table 2 summarizes SNPs capable to influence MPN phenotype and outcome, whose early detection is shaping as an informative tool for personalized patient follow-up and treatment planning.

| SNPs | Gene Function (Relative to Hematopoiesis) | Detection Methods | Allele Variant | MPN Cohort | Disease Subtype Associations | Disease Phenotype Associations | Ref. |
|------|-------------------------------------------|-------------------|---------------|-------------|------------------------------|-------------------------------|------|
| JAK2 46/1 haplotype rs12343867 (T/C) | Hematopoiesis, cytokine receptor signaling | RT-PCR | T allele (wild type) | 130 PMF | n/e | ↓ OS | [55] |
| | | | T allele (wild type) | 414 PMF | n/e | ↓ OS | [56] |
| NR3C1 rs6198 (A/G) | Immune response regulation, erythropoiesis | PCR-SSCP + sequencing | G-allele | 57 MPNs | 22 CTRLs | PV | n/e | [57] |
| | | HRM analysis + sequencing | G-allele (homozygous) | 499 PMF | 2948 CTRLs | PMF | ↑ CD34+ cells, splenomegaly, ↑WBC, ↓ LFS* | [58] |
| CCL2 rs1024611 (A/G) | Chemokine production | RT-PCR | G-allele | 177 MPNs | 149 CTRLs | sMF | ↓ Hb, ↑ IPSS, ↑ blasts, ↑ fibrosis | [59] |
| | | RT-PCR | G-allele (homozygous) | 773 PMF | 323 CTRLs | PMF in males | ↓ OS | [60] |
| MIR146a rs2431697 (C/T) | NF-κB signaling modulation | RT-PCR | T-allele (homozygous) | 967 MPNs | 600 CTRLs | sMF | ↓ MF-free survival in PV and ET | [61] |

Abbreviations: RT-PCR: real-time Polymerase Chain Reaction; OS: Overall Survival; PCR-SSCP: Polymerase chain reaction-single-stranded conformation polymorphism; HRM: High-Resolution Melting; n/e: not evaluated; WBC: White Blood Cells; LFS: Leukemia-free survival; Hb: hemoglobin; IPSS: International Prognostic Scoring System. *only for JAK2V617F+ PMF.

4.1. The rs6198 SNP of the Glucocorticoid Gene

The human glucocorticoid receptor is encoded by NR3C1 located in the 5q31-32 cytoband of chromosome 5 and is composed of nine exons with five splicing variants: GRα, GRβ, GRγ, GR-A, and GR-P. GRα and GRβ are generated by alternative splicing of exons 9α and 9β, respectively. While GRα resides primarily in the cytoplasm and can interact with endogenous or synthetic agonists, GRβ constitutively resides in the nucleus, where it controls transcription primarily by a dominant-negative effect on GRα-induced gene expression [62].

The NR3C1 gene is highly polymorphic. The rs6198 A to G SNP is located within the 3’ untranslated region and it increases the stability of GRβ mRNA, with a half-life up to 6 hours, enhancing, therefore, GRβ expression [63]. In healthy subjects, this SNP is present with an allele frequency between 4% (sub-Saharan Africans) and 20% (Europeans) but its frequency increases in patients with autoimmune disorders, where it also mediates glucocorticoid resistance [64].

The biological role of GRβ and its SNP in MPNs were first investigated by Varricchio and coworkers [57]. The authors found that the dominant negative β isoform of the glucocorticoid receptor is selectively expressed in erythroid cells expanded from patients with polycythemia vera, where it contributes to the development of erythrocytosis. GRβ isoform expression is likely attributable to the increased prevalence of the rs6198 SNP of GR in PV patients, reporting a 55% frequency of the polymorphic allele variant as compared to 9% in healthy control subjects. Given this background, the same group analyzed frequency, genotype–phenotype correlations, and impact on disease progression of the rs6198 SNP of GR in a cohort of 499 PMF. The rs6198 variant, as tagged by the G allele, occurred with a higher frequency in PMF patients as compared to 111 local healthy volunteers and 2837 controls from the UK (genotyped by the WTCCC). Homozygosity for the SNP is associated with higher white blood cell count, splenomegaly, and higher circulating CD34+ cells at the time of diagnosis. Finally, JAK2V617F-positive PMF carrying both risk alleles display shorter
overall and leukemia-free survival as compared to the other genotypes [58]. Therefore, the rs6198 SNP should be considered a host genetic factor for adverse presentation and outcome (when associated to the JAK2V617F mutation) in PMF.

4.2. The rs1024611 SNP of CCL2

The CCL2 gene, encoding for the chemokine CCL2, also known as Monocyte Chemo-attractant Protein-1 (MCP-1), is located in the q11.2-12 cytoband of chromosome 17 and, similarly to other cytokine genes, is highly polymorphic. The rs1024611 SNP, characterized by an A to G substitution in the distal regulatory region of the gene, modulates the transcriptional activity of CCL2, accounting, therefore, for interindividual variability in the levels of circulating chemokine. As a result, G/G homozygous individuals are the highest chemokine producers. In a healthy population, the G allele is over-represented in Asians and Mexicans as compared to Caucasians and African Americans [65,66]. Subjects carrying the rs1024611 SNP of CCL2 display an increased susceptibility to several conditions such as autoimmune disorders, atherosclerosis, and cancer [67–69].

We investigated the prevalence of the rs1024611 SNP of CCL2 in MPNs, finding similar genotypic and allelic frequencies among PV, ET, and control subjects. Focusing on MF, which can be considered the MPN variant characterized by the highest inflammation burden, we found that postPV/ET MF were significantly enriched in polymorphic individuals as compared to (1) overall PMF, (2) prePMF, (3) overtPMF patients, and (4) healthy controls. Additionally, in MF patients, genotype–phenotype correlation studies pointed out a higher frequency of allele-G carriers in (1) intermediate-2/high-IPSS-risk group; (2) patients with severe anemia (Hb < 100 g/L); (3) patients with ≥1% circulating blasts; and (4) patients with ≥ II grading of bone marrow fibrosis [59].

Recently, in a cohort of 773 PMF, we assessed the contribution of this SNP in terms of increased disease risk and its potential effect on disease outcome, demonstrating that male subjects carrying the homozygous genotype G/G had an increased risk of PMF, hinting at a potential genetic interplay between gender and the G-allele variant. We also found that G/G PMF displayed a significantly reduced survival. In combined analysis of the G/G genotype with other well-established clinical prognostic parameters included in the IPSS scoring system we found a significant correlation in both univariate and multivariate analysis. Finally, we determined the functional implication of this SNP in PMF, showing that higher CCL2 circulating levels, determined by the G/G rs1024611 genotype, are biologically relevant since PMF hematopoietic progenitors selectively express CCR2 and activate a pro-survival, Akt-dependent signaling pathway when stimulated with CCL2 [60].

4.3. The rs2431697 of MIR146A

NF-κB signaling hyperactivation has been described in mouse models of MPN as well as in MF and MPN blast phase [70]. Indeed, NF-κB has been identified as a key mediator of inflammation-triggered carcinogenesis [71] and, in MF, it has been suggested to cooperate with JAK2 signaling hyperactivation in supporting cytokine overproduction [70]. On the other side, NF-κB itself mediates the signal transduction downstream of several cytokine and chemokine receptors, including CCR2 [72], which plays a relevant role in PMF pathophysiology, as above described.

NF-κB is regulated by microRNAs (miRNAs), small, single-stranded non-coding RNA molecules capable to regulate gene expression. The miR-146a counteracts the NF-κB-dependent proinflammatory signal via inhibition of TNF Receptor-Associated Factor 6 (TRAF6) and Interleukin 1 Receptor-Associated Kinase 1 (IRAK1). Indeed, miR-146a deficiency accounts for a chronic inflammatory phenotype and enhanced myeloproliferation [73].

MiR-146a levels are regulated by two functional SNPs: rs2910164 and rs2431697, the latter accounting for lower miR-146a expression. Ferrer-Marin et al. investigated whether these SNPs could represent susceptibility factors for MPNs, demonstrating—in a cohort of 967 MPNs—that the rs2431697 T/T genotype (1) is associated to increased pro-inflammatory
cells
rs368234815
2021
Abbreviations:
Response;
rs12979860
rs8099917
INFL4
(T/G)
INFL4
(T/C)
rs368234815 (G/TT)
rs8099917
(T/G)
Table 3. Host genetic variants affecting response to therapy in MPNs.

| SNPs          | Gene Function (Relative to Hematopoiesis) | Detection Methods | Allele Variant | MPN Cohort | Type of Therapy | Response Assessment | Association(s) | Ref.  |
|--------------|------------------------------------------|-------------------|----------------|------------|----------------|---------------------|----------------|-------|
| **INFL4**    | Cytokine production                      | RT-PCR            | C/C            | 100 MPNs   | HR             | Higher HR rate in PVs | [76]          |       |
| rs12979860   |                                          |                   |                |            | peg α-2b       |                     |                |       |
| (T/C)        |                                          |                   |                |            | peg α-2a       |                     |                |       |
| rs8099917    |                                          |                   |                | 122 PVs    | HR             | Higher MR rate       | [77]          |       |
| (T/G)        |                                          |                   |                |            | peg ropeg α-2b |                     |                |       |
| rs368234815  |                                          |                   | TT/TT          | 122 PVs    | HR             | Higher MR rate       | [77]          |       |
| (G/TT)       |                                          |                   |                |            | peg α-2b       |                     |                |       |

Abbreviations: RT-PCR: real-time Polymerase Chain Reaction; HR: Hematologic Response; Inf: interferon; MR: Molecular Response; peg α-2b/-2a: peginterferon α-2b/-2a; ropeg α-2b: ropeginterferon α-2b.

Lindgren and Colleagues, by retrospectively analyzing a cohort of 100 MPN patients (47 PVs, 43 ETs, and 10 MFs), demonstrated that the homozygosity (C/C genotype) for the rs12979860 located in between IFNL3 and IFNL4 was significantly associated with achievement of complete hematologic response (HR) in PVs (79% in C/C patients vs. 48% of other genotypes) [76].

In line with these findings, Jager et al. evaluated how germline variants of INFL3/INFL4 loci influenced the hematologic and molecular response (MR) in a cohort of 122 PV patients enrolled in the PROUD-PV and CONTINUATION-PV trials treated with ropeginterferon alfa-2b, over a follow-up period of 36 months. An initial GWAS screening analysis did not reveal any significant associations (p < 5 × 10^-6) between germline genetic variants and the achievement of HR and MR. However, target association analyses, focused on rs8099917, rs12979860, rs368234815, and rs117648444, showed that rs8099917 T/T, rs12979860 C/C, and rs368234815 TT/TT genotypes individually correlated with higher MR rates. In addition, functional diplotype affecting INF4 production were associated with variable MR rates, with haplotype pairs determining loss of function/impaired function protein variants being associated with higher responses [77].

Therefore, functional INFL locus SNPs may explain heterogeneity in INF-α response in MPN patients.
6. Host Genetic Determinants of Clonal Hematopoiesis of Indeterminate Potential (CHIP)

CHIP refers to an age-related phenomenon characterized by the acquisition of somatic mutations leading to the expansion of clonal hematopoietic stem and progenitor cells. A relevant clinical scenario associated with CHIP is represented by the increased risk of cardiovascular events, likely driven by an increased inflammatory state sustained by mature, mutant myeloid cells [78].

The two most commonly mutated genes in CHIP are DNMT3A and TET2, followed by ASXL1, splicing factors, JAK2, and TP53 [78]. In addition to acquired somatic mutations, two recent genome-wide association studies identified germline genetic variants that predispose to CHIP [79,80].

By whole-genome sequencing on 11,262 Icelanders, Zink et al. [79] described a germline deletion located in intron 3 of TERT (rs34002450) that was significantly associated to CHIP. This finding hinted to a role of telomerase activity in CHIP, both age-related phenomena. Indeed, the Authors demonstrated that telomere length estimates were significantly lower in people with CHIP [79].

Very recently, Bick et al. [80] identified three genome-wide significant loci conferring an increased risk of CHIP involving TERT, TET2, and the intergenic region spanning KPNA4-TRIM59. Concerning TERT, the authors replicated the association with rs34002450 described by Zink. et al. [79] and additionally identified a second intronic TERT variant—rs13167280—independently associated with CHIP status.

TET2 variant, namely, rs1444188061, was found exclusively in samples from individuals with African ancestry, and, despite selectively involving TET2 gene, it was equally robustly associated with DNMT3A-, TET2-, and ASXL1-dependent CHIP; this has been ascribed to the fact that TET2 risk variant disrupts the distal enhancer of the gene, hampering TET2 expression, therefore increasing self-renewal of hematopoietic stem cells [80].

Overall, these seminal studies indicate that germline variants not only affect the risk of developing a hematologic malignancy, but also intervene in shaping the risk of early, pre-malignant states such as CHIP. Of note, both TERT and KPNA4 display germline variants predisposed to CHIP [79,80] and increased MPN risk [13].

7. Conclusions and Perspectives

Germline genetic factors play a relevant role in determining individual predisposition to develop an MPN, and, once the disease is acquired, in affecting disease presentation, course, therapy response, and outcome. This is consistent with the fact that disorders sharing the same somatic driver mutations are nevertheless typified by an extremely heterogenous presentation and behavior. The increasingly widespread use of novel techniques such as Next-Generation Sequencing during the routine diagnostic work-up of MPN patients will allow us to detect prognostically relevant SNPs and potentially include them in a personalized risk stratification, as envisioned, for example, for the JAK2 46/1 haplotype [81].

Indeed, the use of SNP arrays with custom probes for selected, informative gene variants may lead in the future to a germline genetic screening for MPN patients, aiming to identify those being more likely to experience an unfavorable disease course and poor response to therapy, and providing, therefore, physicians with novel tools for adequate patient counseling and personalized disease monitoring.

Clinically relevant SNPs in MPNs discussed in this review basically affect genes involved in the three main biological processes: (1) hematopoiesis; (2) DNA damage repair; and (3) inflammation (Figure 1):
Figure 1. Germline variants associated with MPN risk, phenotype, outcome, and therapy response classified according to their biological function. Genes exerting multiple functions are highlighted in white.

(1) Hematopoiesis defines the tightly regulated process of formation of blood and immune cells. To generate these cells, HSCs give rise—throughout individuals’ life span—to an array of committed progenitors, which proliferate extensively and then differentiate into mature cells. Recent advances in genomics, such as accurate deep sequencing and novel methods of cell tracking, revolutionized the concept of hematopoiesis from a process made of discrete, punctuated phenotypic changes to a “continuum model”, typified by a continuous process of differentiation with blurred demarcation between different stages [82,83]. Genetic studies revealed, also, how mechanisms underlying hematopoiesis are modulated by genetic variations present throughout the population. The importance of these host genetic variations is highlighted by the fact that clinically measured hematopoietic traits typically show extensive interindividual variability and are highly heritable, which means that a relevant part of the observed phenotype variations can be attributed to genetic factors [82,84].

(2) During genome duplication, cells may experience different exogenous and endogenous replication stresses, hampering the progression of DNA replication. Replication stress is a phenomenon exacerbated in cancer cells because of the loss of DNA repair genes or the activation of oncogenic pathways [85]. To counteract replication stress, cells are equipped with DNA damage response, an extensive network of signaling pathways accounting for recognition of DNA damage, DNA remodeling and repair, DNA damage bypass during replication, cell cycle control, and cell fate decisions in response to DNA alterations [86]. More than 450 genes are involved in this network. In addition to MPNs, a variety of polymorphisms in DDR genes have been associated with increased risk of developing acute myeloid leukemia [87] and breast cancer [88].

(3) Inflammation refers to a host defense mechanism orchestrated by the immune system in response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation [89]. Cytokines are key mediators of the inflammatory response, by promoting the recruitment and activation of immune cells. After the human leucocyte antigen (HLA), chemokine genes are probably one of the most polymorphic sets of genes in the immune system, with remarkable effects on the immune response. A number of functionally relevant cytokine SNPs have been found repeatedly associated with disease of
different etiologies but sharing a common pathogenetic aspect such as chronic inflammation [67].

Concerning the functional overview of MPN-related SNPs reported in Figure 1, we can observe that some of the genes involved have multiple functions, with consequent overlap between categories. Moreover, in addition to the above-described overarching categories (hematopoiesis, DNA damage repair, and inflammation), TERT (involved in the cellular aging process) and TET2 (regulating epigenetic changes) must be considered as well.

Examples of functional overlap involve the JAK2 haplotype, playing a role not only in hematopoietic stem/progenitor cell fate by predisposing to the acquisition of autonomous cell growth [9,17,18] but also in systemic inflammation, by fueling an inappropriate myeloid response to cytokines [20]. Similarly, the NR3CI SNP predisposes to both immune dysregulation—by mediating glucocorticoid resistance [90]—and to JAK/STAT signaling abnormalities by associating with STAT3 [57]. Additionally, TERT gene not only regulates cell senescence but also influences IL6 gene expression and hematopoiesis [29]. Finally, CHEK2 gene, a well-characterized component of the DNA damage response pathway, has been endowed with novel regulatory functions in determining hematopoietic stem cell fate [13].

Overall, most of these MPN SNPs share a role in modulating the individual proinflammatory state, being the common host genetic denominator of immune dysregulation, chronic inflammation, and clonal proliferation.

MPNs are a well-established paradigm of oncoinflammatory disorders [30], and it is reasonable to hypothesize that the genetically determined host inflammatory background exerts a relevant role in influencing the features of the disease, thus accounting for phenotypic diversity in MPNs.

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References
1. Arber, D.A.; Orazi, A.; Hasserjian, R.; Thiele, J.; Borowitz, M.J.; Le Beau, M.M.; Bloomfield, C.D.; Cazzola, M.; Vardiman, J.W. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016, 127, 2391–2405.
2. Greenfield, G.; McMullin, M.F.; Mills, K. Molecular pathogenesis of the myeloproliferative neoplasms. J. Hematol. Oncol. 2021, 14, 103, doi:10.1186/s13045-021-01116-z.
3. Grabek, J.; Straube, J.; Bywater, M.; Lane, S.W. MPN: The Molecular Drivers of Disease Initiation, Progression and Transformation and their Effect on Treatment. Cells 2020, 9, 1901, doi:10.3390/cells9081901.
4. Guglielmelli, P.; Lasho, T.L.; Rotunno, G.; Mudireddy, M.; Mannarelli, C.; Nicolosi, M.; Pacilli, A.; Pardanani, A.; Rumi, E.; Rosti, V.; et al. MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis. J. Clin. Oncol. 2018, 36, 310–318, doi:10.1200/JCO.2017.76.4886.
5. Tefferi, A.; Guglielmelli, P.; Lasho, T.L.; Gangat, N.; Ketterling, R.P.; Pardanani, A.; Vannucchi, A.M. MIPSS70+ Version 2.0: Mutation and Karyotype-Enhanced International Prognostic Scoring System for Primary Myelofibrosis. J. Clin. Oncol. 2018, 36, 1769–1770, doi:10.1200/JCO.2018.78.9867.
6. Tefferi, A.; Guglielmelli, P.; Nicolosi, M.; Mannarelli, F.; Mudireddy, M.; Bartalucci, N.; Finke, C.M.; Lasho, T.L.; Hanson, C.A.; Ketterling, R.P.; et al. GIPSS: Genetically inspired prognostic scoring system for primary myelofibrosis. Leukemia 2018, 32, 1631–1642, doi:10.1038/s41375-018-0107-z.
7. McMullin, M.F.; Anderson, L.A. Aetiology of Myeloproliferative Neoplasms. Cancers 2020, 12, 1810, doi:10.3390/cancers12071810.
8. Tashi, T.; Swierczek, S.; Prchal, J.T. Familial MPN Predisposition. Curr. Hematol. Malig. Rep. 2017, 12, 442–447, doi:10.1007/s11899-017-0414-x.
9. Kilpivaara, O; Mukherjee, S; Schram, A.M; Wadleigh, M; Mullally, A; Ebert, B.L; Bass, A; Marubayashi, S; Heguy, A; Garcia-Manero, G; et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. Nat. Genet. 2009, 41, 455–459, doi:10.1038/ng.342.

10. Oddsson, A; Kristinsson, S.Y.; Helgason, G; Guddbjartsson, D.F; Masson, G; Sigurdsson, A; Jonasdottir, A; Jonasdottir, A; Steingrimsdottir, H; Vidarsson, B; et al. The germline sequence variant rs2736100_C in TERT associates with myeloproliferative neoplasms. Leukemia 2014, 28, 1371–1374, doi:10.1038/leu.2014.48.

11. Hinds, D.A; Barnholt, K.E; Mesa, R.A; Kiefer, A.K; Do, C.B; Eriksson, N; Mountain, J.L; Francke, U; Tung, J.Y; Nguyen, H.M; et al. Germ line variants predispose to both JAK2 V617F clonal hematopoiesis and myeloproliferative neoplasms. Blood 2016, 128, 1121–1128, doi:10.1182/blood-2015-06-652941.

12. Tapper, W; Jones, A.V; Kralovics, R; Harutyunyan, A.S; Zoi, K; Leung, W; Godfrey, A.L; Guglielmelli, P; Callaway, A; Ward, D; et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. Nat. Commun. 2015, 6, 6691, doi:10.1038/ncomms7691.

13. Bao, E.L; Nandakumar, S.K; Liao, X; Bick, A.G; Karjalainen, J; Tabaka, M; Gan, O.I; Havelunlin, A.S; Kiiskinen, T.T.J; Lareau, C.A; et al. Inherited myeloproliferative neoplasm risk affects haematopoietic stem cells. Nature 2020, 586, 769–775, doi:10.1038/s41586-020-2786-7.

14. Saliba, J; Saint-Martin, C; Di Stefano, A; Lenglet, G; Marty, C; Keren, B; Pasquier, F; Valle, V.D; Secardin, L; Leroy, G; et al. Germline duplication of ATG2B and GSKIP predisposes to familial myeloid malignancies. Nat. Genet. 2015, 47, 1113–1114, doi:10.1038/nrg.3380.

15. Harutyunyan, A.S; Giambruno, R; Krendel, C; Stukalov, A; Klampfl, T; Berg, T; Chen, D; Millosevic Feenstra, J.D; Jager, R; Giesslinger, B; et al. Germline RBBP6 mutations in familial myeloproliferative neoplasms. Blood 2016, 127, 362–365, doi:10.1182/blood-2015-09-668673.

16. Hirvonen, E.A.M; Pihkanen, E; Hemminki, K; Aaltonen, L.A; Kilpivaara, O. Whole-exome sequencing identifies novel candidate predisposition genes for familial polycythemia vera. Hum. Genom. 2017, 11, 6, doi:10.1186/s40426-017-0102-x.

17. Anelli, L; Zagaria, A; Specchia, G; Albano, F. The JAK2 G617C (46/1) HaploType in Myeloproliferative Neoplasms: Causal or Random? Int. J. Mol. Sci. 2018, 19, 1152, doi:10.3390/ijms19041152.

18. Trif, A.P; Banescu, C; Bojan, A.S; Voina, C.M; Popa, S; Visan, S; Ciubean, A.D; Tripon, F; Dima, D; Popov, V.M; et al. MECOM, HBS1L-MYB, THRB-RARB, JAK2, and TERT polymorphisms defining the genetic predisposition to myeloproliferative neoplasms: A study on 938 patients. Am. J. Hematol. 2018, 93, 100–106, doi:10.1002/ajh.24946.

19. Nahajevszky, S; Andrikovics, H; Batai, A; Adam, E; Bors, A; Csomor, J; Gopcsa, L; Koszarska, M; Kozma, A; Lovas, N; et al. The prognostic impact of germline 46/1 haplotype of Janus kinase 2 in cytogenetically normal acute myeloid leukemia. Haematologica 2011, 96, 1613–1618, doi:10.3324/haematol.2011.043885.

20. Hermouet, S; Vilaine, M. The JAK2 46/1 haplotype: A marker of inappropriate myelomonocytic response to cytokine stimulation, leading to increased risk of inflammation, myeloid neoplasm, and impaired defense against infection? Haematologica 2011, 96, 1573–1579, doi:10.3324/haematol.2011.053592.

21. Shay, J.W; Wright, W.E. Telomerase: A target for cancer therapeutics. Cancer Cell 2002, 2, 257–265, doi:10.1016/s1535-6108(02)00159-9.

22. Blasco, M.A. Telomeres and human disease: Ageing, cancer and beyond. Nat. Rev. Genet. 2005, 6, 611–622, doi:10.1038/nrg1656.

23. Savage, S.A; Alter, B.P. Dyskeratosis congenita. Hematol. Oncol. Clin. N. Am. 2009, 23, 215–231, doi:10.1016/j.hoc.2009.01.003.

24. Maciejewski, J.P; Selleri, C; Sato, T; Anderson, S; Young, N.S. A severe and consistent deficit in marrow and circulating primitive hematopoietic cells (long-term culture-initiating cells) in acquired aplastic anemia. Blood 1996, 88, 1983–1991.

25. Goldman, F.D; Aubert, G; Klingelhoitz, A.J; Hills, M; Cooper, S.R; Hamilton, W.S; Schlueter, A.J; Lambie, K; Eaves, C.J; Lansdorp, P.M. Characterization of primitive hematopoietic cells from patients with dyskeratosis congenita. Blood 2008, 111, 4523–4531, doi:10.1182/blood-2007-10-120204.

26. Zou, P; Gu, A; Ji, G; Zhao, L; Zhao, P; Lu, A. The TERT rs2736100 polymorphism and cancer risk: A meta-analysis based on 25 case-control studies. BMC Cancer 2012, 12, 7, doi:10.1186/1471-2407-12-7.

27. Mocellin, S; Verdi, D; Pooley, K.A; Landi, M.T; Egan, K.M; Baird, D.M; Prescott, J; De Vivo, I; Nitti, D. Telomerase reverse transcriptase locus polymorphisms and cancer risk: A field synopsis and meta-analysis. J. Natl. Cancer Inst. 2012, 104, 840–854, doi:10.1093/jnci/djs222.

28. Thompson, C.A.H; Wong, J.M.Y. Non-canonical Functions of Telomerase Reverse Transcriptase: Emerging Roles and Biological Relevance. Curr. Top. Med. Chem. 2020, 20, 498–507, doi:10.2174/1568026620666020131125110.

29. Wang, F; Fu, P; Pang, Y; Liu, C; Shao, Z; Zhu, J; Li, J; Wang, T; Zhang, X; Liu, J. TERT rs2736100T/G polymorphism upregulates interleukin 6 expression in non-small cell lung cancer especially in adenocarcinoma. Tumour Biol. 2014, 35, 4667–4672, doi:10.1007/s13277-014-1611-z.

30. Masselli, E; Pozzi, G; Gobbi, G; Merighi, S; Gessi, S; Vitale, M; Carubbi, C. Cytokine Profiling in Myeloproliferative Neoplasms: Overview on Phenotype Correlation, Outcome Prediction, and Role of Genetic Variants. Cells 2020, 9, 2136, doi:10.3390/cells9092136.

31. Telomeres Mendelian Randomization, C; Haycock, P.C; Burgess, S; Nounou, A; Zheng, J; Okoli, G.N; Bowden, J; Wade, K.H; Timpson, N.J; Evans, D.M; et al. Association Between Telomere Length and Risk of Cancer and Non-Neoplastic Diseases: A Mendelian Randomization Study. JAMA Oncol. 2017, 3, 636–651, doi:10.1001/jamaoncol.2016.5945.
32. Mangino, M.; Hwang, S.J.; Spector, T.D.; Hunt, S.C.; Kimura, M.; Fitzpatrick, A.L.; Christiansen, L.; Petersen, I.; Elbers, C.C.; Harris, T.; et al. Genome-wide meta-analysis points to CTCF and ZNF676 as genes regulating telomere homeostasis in humans. *Hum. Mol. Genet.* 2012, 21, 5385–5394, doi:10.1093/hmg/dds382.

33. Codd, V.; Nelson, C.P.; Albrecht, E.; Mangino, M.; Deelen, J.; Buxton, J.L.; Hottenga, J.J.; Fischer, K.; Esko, T.; Surakka, I.; et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat. Genet.* 2013, 45, 422–427, doi:10.1038/ng.2528.

34. Levy, D.; Neuhausen, S.L.; Hunt, S.C.; Kimura, M.; Hwang, S.J.; Chen, W.; Bis, J.C.; Fitzpatrick, A.L.; Smith, E.; Johnson, A.D.; et al. Genome-wide association identifies OBF1C as a locus involved in human leukocyte telomere biology. *Proc. Natl. Acad. Sci. USA* 2010, 107, 9293–9298, doi:10.1073/pnas.0911494107.

35. Giachetti, M.; Macauda, A.; Sgherza, N.; Sainz, J.; Gemignani, F.; Maldonado, J.M.S.; Jurado, M.; Tavano, F.; Mazur, G.; Jerez, A.; et al. Genetic polymorphisms associated with telomere length and risk of developing myeloproliferative neoplasms. *Blood Cancer J.* 2020, 10, 89, doi:10.1038/s41408-020-00356-5.

36. Kustikova, O.S.; Schwarzer, A.; Stahlhut, M.; Brugman, M.H.; Neumann, T.; Yang, M.; Li, Z.; Schambach, A.; Heinz, N.; Gerdes, S.; et al. Activation of Evil inhibits cell cycle progression and differentiation of hematopoietic progenitor cells. *Leukemia* 2013, 27, 1127–1138, doi:10.1038/leu.2012.355.

37. Gershman, M.; Ancliff, P.; Estrada, J.; Metzler, P.; Ponstingl, E.; Rutsche, H.; Schwabe, D.; Scott, R.H.; Unal, S.; Wawer, A.; et al. MECOM-associated syndrome: A heterogeneous inherited bone marrow failure syndrome with amegakaryocytic thrombocytopenia. *Blood Adv.* 2018, 2, 586–596, doi:10.1182/bloodadvances.2018016501.

38. Yamazaki, H.; Suzuki, M.; Otsuki, A.; Shimizu, R.; Bresnick, E.H.; Engel, J.D.; Yamamoto, M. A remote GATA2 hematopoietic enhancer drives leukemogenesis in inv(3)(q21;q26) by activating EVI1 expression. *Cancer Cell* 2014, 25, 415–427, doi:10.1016/j.ccr.2014.02.008.

39. Groeschel, S.; Sanders, M.A.; Hoogenboezem, R.; de Wit, E.; Bouwman, B.A.M.; Erpelinc, C.; van der Velden, V.H.J.; Havermans, M.; Avellino, R.; van Lom, K.; et al. A single oncogenic enhancer rearrangement causes concomitant EVI1 and HAVC deregulation in leukemia. *Cell* 2014, 157, 369–381, doi:10.1016/j.cell.2014.02.019.

40. Qayyum, R.; Snively, B.M.; Ziv, E.; Nalls, M.A.; Liu, Y.; Tang, W.; Yanek, L.R.; Lange, L.; Evans, M.K.; Ganesh, S.; et al. A meta-analysis and genome-wide association study of platelet count and mean platelet volume in african americans. *PLoS Genet.* 2012, 8, e1002491, doi:10.1371/journal.pgen.1002491.

41. Menzel, S.; Jiang, J.; Silver, N.; Gallagher, J.; Cunningham, J.; Surdulescu, G.; Lathrop, M.; Farrall, M.; Spector, T.D.; Thein, S.L. The HBS11-MYB intergenic region on chromosome 6q23.3 influences erythrocyte, platelet, and monocyte counts in humans. *Blood* 2007, 110, 3624–3626, doi:10.1182/blood-2007-05-093419.

42. Ganesh, S.K.; Zakai, N.A.; van Rooij, F.J.; Soranzo, N.; Smith, A.V.; Nalls, M.A.; Chen, M.H.; Kottgen, A.; Glazer, N.L.; Dehghan, A.; et al. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. *Nat. Genet.* 2009, 41, 1191–1198, doi:10.1038/ng.466.

43. Soranzo, N.; Spector, T.D.; Mangino, M.; Kuhnel, B.; Rendon, A.; Teumer, A.; Willenborg, C.; Wright, B.; Chen, L.; Li, M.; et al. A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. *Nat. Genet.* 2009, 41, 1182–1190, doi:10.1038/ng.467.

44. Ferreira, M.A.; Hottenga, J.J.; Warrington, N.M.; Medland, S.E.; Willumsen, G.; Lawrence, R.W.; Gordon, S.; de Geus, E.J.; Henders, A.K.; Smit, J.H.; et al. Sequence variants in three loci influence monocyte counts and erythrocyte volume. *Am. J. Hum. Genet.* 2009, 85, 745–749, doi:10.1016/j.ajhg.2009.10.005.

45. Thein, S.L.; Menzel, S.; Peng, X.; Best, S.; Jiang, J.; Close, J.; Silver, N.; Gerovasili, A.; Ping, C.; Yamaguchi, M.; et al. Intergenic variants of HBS11-MYB are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. *Proc. Natl. Acad. Sci. USA* 2007, 104, 11346–11351, doi:10.1073/pnas.0611393104.

46. Uda, M.; Galanello, R.; Sanna, S.; Lettre, G.; Sankaran, V.G.; Chen, W.; Usala, G.; Busonero, F.; Maschio, A.; Albai, G.; et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. *Proc. Natl. Acad. Sci. USA* 2008, 105, 1620–1625, doi:10.1073/pnas.0711566105.

47. Lettre, G.; Sankaran, V.G.; Bezerra, M.A.; Araujo, A.S.; Uda, M.; Sanna, S.; Cao, A.; Schlessinger, D.; Costa, F.F.; Hirschhorn, J.N.; et al. DNA polymorphisms at the BCL11A, HBS1L, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. *Proc. Natl. Acad. Sci. USA* 2008, 105, 11869–11874, doi:10.1073/pnas.0804799105.

48. Stadhouders, R.; Aktuna, S.; Thongjuea, S.; Aghajaniirefah, A.; Pourfarzad, F.; van Ijcken, W.; Lenhard, B.; Roos, H.; Best, S.; Menzel, S.; et al. HBS11-MYB intergenic variants modulate fetal hemoglobin via long-range MYB enhancers. *J. Clin. Invest.* 2014, 124, 1699–1710, doi:10.1172/JCI71520.

49. Sankaran, V.G.; Menne, T.F.; Scepanovic, D.; Vergilio, J.A.; Ji, P.; Kim, J.; Thiru, P.; Orkin, S.H.; Lander, E.S.; Lodish, H.F. MicroRNA-15a and -16-1 act via MYB to elevate fetal hemoglobin expression in human trisomy 13. *Proc. Natl. Acad. Sci. USA* 2011, 108, 1519–1524, doi:10.1073/pnas.1018384108.

50. Lu, J.; Guo, S.; Ebert, B.L.; Zhang, H.; Peng, X.; Bosco, J.; Pretz, J.; Schlanger, R.; Wang, J.Y.; Mak, R.H.; et al. MicroRNA-mediated control of cell fate in megakaryocyte-erythrocyte progenitors. *Dev. Cell* 2008, 14, 843–853, doi:10.1016/j.devcel.2008.03.012.

51. Garcia, P.; Clarke, M.; Vlegiopoulos, A.; Berlanga, O.; Camejo, A.; Lorvellec, M.; Frampton, J. Reduced c-Myc activity compromises HSCs and leads to a myeloproliferation with a novel stem cell basis. *EMBO J.* 2009, 28, 1492–1504, doi:10.1038/emboj.2009.97.
Cells 2021, 10, 2597

52. Pierini, T.; Di Giacomo, D.; Pierini, V.; Gorello, P.; Barba, G.; Lema Fernandez, A.G.; Pellanera, F.; Iannotti, T.; Falzetti, F.; La Starza, R.; et al. MYB deregulation from a EWSR1-MYB fusion at leukemic evolution of a JAK2 (V617F) positive primary myelofibrosis. Mol. Cytogenet. 2016, 9, 68, doi:10.1186/s13039-016-0277-1.

53. Beauchemin, H.; Shooshtharizadeh, P.; Pinder, J.; Dellaire, G.; Moroy, T. Dominant negative Grilb mutations cause moderate thrombocytopenia and an impaired stress thrombopoiesis associated with mild erythrophoietic abnormalities in mice. Haematologica 2020, 105, 2457–2470, doi:10.3324/haematol.2019.222596.

54. Rudd, M.F.; Sellick, G.S.; Webb, E.L.; Catovsky, D.; Houlston, R.S. Variants in the ATM-BRCA2-CHEK2 axis predispose to chronic lymphocytic leukemia. Blood 2006, 108, 638–644, doi:10.1182/blood-2005-12-0022.

55. Tefferi, A.; Laslo, T.L.; Patnaik, M.M.; Finke, C.M.; Hussein, K.; Hogan, W.J.; Elliott, M.A.; Litzow, M.R.; Hanson, C.A.; Pardanani, A. JAK2 germline genetic variation affects disease susceptibility in primary myelofibrosis regardless of V617F mutational status: Nullizygosity for the JAK2 46/1 haplotype is associated with inferior survival. Leukemia 2010, 24, 105–109, doi:10.1038/leu.2009.225.

56. Tefferi, A.; Laslo, T.L.; Mudireddy, M.; Finke, C.M.; Hanson, C.A.; Ketterling, R.P.; Gangat, N.; Pardanani, A. The germline JAK2 GGGCC haplotype and survival among 414 molecularly-annotated patients with primary myelofibrosis. Am. J. Hematol. 2019, 94, 299–305, doi:10.1002/ajh.253497.

57. Vannucchi, A. M.; Zhang, W.; Rondelli, D.; Godbold, J.; Ghinassi, B.; Whitsett, C.; Hoffman, R.; Migliaccio, A. R. The dominant negative beta isoform of the glucocorticoid receptor is uniquely expressed in erythroid cells expanded from polycythaemia vera patients. Blood 2011, 118, (2), 425–36.

58. Poletto, V.; Rosti, V.; Villani, L.; Catarsi, P.; Carolei, A.; Campanelli, R.; Massa, M.; Martinetti, M.; Viarengo, G.; Malovini, A.; et al. A3669G polymorphism of glucocorticoid receptor is a susceptibility allele for primary myelofibrosis and contributes to phenotypic diversity and blast transformation. Blood 2012, 120, 3112–3117, doi:10.1182/blood-2012-05-433466.

59. Masselli, E.; Carubbi, C.; Cambo, B.; Pozzi, G.; Gobbi, G.; Miranda, P.; Follini, E.; Pagliaro, L.; Di Marcontonio, D.; Bonatti, F.; et al. The -2518 A/G polymorphism of the monocyte chemoattractant protein-1 as a candidate genetic predisposition factor for secondary myelofibrosis and biomarker of disease severity. Leukemia 2018, 32, 2266–2270, doi:10.1038/s41375-018-0088-y.

60. Masselli, E.; Carubbi, C.; Pozzi, G.; Percesepe, A.; Campanelli, R.; Villani, L.; Gobbi, G.; Bonomini, S.; Roti, G.; Rosti, V.; et al. Impact of the rs1024611 Polymorphism of CCL2 on the Pathophysiology and Outcome of Primary Myelofibrosis. Cancers 2021, 13, 2552, doi:10.3390/cancers1312552.

61. Ferrer-Marín, F.; Arroyo, A.B.; Bellosillo, B.; Cuenca, E.J.; Zamora, L.; Hernandez-Rivas, J.M.; Hernandez-Boluda, J.C.; Fernandez-Rodriguez, C.; Luno, E.; Garcia Hernandez, C.; et al. miR-146a rs2431697 identifies myeloproliferative neoplasm patients with higher secondary myelofibrosis progression risk. Leukemia 2020, 34, 2648–2659, doi:10.1038/s41375-020-0767-3.

62. Zhou, J.; Cidlowski, J.A. The human glucocorticoid receptor: One gene, multiple proteins and diverse responses. Steroids 2005, 70, 407–417, doi:10.1016/j.steroids.2005.02.006.

63. Derijk, R.H.; Schaaf, M.J.; Turner, G.; Datson, N.A.; Vreugdenhil, E.; Cidlowski, J.; de Kloet, E.R.; Emery, P.; Sternberg, E.M.; Detera-Wadleigh, S.D. A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isofrom mRNA is associated with rheumatoid arthritis. J. Rheumatol. 2001, 28, 2383–2388.

64. Varricchio, L.; Migliaccio, A.R. The role of glucocorticoid receptor (GR) polymorphisms in human erythropoiesis. Am. J. Blood Res. 2014, 4, 53–72.

65. Rovin, B.H.; Lu, S.; Saxena, R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem. Biophys. Res. Commun. 1999, 259, 344–348, doi:10.1006/bbrc.1999.0796.

66. Pham, M.H.; Bonello, G.B.; Castiblanco, J.; Le, T.; Sigala, J.; He, W.; Mummid, S. The rs1024611 regulatory region polymorphism is associated with CCL2 allelic expression balance. PLoS ONE 2012, 7, e94998, doi:10.1371/journal.pone.0094998.

67. Colobran, R.; Pujol-Borrell, R.; Armengol, M.P.; Juan, M. The chemokine network. II. On how polymorphisms and alternative splicing increase the number of molecular species and configure intricate patterns of disease susceptibility. Clin. Exp. Immunol. 2007, 150, 1–12, doi:10.1111/j.1600-2528.2007.03489.x.

68. McDermott, D.H.; Yang, Q.; Kathiresan, S.; Cupples, L.A.; Massaro, J.M.; Kearney, J.F., Jr.; Larson, M.G.; Vasan, R.S.; Hirschhorn, J.N.; O’Donnell, C.J.; et al. CCL2 polymorphisms are associated with serum monocyte chemoattractant protein-1 levels and myocardial infarction in the Framingham Heart Study. Circulation 2005, 112, 1113–1120, doi:10.1161/CIRCULATIONAHA.105.543579.

69. Chen, Z.; Yin, S.; Zheng, L.; Tang, W.; Kang, M.; Wei, W.; Sui, K. Relationship between the Monocyte Chemo-attractant Protein-1 gene rs1024611 A>G Polymorphism and Cancer Susceptibility: A Meta-analysis Involving 14,617 Subjects. Immunol. Invest. 2021, 50, 461–477, doi:10.1080/08820339.2020.1776726.

70. Fisher, D.A.C.; Fowles, J.S.; Zhou, A.; Oh, S.T. Inflammatory Pathophysiology as a Contributor to Myeloproliferative Neoplasms. Front. Immunol. 2021, 12, 683401, doi:10.3389/fimmu.2021.683401.

71. Karin, M.; Greten, F.R. NF-kappaB: Linking inflammation and immunity to cancer development and progression. Nat. Rev. Immunol. 2005, 5, 749–759, doi:10.1038/nri1703.

72. Melgarejo, E.; Medina, M.A.; Sanchez-Jimenez, F.; Urdiales, J.L. Monocyte chemoattractant protein-1: A key mediator in inflammatory processes. Int. J. Biochem. Cell Biol. 2009, 41, 998–1001, doi:10.1016/j.biocel.2008.07.018.
73. Boldin, M.P.; Taganov, K.D.; Rao, D.S.; Yang, L.; Zhao, J.L.; Kalwani, M.; Garcia-Flores, Y.; Luong, M.; Devrekanli, A.; Xu, J.; et al. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *J. Exp. Med.* 2011, 208, 1189–1201, doi:10.1084/jem.20101823.

74. Prokunina-Olsson, L. Genetics of the Human Interferon Lambda Region. *J. Interferon Cytokine Res.* 2019, 39, 599–608, doi:10.1089/jir.2019.0043.

75. Hasselbalch, H.C.; Holmstrom, M.O. Perspectives on interferon-alpha in the treatment of polycythemia vera and related myeloproliferative neoplasms: Minimal residual disease and cure? *Semin. Immunopathol.* 2019, 41, 5–19, doi:10.1007/s00281-018-0700-2.

76. Lindgren, M.; Samuelsson, J.; Nilsson, L.; Knutsen, H.; Ghanima, W.; Westin, J.; Johansson, P.L.; Andreasson, B. Genetic variation in IL28B (IFNL3) and response to interferon-alpha treatment in myeloproliferative neoplasms. *Eur. J. Haematol.* 2018, 100, 419–425, doi:10.1111/ejh.13034.

77. Jager, R.; Gisslinger, H.; Fuchs, E.; Bogner, E.; Milosevic Feenstra, J.D.; Weinzierl, J.; Schischlik, F.; Gisslinger, B.; Schalling, M.; Zorer, M.; et al. Germline genetic factors influence the outcome of interferon-alpha therapy in polycythemia vera. *Blood* 2021, 137, 387–391, doi:10.1182/blood.2020005792.

78. Kohne, T.; Majeti, R. Clonal hematopoiesis: From mechanisms to clinical intervention. *Cancer Discov.* 2021, doi:10.1158/2159-8290.CD-21-0901.

79. Zink, F.; Stacey, S.N.; Norddahl, G.L.; Frigge, M.L.; Magnusson, O.T.; Jonsdottir, I.; Thorgerisson, T.E.; Sigurdsson, A.; Gudjonsson, S.A.; Gudmundsson, J.; et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* 2017, 130, 742–752, doi:10.1182/blood-2017-02-769869.

80. Bick, A.G.; Weinstock, J.S.; Nandakumar, S.K.; Fulco, C.P.; Bao, E.L.; Zekavat, S.M.; Szeto, M.D.; Liao, X.; Leventhal, M.J.; Nasser, J.; et al. Inherited causes of clonal haematopoiesis in 97,691 whole genomes. *Nature* 2020, 586, 763–768, doi:10.1038/s41586-020-2819-2.

81. Vannucchi, A.M.; Guglielmelli, P. The JAK2 46/1 (GGCC) MPN-predisposing haplotype: A risky haplotype, after all. *Am. J. Hematol.* 2019, 94, 283–285, doi:10.1002/ajh.25367.

82. Liggett, I.A.; Sankaran, V.G. Unraveling Hematopoiesis through the Lens of Genomics. *Cell* 2020, 182, 1384–1400, doi:10.1016/j.cell.2020.08.030.

83. Laurenti, E.; Gottgens, B. From haematopoietic stem cells to complex differentiation landscapes. *Nature* 2018, 553, 418–426, doi:10.1038/nature25022.

84. Bao, E.L.; Cheng, A.N.; Sankaran, V.G. The genetics of human hematopoiesis and its disruption in disease. *EMBO J.* 2019, 38, e10316, doi:10.15252/embr.201910316.

85. Yoshida, K.; Fujita, M. DNA damage responses that enhance resilience to replication stress. *Cell Mol. Life Sci.* 2021, doi:10.1007/s00018-021-03926-3.

86. McPherson, K.S.; Korzhnev, D.M. Targeting protein-protein interactions in the DNA damage response pathways for cancer chemotherapy. *RSC Chem. Biol.* 2021, 2, 1167–1195, doi:10.1039/d1cb00101a.

87. Esposito, M.T.; So, C.W. DNA damage accumulation and repair defects in acute myeloid leukemia: Implications for pathogenesis, disease progression, and chemotherapy resistance. *Chromosoma* 2014, 123, 545–561, doi:10.1007/s00412-014-0482-9.

88. Mehmoood, A.; Kayani, M.A.; Ahmed, M.W.; Nisar, A.; Mahjabeen, I. Association between single nucleotide polymorphisms of DNA damage response pathway genes and increased risk in breast cancer. *Future Oncol.* 2020, 16, 1977–1995, doi:10.2217/fon-2020-0086.

89. Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 2018, 9, 7204–7218, doi:10.18632/oncotarget.23208.

90. Ramamoorthy, S.; Cidlowski, J.A. Exploring the molecular mechanisms of glucocorticoid receptor action from sensitivity to resistance. *Endocr. Dev.* 2013, 24, 41–56, doi:10.1159/000342502.