Polymorphisms in Drug Transporter and Metabolism Genes Associated with Resistance to Imatinib in Chronic Myeloid Leukemia: A Systematic Review and Meta-Analysis

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Abstract: The aim of this study was to establish the relationship between different polymorphisms in drug transporter and metabolizer genes and resistance to imatinib in chronic myeloid leukemia (CML). For this purpose, an exhaustive search was carried out in the Scopus, Web of Science, and PubMed databases using different combinations of keywords with different inclusion and exclusion criteria. The meta-analysis included nine studies that met the established criteria. The results of the study showed that the polymorphic variants AG and GG of CYP3A5*3 are associated with response to treatment, presenting a significantly lower risk with resistance to imatinib. Likewise, the variants T1236C and G2677T/A of the ABCB1 gene show a significant association with treatment efficacy. In addition, the genetic polymorphism 1236T, homozygous CC of the MDR1 gene, significantly influences the increased risk of cytogenetic relapse and the polymorphic variant 361G>A GA of the SLCO1A2 gene significantly affects the complete molecular response.

Keywords: LMC; Philadelphia chromosome; polymorphism; translocation; resistance; meta-analysis

1. Introduction

Chronic myeloid leukemia, hereinafter CML, is a chronic myeloproliferative neoplasm detected by conventional and molecular cytogenetic studies. It is caused by a translocation between chromosomes 9 and 22, generating a BCR-ABL oncogene characterized as “Philadelphia chromosome”, Ph+ chromosome, which generates a BCR-ABL chimeric fusion protein (kinase, constitutively active) [1,2]. This BCR–ABL fusion causes uncontrolled proliferation of the myeloid cell line in the bone marrow.

Tyrosine kinase inhibitors (TKIs), such as imatinib, represent today the therapeutic standard for CML, making it possible to significantly improve patient survival. However, other factors involved in the response to CML monotherapy are the CYP450 family enzymes, where the different isoforms of CYP3A4, CYP2C8, and CYP3A5 help in the biotransformation of imatinib.

Currently, considerable differences in the pharmacokinetics of imatinib have been observed between individuals, implying a variable therapeutic response. Imatinib is metabolized in the liver predominantly by cytochrome P450 isoforms CYP3A4, CYP2C8, and CYP3A5, and, to a lesser extent, CYP1A2, CYP2D6, CYP2C9, and CYP2C19.

One of the treatments that offers remission of the disease is bone marrow transplantation, which is available only for a minority of patients with very specific conditions, and the treatment depends on the availability of a donor [3]. It is, therefore, necessary to adequately
manage treatment to offer an opportunity to patients who do not have this treatment option to improve their clinical conditions against the disease.

In that sense, it is of great importance to better understand the molecular and cytogenetic mechanisms involved and the clinical evolution of each patient. This is because everyone presents a different genetic variability in relation to the response to CML treatments and also presents genetic susceptibility to develop other diseases and/or the probability of responding to treatments.

The majority of patients with CML obtain clinical response under treatment with imatinib, however, a significant proportion of them do not achieve such a response either because they are resistant to treatment or the variability of the socioeconomic characteristics of the patients influences the accessibility that they may have to treatment when they present a failure to this tyrosine kinase inhibitor. On the other hand, imatinib administered to different patients with the same pathology can obtain different responses; an effective and safe dose in one individual can be subtherapeutic or toxic in another. This makes it necessary to analyze the causes of these different responses in patients.

Currently, it is necessary to explore action alternatives for the management of those patients who present resistance to imatinib, both in the chronic phase and in the accelerated and blastic phases [4]. This is because mutations implicated in the progression of the disease towards more advanced phases and polymorphisms in genes associated with drug metabolism have been found.

Considering the above, this article contains a systematic literature review and meta-analysis on gene polymorphisms associated with the metabolism of imatinib in CML patients and their response to treatment. This was for the purpose of understanding the metabolic process and/or biotransformation of the first-line drug imatinib given to CML patients which could help in establishing a better treatment option.

State of the Art

In [5], they demonstrate that imatinib interacts with the ATP binding site in ABL only when the protein is in its inactive conformation. Thus, the inhibitor prevents the conformational transition to the active form, which is responsible for binding and/or phosphorylation of signal transduction molecules. Consequently, downstream effector molecules are not phosphorylated, and signal transduction is not activated. In the era of imatinib, monitoring by RT-QPCR has become an important tool to measure BCR–ABL transcript levels and to assess long-term response to treatment. Many attempts have been made to standardize procedures to monitor BCR–ABL transcription [6].

In [7], they show that the first clinical evaluations of the efficacy of imatinib (Phase I studies) in the treatment of CML showed hematologic remission in 50% of patients in chronic phase and evidence of some cytogenetic response in all cases. In addition, in patients with CML in blast crisis, a disappearance of the number of circulating blasts and extramedullary infiltrates was detected. Phase II studies showed that after daily administration of 400 mg/kg total, in patients who had been refractory or intolerant to IFN-α, 95% presented a complete hematological response (CHR), and in 41% of them a CCR (complete cytogenetic response) was evidenced.

In vitro studies have shown that imatinib is able to inhibit the growth of BCR–ABL expressing cell lines, an effect that in some of them is achieved by inhibiting signaling pathways such as JAK5-STAT and PI3 kinase. Several studies have shown that imatinib can inhibit CML mononuclear cells obtained in the chronic phase and in blast crisis, as well as reduce the number of colonies from peripheral blood and bone marrow, inhibit proliferation and cell cycle of CD34+CD38- primitive progenitors and CD34+CD38+ committed cells, without altering the behavior of normal cells [8,9]. Consequently, in vivo administration of imatinib is able to restore hematopoietic behavior (proliferation and expansion) of hematopoietic stem and progenitor cells, but does not eliminate all malignant progenitor cells, which remain in patients even after complete cytogenetic responses.
have been obtained [7,10]. Therefore, the use of this inhibitor allows a fairly satisfactory management of patients, but does not guarantee cure of the disease [7,11].

It was established in [12] that, due to tyrosine kinase inhibitor therapy, a newly diagnosed individual with chronic phase CML is now more likely to die from any other medical condition, but not from leukemia. In addition, numerous studies consistently report that a durable deep molecular response (DMR) is achieved in approximately 40–50% of treated individuals, allowing treatment to be safely discontinued.

Worldwide, imatinib continues to be recommended as a first-line treatment for patients with chronic phase CML at a standard dose of 400 mg/day [13]. Higher doses (between 600 and 800 mg/day) have been recommended for individuals with initial resistance, in combination therapies, as well as in patients in the blastic phase or with residual disease. Prior to the advent of second generation ITKs, some patients resistant to imatinib treatment benefited from receiving a step increase in dose (up to 800 mg/day). The administration of high doses to newly diagnosed patients, to improve molecular and cytogenetic response, has not yielded the expected results because although the response rate increases, the difference has not been significant [14].

Accumulating evidence over time regarding the administration of imatinib as a first-line therapy in newly diagnosed patients with chronic phase CML indicates a 65–70% chance of CRC at 12 months, 50–55% chance of MMR at 2 years, and 45–50% chance of MR4 at 5 years. Data derived from sequential laboratory results indicate that the proportion of patients achieving an MMR with first-line imatinib continues to increase after 8 years [12]. Imatinib treatment has also improved the course of the disease in children and adolescents, in whom it tends to present more aggressive features compared to adults [15].

In some situations, the dose increase could be successful and temporarily overcome the resistance mechanisms, especially in cases of genomic amplification of the ABL gene fragmentation site or in patients with an over-expression of BCR–ABL. However, in patients with mutations in the BCR–ABL gene, the affinity of imatinib for tyrosine kinase is reduced and thus further resistance is induced. The most frequent mutations are E255K, E255V, Y253F, Y253H in the P-loop of the BCR–ABL protein and mutations in T315, M351 and E355. This mechanism has two major effects: preventing the binding of imatinib to BCR–ABL and thereby inducing stable activation of BCR–ABL insensitive to this inhibitor [16].

Molecular responses monitored by RT-qPCR have important clinical implications. At the individual level, RT-qPCR studies can identify the degree of molecular response that predicts long-term stability and patterns of response that indicate relapse and resistance to Imatinib. Detection of BCR–ABL mutations in patients with acquired resistance is recommended to guide future therapies. Indeed, different mutations are associated with different degrees of resistance, some of which could be overcome by increasing the dose of imatinib or using one of the second-generation TK inhibitors [17].

Resistance to imatinib in CML patients has been extensively studied, as it can be caused by several mechanisms. Some of the most frequently reported are mutations in the kinase domain of BCR–ABL, high levels of the protein, inadequate intracellular levels of imatinib because of PGP expression and physiological growth factor signaling. Integrin signaling can maintain viability even with completely inhibited kinase activity or quiescent cells protected against imatinib [18].

It has been reported that one of the causes of resistance to treatment could be the polymorphic variants present in the CYP450 family genes, such as CYP3A4*1B, CYP3A4*2, and CYP3A5*3, responsible for drug metabolism and biotransformation; however, the results reported in the literature on this subject are controversial. In 2006, Gardner et al. [19] studied the relationship between CYP3A4 gene polymorphisms and six other genes associated with imatinib metabolism in human embryonic kidney cells. The study revealed only a small influence of allelic variants of the genes on the metabolism and pharmacokinetics of imatinib, so the authors pointed out the need for further research.

For their part, Camargo et al. [20] in 2008, set out to study the influence of CYP3A4 gene polymorphisms in patients treated with imatinib and in a control group. The authors
showed a direct relationship between the percentage of blast cell decrease and the time
of drug administration, but failed to demonstrate any influence between the genotype
that was identified as most relevant in that population, CYP3A4*1B, and the cytogenetic
response of the patients.

Sailaja et al. [21] in 2010, studied the influence of CYP3A5*3 and *6 genetic polymor-
phisms in 265 CML patients treated with imatinib and 241 controls with no history of
cancer, in Hyderabad (India). They analyzed the possible involvement of these polymor-
phisms in the origin of CML, as well as their impact on the response to imatinib. The
analysis of the polymorphisms was performed by PCR-RFLP. Their results suggest that the
CYP3A5*3 genetic polymorphism in CML patients could be associated with an increased
risk of developing CML and disease progression, but not necessarily with a lower response
to imatinib.

For their part, Bedewy et al. [22] studied, in 2013, CYP3A5*3 polymorphisms in
78 Egyptian patients with newly diagnosed CML in critical phase, hereinafter CP, who
received imatinib therapy. The polymorphism for this gene was shown to be significantly
associated with a less satisfactory clinical course in response to treatment. However, the
authors acknowledge the need for further study of the effects of this polymorphism on a
larger scale.

Vaidya et al. [23] studied, in 2015, the influence on the clinical course of a cohort of
106 individuals with newly diagnosed chronic myeloid leukemia, that 12 allelic variants
of the CYP3A4, CYP3A5, and hOCT1 genes could exert. In that cohort of patients, only six of
the genetic variants studied were detected. The CYP3A5*3 and hOCT1 M408V polymor-
phisms were associated with CCR at 6 months and MMR (major molecular response) at
12 months. The authors conclude that these polymorphisms may contribute to determining
and predicting the clinical response to imatinib and the clinical course of patients with
chronic myeloid leukemia.

In 2016, Maddin et al. [24] studied 270 patients with CML in accelerated phase, AP, or
blastic phase, BP, treated for at least 12 months with imatinib as first-line therapy, cared
for in various hospitals in Malaysia, in order to analyze the genetic polymorphisms for
CYP3A4*18 and CYP3A5*3 by PCR-RFLP. Their findings were surprising in that they
detected a significant association between the CYP3A5*3 polymorphism and a lower
risk of developing resistance to imatinib treatment. The homozygous genotype for this
polymorphism was shown to be associated with a decrease in the activity of the enzyme,
resulting in a lower clearance rate and higher bioavailability of the drug. The authors
suggested that genotyping for these SNPs before initiating therapy may be important in
predicting response to imatinib in patients with CML.

Saiz-Rodríguez et al. [25], in 2020, determined the influence of several CYP3A cyto-
chrome P450 polymorphisms on the pharmacokinetic parameters of various substrates
for those proteins, including imatinib, in 251 healthy volunteers who received a single dose
of each drug. Polymorphisms for the CYP3A4 and CYP3A5 genes were genotyped in the
volunteers by qualitative PCR. The authors reported that neither the CYP3A phenotype
nor the individual polymorphisms for CYP3A4 or CYP3A5 were significantly associated
with changes in drug pharmacokinetics.

Likewise, the possible participation of some polymorphisms in genes coding for drug
transporter proteins such as SLC22A1/OCT1 (rs35191146), ABCB1/MDR1 (rs1128503)
and ABCG2/BCRP2 (rs2231137), in determining individual variations in the response to
treatment with imatinib, has also been proposed [25].

In 2017, Harivenkatesh et al. [26] conducted a study in individuals with CML in FC
(173), diagnosed at a mean age of 36 years, of whom only 41% responded to treatment. The
Hardy–Weinberg equilibrium was met for almost all alleles, except for MDR1-C1236T. The
minimal levels of imatinib detected in patients varied widely across genotypes. Among
patients who responded to treatment, a higher frequency of genotypes GG for CYP3A5-
A6986G and TT for MDR1-C1236T, C3435T and G2677T/A was evident, whereas in non-
responders, genotypes AA for CYP3A5-A6986G, CC for MDR1-C1236T, and C3435T and
GG for MDR1-G2677T/A predominated. The authors concluded that variations in CYP3A5 and MDR1 genes are responsible for interindividual differences in circulating levels of imatinib and contribute to determining treatment response in CML patients. The detection of polymorphic variants for these genes could be very useful to individualize treatment regimens to achieve a satisfactory clinical course.

On the other hand, as far as molecular aspects are concerned, 95% of CML cases present a BCR-ABL gene resulting from the junction of exon 13 (e13) or 14 (e14) of the BCR gene with exon 2 (a2) of the ABL1 gene. Therefore, the usual rearrangements will be e14a2 (b3a2) and e13a2 (b2a2), which code for a 210 kD BCR-ABL1 protein (p210). The p210 proteins derived from these two genetic rearrangements are associated with different clinical courses; the one derived from b3a2 has lower kinase activity, and patients who carry it respond better to treatment, achieve DMR more frequently, and achieve higher survival rates. In the remaining 5% of patients, other different fusions between BCR and ABL1 called atypical transcripts are detected, including: e19a2 (p230) and e1a2 (p190), in addition to others such as e13a3, e14a3, e6a2, and e8a2. Each of these is detected at frequencies of less than 1%. Although CML with the presence of atypical rearrangements is a rare entity, it should be kept in mind and cytogenetics/FISH should continue to be used at the time of diagnosis. To avoid false negatives, it is very useful to use molecular techniques capable of detecting these variants in the presence of a positive cytogenetic/FISH and a negative BCR-ABL1 rearrangement [18].

In 2017, Akram et al. [27] investigated the presence of BCR–ABL compound mutations in patients with advanced and late chronic phase imatinib-sensitive CML as a potential driver of progression. However, there are limited reports on the association of BCR–ABL compound mutations with disease progression in imatinib-sensitive CML patients. They investigated the presence of ABL-K D mutations in chronic phase in 41 patients, 31 patients in late chronic phase, and 16 in accelerated phase responders to imatinib. Direct sequencing analysis was used for this purpose. Eleven patients (12.22%) with late CP CML were detected to have a total of 24 types of point mutations, of which eight (72.72%) harbored compound mutation sites.

In 2020, Hoemberger et al. [28] studied the cumulative mechanisms of several important mutations in ABL kinase conferring resistance to imatinib, since despite the success of the anticancer drug, the main obstacle in prolonged treatment is the occurrence of mutations within the ABL kinase domain; finding that many resistance mutations occur in the dynamic hotspots was recently identified as responsible for the high selectivity of imatinib towards ABL.

Regular assessment of molecular response can help clinicians to discover the possibility of remission or any new decision to change therapeutic strategies, concluding in this study that the identification of ABL kinase domain mutations can be used as a suitable method and is useful to improve therapeutic strategies, avoiding treatment delays, and excessive expenses in CML patients with imatinib resistance [28].

It has been observed that other molecular and cytogenetic changes related to disease progression are present in the vast majority of CML patients. These changes are also orchestrated by the BCR-ABL1 oncoprotein, which contributes to the acquisition of additional genetic abnormalities, probably by promoting an increase in genomic instability. This process of genetic diversification, selection, and clonal expansion, which has been termed clonal evolution, is associated with disease progression to blast crisis (BC), as well as increased incidence of relapse, poor prognosis, and resistance to imatinib treatment. Specifically, changes involving chromosomes 8, 17, 9, and 22 are detected in 70–80% of patients. Additional genetic abnormalities frequently detected during blast crisis are trisomy 8; isochromosome 17; additional deletions on chromosome 9; duplication of the Ph chromosome (an event leading to increased expression of BCR–ABL protein and its associated effects), or of chromosomes 19, 21, or 17; or loss of the Y chromosome or monosomy 7, 10, 23, 68. Another alteration identified during the BC phase is the loss of p53, which is associated with increased resistance to apoptosis [29].
2. Materials and Methods

2.1. Data Collection

An exhaustive search was carried out with the purpose of identifying published studies that related to the different polymorphisms in drug transporter and metabolizer genes associated with resistance to imatinib in chronic myeloid leukemia. The search included the conformation of search strings that were validated in different scientific databases (PubMed, Scopus, Web of Science).

The following search strings were used:

((Chronic myeloid leukemia) AND (Imatinib) AND (Polymorphism))

((Chronic myeloid leukemia) AND (Imatinib) AND (CYP3A4) AND (CYP2C8))

Search: (imatinib) AND (Chronic myeloid leukemia)


2.2. Article Selection

As a result of the application of the search strings in the scientific databases, 192 articles were obtained and analyzed by two unblinded reviewers who oversaw examining the articles obtained through searches according to specific inclusion criteria.

The inclusion criteria established were as follows:

- Published studies related to the use of imatinib in patients diagnosed with chronic myeloid leukemia.
- Studies on polymorphisms in drug transporter and metabolizer genes associated with imatinib resistance.
- Studies with results validated by clinical trials.
- Studies that included odds ratio (OR) as an outcome measure with a 95% confidence interval.

To be included, they must meet all four criteria. If there were articles that met one, two or three of the inclusion criteria, they were not considered.

2.3. Data Extraction and Assessment of Quality and Risk of Bias

A table was designed to synthesize the information from the selected studies, the fields of which are shown in Table 1.

Table 1. Information collected from the studies analyzed.

<table>
<thead>
<tr>
<th>Field</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Id_Study ni</td>
<td>Number assigned to the study</td>
</tr>
<tr>
<td>Title</td>
<td>Title of the article</td>
</tr>
<tr>
<td>Authors</td>
<td>Authors</td>
</tr>
<tr>
<td>Year</td>
<td>Year of publication of the article</td>
</tr>
<tr>
<td>Country ni</td>
<td>Country where the clinical trial was applied</td>
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<tr>
<td>Age</td>
<td>Average age of patients</td>
</tr>
<tr>
<td>Treatment Phase</td>
<td>Disease stage (Chronic, Blast, Accelerated)</td>
</tr>
<tr>
<td>Intervention</td>
<td>Imatinib dose</td>
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<tr>
<td>Duration (Months)</td>
<td>Duration of treatment in months</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>Type of polymorphism studied</td>
</tr>
<tr>
<td>Nucleotide</td>
<td>Type of nucleotide studied</td>
</tr>
<tr>
<td>Gene</td>
<td>Type of gene studied</td>
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</tbody>
</table>
Each of the reviewers worked independently on the articles analyzed, as a primary result, then the information was compiled and the articles that were included in the review and meta-analysis were selected. Each selected article was subjected to an evaluation of its methodological quality using a scale of 1 to 4, where those studies obtaining the highest scores indicate a higher quality, as established in [30].

Three methods were used to estimate publication bias, the trim and fil method, which is a non-parametric technique for augmenting data [31]; the Vevea and Hedges weight function model [32]; and the Egger regression using a funnel plot [33].

2.4. Meta-Analysis Methodology

As a result of the manual review, the initial results were constructed, taking direct information from the articles using Table 1. These data were organized and prepared to be entered into the RStudio software where the meta-analysis was performed.

First, the data were transformed so that they were accepted by the software (the Pearson correlation coefficient p taken from each study as transformed into Fisher’s z coefficient so that there was no problem when calculating the variances) [34].

Then, we proceeded to develop the meta-analysis using RStudio libraries and functions. The heterogeneity (variation in the results among the included studies) was measured and reported, and the influence diagnosis was performed to establish which studies had the greatest influence on the results.

In the development of the meta-analysis, different statistical calculations focused on establishing the resistance of some polymorphisms to imatinib were performed.

3. Results

3.1. Description of the Studies

The search yielded 192 articles, where 58 were selected as relevant to the research, of which 49 were excluded because they did not meet the four inclusion criteria mentioned above. Figure 1 shows the article selection process.

Among the excluded articles were some that studied patients with CML but subjected to another type of treatment and/or did not include polymorphisms or their outcome data was not possible to calculate the OR (odds ratio).

Table 2 shows the studies selected for the meta-analysis. In each of the studies the results were individualized, taking into account each polymorphism studied. Although nine articles were selected in the study, for the purpose of a better understanding of each one, the studies were divided into each of the polymorphism genes studied.

<table>
<thead>
<tr>
<th>Field</th>
<th>Content</th>
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<tbody>
<tr>
<td>Genotyping Technique</td>
<td>Genotyping technique used in the study</td>
</tr>
<tr>
<td>Genotyping</td>
<td>Associated genotype</td>
</tr>
<tr>
<td>Evaluation of response to treatment</td>
<td>Evaluation technique, in this case odds ratio (OR) was chosen as an indicator.</td>
</tr>
<tr>
<td>OR</td>
<td>OR value for each</td>
</tr>
<tr>
<td>CI (%)</td>
<td>95% confidence interval</td>
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<tr>
<td>CI: Lower Limit</td>
<td>Lower limit of the confidence interval</td>
</tr>
<tr>
<td>CI: Upper Limit</td>
<td>Upper limit of the confidence Interval</td>
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<tr>
<td>p</td>
<td>Pearson correlation coefficient</td>
</tr>
<tr>
<td>Association Found (polymorphisms vs. resistance to Im)</td>
<td>Establishes the association found between polymorphisms and resistance to imatinib.</td>
</tr>
</tbody>
</table>
### Table 2. Studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Id</th>
<th>Study</th>
<th>References</th>
<th>Year</th>
<th>Country</th>
<th>Database</th>
<th>Journal</th>
<th>Journal Categorization</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Impact of CYP3A4<em>18 and CYP3A5</em>3 Polymorphisms on Imatinib Mesylate Response Among Chronic Myeloid Leukemia Patients in Malaysia</td>
<td>[24]</td>
<td>2016</td>
<td>Malaysia</td>
<td>PubMed</td>
<td>Oncology and Therapy</td>
<td>Q2</td>
<td>Switzerland</td>
</tr>
<tr>
<td>2</td>
<td>Transporter1 (C480G) in Egyptian Chronic Myeloid Leukemia Patients on Imatinib Do polymorphisms in MDR1 and CYP3A5 genes influence the risk of cytogenetic relapse in patients with chronic myeloid leukemia on imatinib therapy? Association of MDR1 gene polymorphism(G2677T) with imatinib response in Egyptian chronic myeloid leukemia patients</td>
<td>[35]</td>
<td>2018</td>
<td>Egypt</td>
<td>WoS</td>
<td>American Journal of Molecular Biology</td>
<td>Not categorized</td>
<td>USA</td>
</tr>
<tr>
<td>3</td>
<td>Do polymorphisms in MDR1 and CYP3A5 genes influence the risk of cytogenetic relapse in patients with chronic myeloid leukemia on imatinib therapy? Association of MDR1 gene polymorphism(G2677T) with imatinib response in Egyptian chronic myeloid leukemia patients</td>
<td>[26]</td>
<td>2017</td>
<td>India</td>
<td>PubMed</td>
<td>Leukemia and Lymphoma</td>
<td>Q2</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>4</td>
<td>Association of MDR1 gene polymorphism(G2677T) with imatinib response in Egyptian chronic myeloid leukemia patients</td>
<td>[36]</td>
<td>2013</td>
<td>Egypt</td>
<td>PubMed</td>
<td>Hematology</td>
<td>Q3</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>5</td>
<td>Effects of Trough Concentration and Solute Carrier Polymorphisms on Imatinib Efficacy in Chinese Patients with Chronic Myeloid Leukemia Association of genotypes and haplotypes of multi-drug transporter genes ABCB1 and ABCG2 with clinical response to imatinib mesylate in chronic myeloid leukemia patients Reduced ABCG2 and increased SLC22A1 mRNA expression are associated with imatinib response in chronic myeloid leukemia Genetic Variants of ABC and SLC Transporter Genes and Chronic Myeloid Leukaemia: Impact on Susceptibility and Prognosis Effects of ABCG2 C421A and ABCG2 G34A genetic polymorphisms on clinical outcome and response to imatinib mesylate, in Iranian chronic myeloid leukemia patients</td>
<td>[37]</td>
<td>2020</td>
<td>Chinese</td>
<td>PubMed</td>
<td>Journal of Pharmacy and Pharmaceutical Sciences</td>
<td>Q2</td>
<td>Canada</td>
</tr>
<tr>
<td>6</td>
<td>Association of genotypes and haplotypes of multi-drug transporter genes ABCB1 and ABCG2 with clinical response to imatinib mesylate in chronic myeloid leukemia patients Reduced ABCG2 and increased SLC22A1 mRNA expression are associated with imatinib response in chronic myeloid leukemia Genetic Variants of ABC and SLC Transporter Genes and Chronic Myeloid Leukaemia: Impact on Susceptibility and Prognosis Effects of ABCG2 C421A and ABCG2 G34A genetic polymorphisms on clinical outcome and response to imatinib mesylate, in Iranian chronic myeloid leukemia patients</td>
<td>[38]</td>
<td>2014</td>
<td>Malaysia</td>
<td>PubMed</td>
<td>Biomedicine &amp; Pharmacotherapy</td>
<td>Q1</td>
<td>France</td>
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<tr>
<td>7</td>
<td>Reduced ABCG2 and increased SLC22A1 mRNA expression are associated with imatinib response in chronic myeloid leukemia Genetic Variants of ABC and SLC Transporter Genes and Chronic Myeloid Leukaemia: Impact on Susceptibility and Prognosis Effects of ABCG2 C421A and ABCG2 G34A genetic polymorphisms on clinical outcome and response to imatinib mesylate, in Iranian chronic myeloid leukemia patients</td>
<td>[39]</td>
<td>2014</td>
<td>Brazil</td>
<td>Scopus</td>
<td>Medical Oncology</td>
<td>Q2</td>
<td>USA</td>
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<tr>
<td>8</td>
<td>Genetic Variants of ABC and SLC Transporter Genes and Chronic Myeloid Leukaemia: Impact on Susceptibility and Prognosis Effects of ABCG2 C421A and ABCG2 G34A genetic polymorphisms on clinical outcome and response to imatinib mesylate, in Iranian chronic myeloid leukemia patients</td>
<td>[40]</td>
<td>2022</td>
<td>Portugal</td>
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<td>International Journal of Molecular Sciences</td>
<td>Q1</td>
<td>Switzerland</td>
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<tr>
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<td>Genetic Variants of ABC and SLC Transporter Genes and Chronic Myeloid Leukaemia: Impact on Susceptibility and Prognosis Effects of ABCG2 C421A and ABCG2 G34A genetic polymorphisms on clinical outcome and response to imatinib mesylate, in Iranian chronic myeloid leukemia patients</td>
<td>[41]</td>
<td>2023</td>
<td>Irán</td>
<td>Scopus</td>
<td>Egyptian Journal of Medical Human Genetics</td>
<td>Q4</td>
<td>Egypt</td>
</tr>
</tbody>
</table>
3.2. Primary Results

Table 3 summarizes the types of polymorphisms studied where the genes, nucleotides, and genotyping techniques are recognized, as well as the statistical calculation applied in the study.

Considering Table 3, the main techniques used for genotyping were the PCR-RFLP method, validated by direct gene sequencing [24,26,36,38,41] and polymerase chain reaction (PCR) and Sanger sequencing [37,40].

Table 4 shows the statistical results of the analyzed studies, where it can be observed that CYP3A5*3 polymorphisms in their variants heterozygous (AG) and homozygous (GG) present a significantly lower risk of developing resistance to imatinib, while ABCB1 may influence resistance to imatinib.

3.3. Meta-Analysis Results

To perform the meta-analysis in R Study, the rma function of the metafor package was used, giving the following result:

Random-Effects Model (k = 21; tau^2 estimator: REML)

\[ \tau^2 (\text{estimated amount of total heterogeneity}): 0.0038 \ (SE = 0.0032) \]
\[ \tau (\text{square root of estimated } \tau^2 \text{ value}): 0.0515 \]
\[ \hat{r}^2 (\text{total heterogeneity / total variability}): 37.68\% \]
\[ H^2 (\text{total variability / sampling variability}): 1.60 \]

Test for Heterogeneity:
\[ Q(df = 20) = 31.7886, p-val = 0.0456 \]

Model Results:

<table>
<thead>
<tr>
<th>estimate</th>
<th>se</th>
<th>zval</th>
<th>pval</th>
<th>ci.lb</th>
<th>ci.ub</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0501</td>
<td>0.0223</td>
<td>2.2471</td>
<td>0.0246</td>
<td>0.0064</td>
<td>0.0939</td>
</tr>
</tbody>
</table>

---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

At the time of the basic meta-analysis, the model was adjusted by random effects with a k = 21.
Table 3. Meta-analysis meta primary results—analysis.

<table>
<thead>
<tr>
<th>References</th>
<th>n</th>
<th>Age</th>
<th>Treatment Phase</th>
<th>Intervention</th>
<th>Duration (Months)</th>
<th>Polymorphism</th>
<th>Nucleotide</th>
<th>Gene</th>
<th>Genotyping Technique</th>
<th>Genotyping</th>
<th>Statistical Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>[24]</td>
<td>270</td>
<td>42.515</td>
<td>Chronicle (220) Accelerated (36) Blastic (14)</td>
<td>Imatinib (400 mg/day) Imatinib (400 mg/day) Imatinib (400 mg/day)</td>
<td>12</td>
<td>CYP3A5<em>3 CYP3A5</em>3 CYP3A4*18</td>
<td>SNP (1)</td>
<td>CYP3A5<em>3 CYP3A5</em>3 CYP3A4*18</td>
<td>Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).</td>
<td>Heterozygous (AG) Homozygous (GG) Heterozygous (TG)</td>
<td>Binary logistic regression</td>
</tr>
<tr>
<td>[35]</td>
<td>50</td>
<td>46</td>
<td>Chronicle</td>
<td>Response criteria were assessed according to the NCCN guidelines Accessed 12/09/2023 <a href="https://www.nccn.org/professionals/physician_gls/PDF/cml.pdf">https://www.nccn.org/professionals/physician_gls/PDF/cml.pdf</a></td>
<td>10</td>
<td>C480G</td>
<td>C480G</td>
<td>Real-time PCR using TaqmanTM assays</td>
<td>Homozygous (CC)</td>
<td>N/E</td>
<td></td>
</tr>
<tr>
<td>[26]</td>
<td>104</td>
<td>36</td>
<td>Chronicle</td>
<td>Imatinib (400 mg/dia)</td>
<td>60</td>
<td>C1236T C3535T G2677T/A</td>
<td>SNP (1)</td>
<td>MDR1</td>
<td>PCR-RFLP method and validated by direct gene sequencing</td>
<td>CC TT</td>
<td>N/E</td>
</tr>
<tr>
<td>[36]</td>
<td>96</td>
<td>44.44 ± 12.37</td>
<td>Chronicle (66) Accelerated (18) Blastic (12)</td>
<td>Imatinib 400 a 600 mg</td>
<td>12</td>
<td>G2677T</td>
<td>SNP (1)</td>
<td>MDR1</td>
<td>Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP)</td>
<td>GT TT</td>
<td>Unconditional logistic regression.</td>
</tr>
<tr>
<td>[37]</td>
<td>171</td>
<td>43.61 ± 12.27</td>
<td>Chronicle</td>
<td>Imatinib (400 mg/dia)</td>
<td>12</td>
<td>SLCO1A2</td>
<td>SNP (1)</td>
<td>361G&gt;A</td>
<td>Polymerase chain reaction (PCR) and Sanger sequencing</td>
<td>GA</td>
<td>Unconditional logistic regression.</td>
</tr>
<tr>
<td>[38]</td>
<td>215</td>
<td>41.5</td>
<td>Chronicle</td>
<td>Imatinib (400 mg/dia)</td>
<td>N/E</td>
<td>T1236C G2677T/A C421&gt;A</td>
<td>SNP (1)</td>
<td>ABCB1</td>
<td>Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP)</td>
<td>Homozygous CC TT/AT/AA variant</td>
<td>N/E</td>
</tr>
<tr>
<td>[39]</td>
<td>118</td>
<td>50.3</td>
<td>Chronicle</td>
<td>Imatinib (400 mg/dia)</td>
<td>N/E</td>
<td>ARNMs</td>
<td>SNP (1)</td>
<td>ABCG2</td>
<td>PCR-RFLP and real-time PCR using TaqmanTM assays</td>
<td>N/E</td>
<td>N/E</td>
</tr>
<tr>
<td>[40]</td>
<td>198</td>
<td>54</td>
<td>Chronicle</td>
<td>Imatinib (400 mg/dia)</td>
<td>N/E</td>
<td>ABCB1</td>
<td>SNP (1)</td>
<td>ABCB1</td>
<td>PCR method and validated by direct gene sequencing.</td>
<td>CC TT</td>
<td>Unconditional logistic regression.</td>
</tr>
<tr>
<td>[41]</td>
<td>72</td>
<td>50.27 ± 12.72</td>
<td>Chronicle</td>
<td>Imatinib (400 mg/dia)</td>
<td>21</td>
<td>ABCG2</td>
<td>SNP (1)</td>
<td>G34A C421A</td>
<td>PCR-RFLP method and validated by direct gene sequencing.</td>
<td>AG California</td>
<td>Logistic regression.</td>
</tr>
</tbody>
</table>
Table 4. Statistical results of the meta-analyzed studies.

<table>
<thead>
<tr>
<th>References</th>
<th>Polymorphism</th>
<th>Genotyping</th>
<th>OR</th>
<th>CI 95%</th>
<th>p</th>
<th>Association Found (Polymorphisms vs. Resistance to Im)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP3A5*3</td>
<td>Heterozygous (AG)</td>
<td>0.171</td>
<td>0.09 - 0.324</td>
<td>0.001</td>
<td>Significant minor risk</td>
</tr>
<tr>
<td>[24]</td>
<td>CYP3A5*3</td>
<td>Homozygous (GG)</td>
<td>0.257</td>
<td>0.126 - 0.525</td>
<td>0.001</td>
<td>Significant minor risk</td>
</tr>
<tr>
<td></td>
<td>CYP3A4*18</td>
<td>Heterozygous (TC)</td>
<td>0.648</td>
<td>0.277 - 1.515</td>
<td>0.316</td>
<td>Negative</td>
</tr>
<tr>
<td>[35]</td>
<td>C480G</td>
<td>Homozygous (CC)</td>
<td></td>
<td>0.089 - 0.312</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1236T</td>
<td>CC</td>
<td>4.382</td>
<td>1.145 - 16.774</td>
<td>0.022</td>
<td>Significantly increased risk of cytogenetic relapse.</td>
</tr>
<tr>
<td>[26]</td>
<td>C435T</td>
<td>TT</td>
<td>0.309</td>
<td>0.134 - 0.708</td>
<td>0.005</td>
<td>Significantly lower risk of cytogenetic relapse.</td>
</tr>
<tr>
<td></td>
<td>G2677T/A</td>
<td>A</td>
<td>0.266</td>
<td>0.111 - 0.636</td>
<td>0.003</td>
<td>Significantly lower risk of cytogenetic relapse.</td>
</tr>
<tr>
<td>[36]</td>
<td>G2677T</td>
<td>GT</td>
<td>2.519</td>
<td>1.059 - 5.99</td>
<td>0.037</td>
<td>May be useful in predicting response to treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>0.166</td>
<td>0.044 - 0.627</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>[37]</td>
<td>SLCO1A2</td>
<td>GA</td>
<td>4.32</td>
<td>0.924 - 20.206</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1236C</td>
<td>Homozygous CC</td>
<td>2.79</td>
<td>1.217 - 6.374</td>
<td>0.01</td>
<td>Significant association with treatment efficacy</td>
</tr>
<tr>
<td>[38]</td>
<td>G2677T/A</td>
<td>TT/AT/AA variant</td>
<td>0.48</td>
<td>0.239 - 0.957</td>
<td>0.03</td>
<td>Significant association with treatment efficacy</td>
</tr>
<tr>
<td></td>
<td>ABCB1</td>
<td></td>
<td>0.49</td>
<td>0.248 - 0.974</td>
<td>0.04</td>
<td>Can influence with resistance</td>
</tr>
<tr>
<td>[39]</td>
<td>C421&gt;A</td>
<td></td>
<td>2.2</td>
<td>1.273 - 3.811</td>
<td>0.004</td>
<td>Can influence with resistance</td>
</tr>
<tr>
<td></td>
<td>ABCG2</td>
<td>N/E</td>
<td>24</td>
<td>1.74 - 330.8</td>
<td>0.018</td>
<td>May be associated with resistance</td>
</tr>
<tr>
<td>[40]</td>
<td>ABCB1</td>
<td></td>
<td>1.483</td>
<td>1.154 - 1.906</td>
<td>0.002</td>
<td>Findings on SNVs may be a useful tool for understanding interindividual variability and improving therapeutic decisions, including treatment selection.</td>
</tr>
<tr>
<td></td>
<td>rs2231142</td>
<td>CC</td>
<td>0.589</td>
<td>0.388 - 0.892</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs683369</td>
<td></td>
<td>0.598</td>
<td>0.469 - 0.762</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs2631365</td>
<td>TT</td>
<td>0.682</td>
<td>0.534 - 0.869</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>[41]</td>
<td>ABCG2</td>
<td>AG</td>
<td>1.89</td>
<td>0.66 - 5.39</td>
<td>0.235</td>
<td>Not useful for predicting IM response</td>
</tr>
<tr>
<td></td>
<td>CYP3A5*3</td>
<td>California</td>
<td>2.78</td>
<td>0.7 - 11.02</td>
<td>0.146</td>
<td></td>
</tr>
</tbody>
</table>
The above code taken from the R console shows the initial results of the meta-analysis, which mean the following:

$I^2$: A heterogeneity meter, sensitive to sample sizes [42]. In this case, its result was 37.68%. This heterogeneity indicator can be low (up to 25%), moderate (up to 50%), and high (up to 75%) [43]. In this sense, heterogeneity had a variability of 37.68%, i.e., it is considered moderate.

$Q$: Another heterogeneity estimator that is not sensitive to the sample size but is sensitive to the number of meta-analyzed studies; moreover, since it is a hypothesis test, it is interpreted considering its $p$-value [42]. For this study, its result was 30.6959 and its $p$-value corresponded to 0.0456, which indicates that there was significant heterogeneity among the meta-analyzed studies.

$\tau^2$: Corresponds to an estimate of the variance of the true effect sizes among the meta-analyzed studies [42,43]. In this case, the estimated $\tau$ was 2. The restricted maximum likelihood method was used, which was located at 0.0501 [43].

The estimated correlation between the variables studied was calculated and the result was 0.0597 with a standard error of 0.0223 and a significance level of 0.0246, where a $p$-value between 0.01 and 0.05 is not significant [43].

Considering Figure 2, reference [3,24] was the most influential in the study.

To show the results of the meta-analysis, the Forest plot is shown, where the correlation in Fisher’s $z$-values and confidence intervals are indicated (Figure 3).

![Forest plot](Figure 3. Forest plot. References [1–8].)
This meta-analysis studied different polymorphisms in drug transporter and metabolic genes associated with resistance to imatinib in CML, including 21 results from 9 studies analyzed. The results showed no correlation between the studied polymorphisms and imatinib resistance ($z \pm se = 0.051 \pm 0.0223, IC 95\% [0.0064, 0.0939]; Z = 2.2471, p = 0.0246$). Moderate heterogeneity was found among the mean effect size among the included studies ($\tau^2 \pm se = 0.0038 \pm 0.0032, IC 95\% [0.000, 0.0133]; \tau = 0.0615; Q(20) = 31.7886, p > 0.001; I^2 = 37.68\%, IC 95\% [0.00\% - 68.06\%]$).

3.4. Publication Bias

For this study, three techniques were used to test for publication bias, using Egger’s test, and presenting the results in a funnel plot (Figure 4), where it can be established that there was no evidence of publication bias in the study.

```
regtest(res)
```

**Regression Test for Funnel Plot Asymmetry**

*Model:* mixed-effects meta-regression model

*Predictor:* standard error

*Test for Funnel Plot Asymmetry:* $z = 1.0845$, $p = 0.2781$

*Limit Estimate (as sei → 0):* $b = -0.0507$ (CI: $-0.2387$, $0.1372$)

![Egger publication bias](image)

**Figure 4.** Egger publication bias.

This result is in contrast with the result of applying the trim-and-fill method where the studies assumed to be absent in the funnel plot were estimated and the estimate of the actual effect was adjusted [42]. The following code shows the result in the R console:
Figure 4. Egger publication bias. This result is in contrast with the result of applying the trim-and-fill method where the studies assumed to be absent in the funnel plot were estimated and the estimate of the actual effect was adjusted [42]. The following code shows the result in the R console:

As a result of the application of the trim-and-fill method, it was initially established that no study was missing from the left side, which confirms that there was no publication bias. Finally, the Vevea and Hedges weight function model was applied where a likelihood ratio test was applied, and the result is shown in the R console code:

Taking into account this result, it was verified that there was no publication bias in the study, since the \( p \)-value of the test was 0.96909, greater than 0.1 and, therefore, it was not significant.

4. Discussion

The present meta-analysis investigated the association of different polymorphisms with the response to imatinib at different doses (400–600 mg/day) in patients diagnosed with CML. Nine studies that met the inclusion criteria were analyzed. Various studies evaluated the effect of polymorphisms of genes involved in drug metabolism and transport on the therapeutic response by measuring imatinib levels in patients with chronic myeloid leukemia. Imatinib mesylate is considered one of the first-line...
Taking into account this result, it was verified that there was no publication bias in the study, since the p-value of the test was 0.96909, greater than 0.1 and, therefore, it was not significant.

4. Discussion

The present meta-analysis investigated the association of different polymorphisms with the response to imatinib at different doses (400–600 mg/day) in patients diagnosed with CML. Nine studies that met the inclusion criteria were analyzed.

Various studies evaluated the effect of polymorphisms of genes involved in drug metabolism and transport on the therapeutic response by measuring imatinib levels in patients with chronic myeloid leukemia. Imatinib mesylate is considered one of the first-line drugs used for chronic myeloid leukemia whose mechanism of action is to inhibit the effect of the fusion protein tyrosine kinase, product of the BCR/ABL1 fusion gene, with the cytogenetic relapse and molecular response of myeloid leukemia [6].

Studies reported the relationship of the C480G variant of the HOCT1 gene on the effects of imatinib and clinical response, finding no associations with clinical response [44]. This coincided with [39], where there was no correlation between the C480G polymorphism with hematological and cytogenetic studies. On the other hand, they also reported this variant with drug resistance where patients with the GG genotype required higher doses of imatinib to achieve a molecular response, but the differences were not significant [45]. Likewise, Kim et al. mentioned that this GG genotype was found to be associated with failure to treatment with imatinib compared to the CC and CG genotypes associated with a better response [46].

The MDR1 or ABCB1 gene, considered a Pgp transporter, which functions as an efflux pump, is associated with resistance to drugs including imatinib [47]. Studies indicate that patients who presented the CC genotype of the C1236T polymorphic variant presented a greater relapse of the disease, with low levels of imatinib compared to the TT genotype, which was found to be associated with a lower risk of relapse to CML [26]. Likewise, studies reported that the CC genotype for the C1236T and C3435T polymorphisms in the MDR1 gene had a higher risk of relapse compared to patients with the TT genotype [38].

Genes of the CYP450 family are of importance, especially the CYP3A5 gene, playing a key role in the biotransformation of imatinib and other xenobiotics. A study reported that carriers of the (*1/*3) and (*3/*3) variants of CYP3A5 were associated with a lower risk of having resistance against imatinib, suggesting that they could be protective alleles for patients with CML. Those with the CYP3A5*1/*1 genotype had a higher risk of developing resistance to treatment [46].

On the other hand, the mutations present in the kinase domain are predictive for cytogenetic relapse upon treatment of patients [48] and the overexpression of transporter genes such as the glycoprotein PGP could be involved in resistance to treatments [49]. Although some studies indicate that some results may be attributed to study heterogeneity, various clinical endpoints, limited sample number [24].

This study provides important information on future treatments for CML and may serve as a reference for future work.

5. Conclusions

In conclusion, the genetic polymorphism 1236T, homozygous CC of the MDR1 gene significantly influences the increased risk of cytogenetic relapse and the polymorphic 361G>A GA variant of the SLCO1A2 gene significantly affects the complete molecular response.

On the other hand, polymorphic variants AG and GG of CYP3A5*3 are associated with treatment response, presenting a significantly lower risk of imatinib resistance, as well as variants T1236C and G2677T/A of the ABCB1 gene showing a significant association with treatment efficacy; this is in comparison with CYP3A4*18TC, which did not show a significant association with treatment response. It is important to know the pharmacogenetic variants associated with interindividual variability in patients in the choice of treatments.

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Conflicts of Interest: The authors declare no conflicts of interest.

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