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Review Article

Single Nucleotide Polymorphisms as Genetic Determinants of Azathioprine Efficacy & Toxicity

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Abstract

Azathioprine (AZA) is among the most widely prescribed immunosuppressive agents of the past five decades. It finds its application in organ transplantation therapy, autoimmune disorders, and various inflammatory conditions. However, its therapeutic efficacy is often marred by unwanted side effects or adverse drug reactions (ADRs). Polymorphism in genes like thiopurine methyltransferase (*TPMT*), inosine triphosphate pyrophosphatase (*ITPA*), nudix hydrolase 15 (*NUDT15*), and later influences on enzymatic activity play a crucial role in modulating AZA's response and toxicity. Additionally, racial, and ethnic factors further complicate the metabolic outcome of this drug. These changes can lead to undesirable outcomes like myelotoxicity, hematopoietic toxicity, neutropenia, and leukocytopenia. Due to the unpredictability of AZA outcomes, elicited by genetic variations, the Food and Drug Administration (FDA) has recommended genetic screening prior to AZA therapy initiation, highlighting the move towards precision medicine. While pharmacogenetic screening is recommended, accessibility, cost, and ethical considerations are still hurdles in its implementation. This review explores deep into the studies and literature available, reporting the intricate interaction between single nucleotide polymorphisms (SNPs) and the resulting impact on AZA efficacy. The article also recommends how to effectively use pharmacogenetic tools for improving its usage. By incorporating genetic information in treatment plans involving AZA, a patient-centered approach can be achieved, potentially improving the efficacy, safety, and cost-effectiveness of AZA therapy.

Keywords: Genetic variation, azathioprine, adverse effects, efficacy, genetic polymorphism

1. Introduction

Azathioprine (AZA) is one of the classical immunosuppressive agents in use today. It has been used widely for more than 50 years, thus becoming the most commonly used immunosuppressor (Lazarević et al. 2022). It is used in multiple indications spanning post-organ transplant therapy, multiple sclerosis, sarcoidosis, rheumatoid arthritis, and inflammatory bowel disease (IBD) to dermatological conditions. AZA, a thiopurine analog, is rapidly converted non-enzymatically into 6-mercaptopurine which, in turn, is converted into the active moiety, 6-thioguanine nucleotide, by the enzymatic hypoxanthine phosphoribosyl transferase pathway (Dewit et al. 2002). It inhibits purine synthesis by incorporating its metabolites into the replicating DNA (Mohammadi and Kassim 2022).

However, this drug is also associated with various adverse effects like myelosuppression, neutropenia, and drug intolerance. Notably, the risk of neutropenia has restricted the effective use of AZA (Fargher et al. 2007). Some of these adverse outcomes may be attributed to genetic polymorphism of genes like thiopurine methyltransferase (*TPMT*), inosine triphosphate pyrophosphatase (*ITPA*), nudix hydrolase

15*(NUDT15)*, aldehyde oxidase 1 *(AOX1)*, atypical chemokine receptor 1 *(ACKR1)*, and methylenetetrahydrofolate reductase (*MTHFR)*. Various SNPs may either enhance or reduce the efficacy of AZA, making dose calibration, and medication substitution, along with careful monitoring, through blood tests and other biomarkers, imperative. Nevertheless, the therapeutic response and toxicity of thiopurine therapy are highly variable among individuals and remain unpredictable. Racial and ethnic genetic variations also come into play in affecting AZA efficacy and toxicity profile. For example, in Caucasians, about 1 in 300 has a high risk due to *TPMT* polymorphism-associated enzyme deficiency. Moreover, polymorphisms in the *ITPA* gene may account for adverse effects in Asian populations.

The Food and Drug Administration (FDA) has put AZA on the list of drugs that should be prescribed by using a pharmacogenetic guideline, a major breakthrough in precision medicine (Haga, Thummel, and Burke 2006). Additionally, a pharmacoeconomic analysis through a trial-based evaluation of *TPMT* genotyping for AZA resulted in a moderate lowering of treatment-associated costs. While this study has its limitations, further research holds the potential to revolutionize the field of personalized medicine (Thompson et al. 2014; Priest et al. 2006).

2. Indications of Azathioprine

AZA was initially developed to be an immunosuppressant for the prevention of post-transplant organ rejection (Stratta 1998). Today, the drug indication landscape has expanded remarkably with numerous applications that go beyond their intended use (Anstey and Lear 1998). The foremost indication of AZA is as adjunctive therapy for post-renal transplant immunosuppression(Ladrière 2013), pivotal for the success of the procedure. Furthermore, It is most frequently prescribed for its immunomodulatory and steroid-sparing actions, especially in the treatment of inflammatory skin and lupus (TAN et al. 1997; Bracho-Borro, Franco-Ruiz, and Magaña 2022).

Many attribute the versatility of AZA indications to an in-depth comprehension of its intricate metabolic pathway, which has enabled its strategic use against various maladies, like immunomodulation in dermatology, etc. (ANSTEY, WAKELIN, and REYNOLDS 2004).

Transcending its immunosuppressive origins, there is growing evidence of AZA efficacy for myasthenia gravis treatment (Kuks, Djojoatmodjo, and Oosterhuis 1991; Mertens, Balzereit, and Leipert 1969; Morren and Li 2020). As we explore deeper into understanding the pharmacokinetics and pharmacodynamics of this drug, its applications seem boundless with off-label uses continuing to appear. For instance, researchers have been trying to use this medication for rare conditions like idiopathic retroperitoneal fibrosis, interestingly, AZA showed promise on that front as well (Cogan and Fastrez 1985).

However, caution is still recommended owing to the unpredictable and under-researched impact of genetic polymorphism. Therapeutic drug monitoring is employed for patients on AZA to prevent undesirable outcomes. Moreover, prescribing AZA in dermatological conditions has been met with skepticism in certain studies shedding light on the complexity of its mechanisms in specific contexts (Schram et al. 2011; Drucker et al. 2020)

3. Challenges with AZA Treatment

Despite numerous indications of AZA, its mode of action remains unexplored, and here lies the main challenge with using this drug. This challenge adds to the complexity of treatment and the unpredictability of therapeutic outcomes. This drug should be ruled out for the patients who are either unable to or do not consent to following close monitoring of drug levels and other biomarkers, during treatment. Moreover, its slow onset of action (2-3 months)

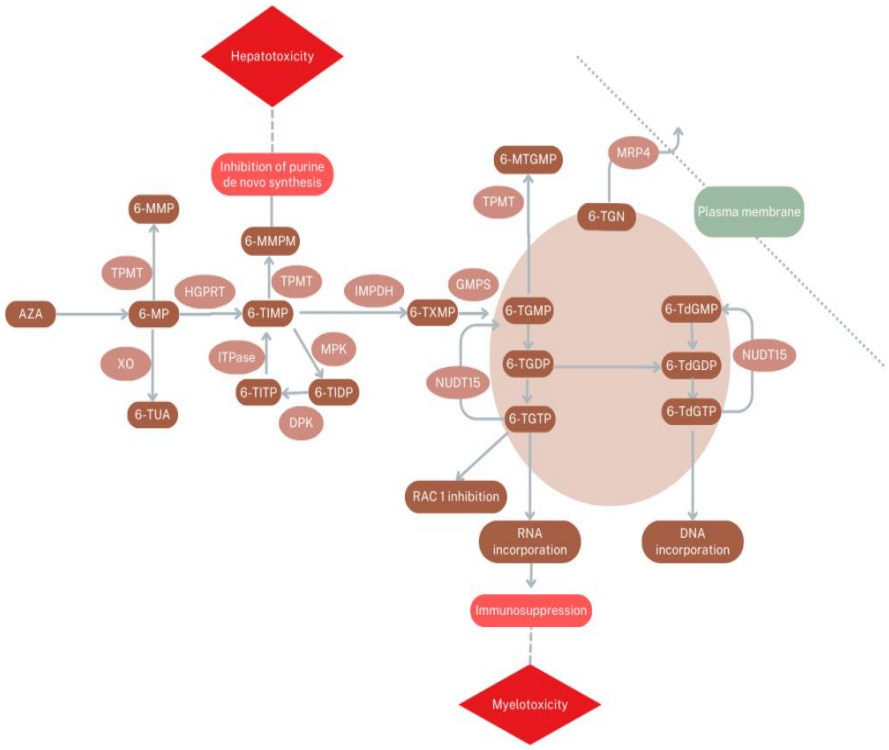

Figure 1: AZA transportation, metabolism, and ADRs. Metabolites are shown in brown boxes:6-mercaptopurine **(6-MP),** 6-Methylmercaptopurine **(6-MMP)**, 6-thiouric acid**(6-TUA)**, 6-methylmercaptopurine ribonucleotides **(6-MMPR)**, 6-thioinosine monophosphate **(6-TIMP)**, 6-thioinosine diphosphate **(6-TIDP)**, 6-thioinosine triphosphate**(6-TITP)**, 6-thioxanthosine monophosphate**(6-TXMP)**, 6-thioguanine monophosphate **(6-TGMP)**, 6-thioguanine diphosphate **(6-TGDP)**, 6-thioguanine triphosphate **(6-TGTP)**, 6-thio-deoxyguanine monophosphate **(6-TdGMP)**, 6-thio-deoxyguanine diphosphate **(6-TdGDP)**, 6-thio-deoxyguanine triphosphate **(6-TdGTP)**, 6-methylthioguanine monophosphate**(6-MTGMP)**, 6-thioguaninenucleotides**(6-TGN)**. Enzymes or transporters are shown in pink boxes: XO, TPMT, hypoxanthine phosphoribosyl transferase **(HGPRT)**, inosine monophosphate dehydrogenase **(IMPDH)**, guanosine monophosphate synthetase **(GMPS)**, monophosphate kinase **(MPK)**, diphosphate kinase **(DPK)**, inosine triphosphate pyrophosphatase **(ITPase)**, multidrug resistance-associated protein 4 **(MRP4)**.

might further hinder patient compliance. It must be kept in mind that immunosuppression is a double-edged sword; in addition to preserving the transplanted organ, the AZA-corticosteroid immunosuppressive activity can lead to an increased susceptibility to viral, bacterial, and fungal infections. In elderly patients, an AZA-prednisolone combination can even lead to mortality (ANSTEY, WAKELIN, and REYNOLDS 2004). Additionally, AZA-induced pancreatitis is a dose-independent side effect, and the condition may turn fatal in case of continued use. The exact underlying mechanism behind this remains unknown (Shah et al. 2022). The risk of neutropenia has restricted the effective use of AZA (Fargher et al. 2007)

Furthermore, inter-patient variability in TPMT activity is another factor that further adds to the intricacies of AZA therapy. As mentioned previously, the FDA has added AZA to the list of drugs that require prior pharmacogenetic assessment of a patient. Knowledge of *TPMT*

polymorphism prevents those with exceptionally low or undetectable TPMT from receiving AZA and may prevent fatal bone marrow suppression.

In addition to *TPMT*, *ITPA* genetic polymorphism also predisposes patients to AZA-induced ADRs (Arenas et al. 2007). In another study done on the Chinese population, *NUDT15* was also found to be a significant factor in AZA-related adverse events. The presence of the *NUDT15* polymorphism (415C>T, rs116855232) is notably associated with AZA-induced myelosuppression. Therefore, the study recommended assessing these two genes prior to AZA administration (Chen et al. 2021).

Genetic polymorphism, racial differences, and immunological status of a patient further compound the AZA therapy-associated issues. Although there are studies that back genetic screening before starting AZA therapy as an economically sage decision, the accessibility, cost, and awareness surrounding this practice add difficulty to prescribing this drug (Marra, Esdaile, and Anis 2002).

4. Genetic Polymorphism Impact on AZA Therapeutic Outcome

Genetic polymorphism might be considered as one of the determinants of AZA therapeutic response. These genetic variations may increase potency even at lower doses or potentially make the therapy ineffective. To understand these responses, this article aims to extensively review existing literature on genetic variations and their implications for the therapeutic effects of AZA.

4.1. *TPMT*

TPMT gene codes for enzymes that catabolize thiopurines. Individual sensitivity and toxicity to such medications are connected with genetic variants that alter its enzymatic activity, leading to TPMT insufficiency(committee 2023f). In AZA metabolism, TPMT adds a methyl group to the thiopurine molecules, which helps to deactivate them and make them more water-soluble for elimination from the body. So, variations in the *TPMT* gene can lead to distinct levels of TPMT enzyme activity.

Several SNPs in the *TPMT* gene—encoding for the main enzyme, TPMT, involved in azathioprine metabolism—have been identified in the past 20 years (Cattaneo, Baldelli, and Perico 2008).

A study predicted that Individuals with TPMT deficiency, receiving standard doses of thiopurines, are at significantly high risk of toxicity, primarily due to unchecked production of thioguanine nucleotides (TGNs). To evaluate this claim, in a six-month-long study in London, 207 patients (aged 18-80) were analyzed. Among them, 117 had CD and 90 had ulcerative colitis. Patients were genotyped for the three common *TPMT* polymorphisms: *TPMT**3B (460 G>A, rs1800460), *TPMT**3C (719 A>G, rs1142345), and *TPMT**3A (presence of both polymorphisms). 83 patients (39%) withdrew due to adverse effects on an intention-to-treat analysis. In addition, 12 (6%) patients reported side effects but continued treatment over the six-month period. Gastrointestinal issues caused more heterozygous people to stop treatment, as compared to those with normal TPMT. Myelotoxicity was also more frequent in those with a heterozygous *TPMT* genotype (26% vs. 0.5%, $P < 0.01$). Among those with a heterozygous *TPMT* genotype (9% of patients), 79% could not tolerate AZA for 6 months compared to 35% of those with normal *TPMT*. However, a baseline TPMT activity below 35pmol/h/mg/Hb was associated with a greater chance of clinical response compared with a TPMT above 35pmol/h/mg/Hb (81% vs. 43% respectively, $P<0.001$) (ANSARI et al. 2008).

Moreover, the researchers selected a total of 333 CD patients on AZA (based in the Northwest of England) and divided them into two groups, for a 4-month long study. One group underwent *TPMT* genotyping prior to AZA and the other was to begin treatment without genotyping. Drug Metabolism Genotyping assays were performed for *TPMT**2 and *TPMT**3(A, B, and

C) alleles. The variant allele *TPMT**2 is defined by a single missense change c.238G>C (rs1800462). *TPMT**3A is defined by a haplotype of two SNPs (c.460G>A, rs1800460 and c.719A>G, rs1142345) resulting in non-synonymous changes p.Ala154Thr and p.Tyr240Cys, respectively, whereas *TPMT**3C (rs1142345) is characterized by c.719A>G, without the c.460G>A variant. Individuals were categorized as wild type if they did not have one or more of these three variant alleles. While results showed no difference between the two groups as no ADRs occurred in younger people (ADR, p = 0.59); however, ADRs were more common in older patients (p = 0.01). One person with *TPMT* variation i.e., having one normal and one altered gene copy (homozygosity) developed severe neutropenia, while *TPMT* heterozygotes were not at an elevated risk for ADR. According to this, heterozygotes are safe when given conventional AZA doses but homozygous variations have a high risk of neutropenia (Newman et al. 2011). This study also concluded that *TPMT* screening does not improve AZA efficacy.

Similarly, In a study by (Charles R. Yates 1997), *TPM* phenotype analysis was conducted on 282 unrelated individuals of white ethnicity. Genotypes were examined in intermediate TPMT activity individuals (heterozygotes) and a matched number of high-activity individuals. Additionally, 6 TPM-deficient patients' genotypes were analyzed. PCR assays showed *TPMT**2 G238C (rs1800462) and *TPMT3* (G460A (rs1800460), A719G (rs1142345)) mutations, while TPMT activity was measured using radiochemical assays. The Concordance between *TPMT* genotype and phenotype was determined. The *TPMT* genotype was assessed in individuals showing intermediate TPMT activity (heterozygotes) and an equivalent number of randomly chosen individuals with high activity. Furthermore, the genotype was examined in 6 patients with TPMT deficiency. Around 10% of patients had intermediate TPMT enzyme activity due to heterozygosity. When these individuals were administered the standard thiopurine dose (75 mg/m2 body surface area per day), those with TPMT deficiency accumulated 6-TGN in their blood cells, leading to potentially fatal suppression of bone marrow function. In this study, the most common altered gene was *TPMT**3A (G460→A (rs1800460), A719→G (rs1142345), found in 18 out of 21 patients (85%). Less common were TPMT*2 (rs1800462) and TPMT*3C (rs1142345), each present in about 5% of patients.

Racial and ethnic genomic differences should also be considered to predict AZA effects on the body. Numerous studies recommend genetic screening of patients before prescribing AZA, a practice codified by the FDA (Haga, Thummel, and Burke 2006).

In another effort to establish a link between the *TPMT* allele's presence and withdrawal of AZA, as a therapeutic agent, in RA patients, a study was conducted in Barcelona. 111 RA (white) patients from Spain were recruited and genotyped. Out of 111, 6.3% (7 patients) carried the mutant *TPMT**3A allele, with the most frequent mutation being *TPMT**3A (G460→A (rs1800460), A719→G (rs1142345)). In a subset of 40 patients treated with AZA, therapy discontinuation of six occurred due to side effects and 26 due to lack of effectiveness. Within this group, three patients with normal *TPMT* alleles experienced moderate side effects, while three *TPMT**3A carriers faced severe gastrointestinal effects like nausea and vomiting. The study suggested that genotyping *TPMT* could allow higher AZA doses in patients with normal *TPMT* alleles, boosting the drug's immune-suppressing effectiveness (Corominas et al. 2003).

In a study, conducted on 209 white healthy subjects and 196 Black healthy subjects (202 women and 303 men), the data shows that TPMT activity is similarly polymorphic in American Black subjects and white subjects, although median TPMT activity is approximately 17%

Table1. Summary of SNPs and Their Impact on AZA Efficacy

SNP	POPULATION	SAMPLE SIZE	SNP ASSOCIATION AND EFFECT	REFERENCE
TPMT				
(rs1800460, rs1142345)	British	207	Myelotoxicity	(ANSARI et al. 2008)
	White Americans	282	Drug accumulation	(Charles R. Yates 1997)
	Spanish	111	Severe side effects	(Corominas et al. 2003)
(rs1800462)	British	333	No ADRs	(Newman et al. 2011)
	Egyptian	150	Myelosuppression	(Abuelsoud, Fayed, and Elkateeb 2021)
ITPA				
(rs1127354)	British	89	ADRs (e.g., pancreatitis)	(Marinaki et al. 2004)
	Dutch	72	No ADRs	(DE RIDDER et al. 2006)
(rs7270101)	German	71	Severe side effects	(von Ahsen et al. 2005)
	Scottish	65	No toxicity	(Breen et al. 2005)
NUDT15				
(rs746071566, rs116855232)	German	107	Hematotoxicity	(Schaeffeler et al. 2019)
	Americans	1403	Myelotoxicity	(Dickson et al. 2022)
rs116855232	Korean	978	Leukopenia	(Yang et al. 2014)
	Chinese	253	Leukopenia	(Zhu et al. 2016)
	Japanese	92	Myelotoxicity	(Tanaka et al. 2015)
(rs116855232)	Indian	69	Leukopenia	(Shah et al. 2017)
ACKR1				
rs2814778-CC	Americans	1466	Hematopoietic toxicity	(Dickson and Daniel 2022)
	African	94	Leukemia	(Dickson and Daniel 2022)
MTHFR				
(rs1801133)	Serbian	102	Elevated right ventricular systolic pressure	(Jelovac et al. 2023)
		219	Decreased AZA efficacy	(Arenas et al. 2005)
		65	AZA intolerance	(Breen et al. 2005)
XDH				
(rs4407290)	Polish	156	Weak protective effect against ADRs	(Kurzawski et al. 2012)
(rs45547640)	Korean	964	Leukopenia	(Park et al. 2016)
MOCOS				
(rs3744900)	Korean	139	Leukemia	(Choi et al. 2019)
(rs594445, rs1057251)	Iranian	406	ADRs	(Taheri et al. 2020)
(rs594445)	British	192	Weak protective effect against ADRs	(SMITH et al. 2009)
	non-Caucasian British	14	No toxicity	(SMITH et al. 2009)
	Polish	156	Classic xanthinuria ty	(Kurzawski et al. 2012)
AOX1				
(rs55754655)	British	192	No therapeutic response produced	(SMITH et al. 2009)
	Polish	156	Hematological toxicity	(Kurzawski et al. 2012)
Increased AOX1	Americans	1201	Myelotoxicity	(Daniel et al. 2022)
	Non-White Americans	517	Myelotoxicity	(Daniel et al. 2022)
(rs55754655)	Jordanian	100	No efficacy	(Mahasneh 2020)

lower in Black subjects. The study did not find significant variation in AZA response upon gender-based result evaluation. This is crucial for understanding varied AZA responses in different races (McLeod et al. 1994). However, the study did not try to link this response to *TPMT* genetic polymorphism.

Furthermore, a study involving 150 autoimmune patients (average age: 35.85 years), aimed at detecting the frequencies of allelic variants (*TPMT**3A (G460→A (rs1800460), A719→G (rs1142345), *TPMT**3C (719 A>G, rs1142345), and *TPMT**3G (rs1800460)) and their association with AZA clinical efficacy and ADRs, was conducted in Egypt. They found that AZA treatment failed in 50% of subjects, leading to drug discontinuation or substitution (45%). Myelosuppression rates were higher in certain genetic variants *TMPT**2 G238C (rs1800462). Interestingly, females faced higher immunosuppression risk. This study recommended *TPMT* genetic screening before starting AZA therapy (Abuelsoud, Fayed, and Elkateeb 2021)

4.2. *ITPA*

ITPA enzyme is encoded by *ITPA.* The encoded protein hydrolyzes inosine triphosphate and deoxyinosine triphosphate to the monophosphate nucleotide and diphosphate. Any defect in this protein can result in inosine triphosphate pyrophosphorylase (ITPase) deficiency, causing ITP accumulation in red blood cells. Multiple transcript variants are usually produced as a consequence of alternate splicing (committee 2023c). 6-thioinosine triphosphate (6-TITP) gets converted to phosphorylated 6-thioinosine monophosphate (6-TIMP), an intermediate metabolite of AZA/6-mercaptopurine, with the help of ITPA enzyme. In ITPA-deficient patients, 6-TITP accumulation leads to ADRs, including myelosuppression. So far, Five functional *ITPA* gene SNPs have been identified (Eltantawy et al. 2023).

A study conducted in the Egyptian population, aimed to evaluate specific SNPs, involved in the toxicity and efficacy of AZA, and find the correlation between the NUDT15(rs116855232), TPMT (rs1800460), and ITPA (rs1127354). ITPA (rs1127354) was found to be significantly associated with adverse effects among IBD patients on AZA treatment (Eltantawy et al. 2023).

In a study, (Marinaki et al. 2004) established an association between *ITPA* 94C>A (rs1127354) SNP and ADRs to AZA therapy. The study was done by recruiting IBD patients, 62 of those who experienced adverse reactions to AZA and 68 control patients who tolerated AZA treatment well. The researchers set out to analyze the genetic profile of patients for *TMPT* and *ITPA* variants. The results illustrated that while TPMT deficiency was associated with certain side effects, such as nausea and vomiting, *ITPA* 94C>A (rs1127354) polymorphism was significantly associated with ADRs to AZA therapy, including influenza-like symptoms, rash, and pancreatitis. However, they also suggested that TPMT and ITPA deficiency might account for less than half of the observed AZA toxicity.

In another study, the researchers did a 6-month prospective study including 71 patients (German Caucasians) with CD, undergoing first-time AZA treatment. The *ITPA* gene variant under study was IVS2 + 21A>C (rs7270101), which affects splicing efficiency. It was found to be more frequent (15%) than 94C>A (rs1127354) mutation (9%) in this study. Individuals with one copy of IVS2 + 21A>C had 60% of the normal ITPA activity. This suggests that homozygotes for IVS2 + 21A>C may have activity like heterozygotes for 94C>A (rs1127354). Importantly, either having one copy of *ITPA* 94C>A or being homozygous for IVS2 + 21A>C was significantly linked to early discontinuation of AZA therapy (within two weeks) due to side effects (von Ahsen et al. 2005).

On the other hand, another paper published in the same year contradicted the earlier findings. The research on 65 liver transplant recipients,

enrolled from the Liver Transplant Out-Patient Clinic at the Royal Infirmary of Edinburgh between March and May 2003, the variation *ITPA* 94C>A (rs1127354), which results in <25% residual activity, concluded that it did not predict side effects to AZA therapy (dominant model, P = 1.000). Similarly, the *ITPA* IVS2+21A>C (rs7270101), which is associated with 60% residual enzyme activity, was not significantly associated with AZA intolerance (dominant model, P = 0.7766; recessive model, P = 0.1688). Moreover, A second polymorphism in the *ITPA* gene (IVS2+21A>C) results in approximately 60% wild-type activity in homozygotes but has not been reported to be associated with AZA toxicity (Breen et al. 2005).

A Dutch study conducted in 72 pediatric IBD patients, who were treated with AZA, reached a similar conclusion. Interestingly, only one patient with adverse events had a heterozygous *TPMT**3C (rs1142345) allele, and two patients with homozygous *ITPA* 94C>A (rs7270101) tolerated standard AZA doses without toxicity, subverting the expectation that such a variation might lead to AZA toxicity. Although adverse events led to AZA discontinuation in 11 (15.3%) cases, no significant association of functional *ITPA* and *TPMT* polymorphisms and the occurrence of AZA-related adverse events could be established. This study concluded that Pharmacogenetic assessment prior to thiopurine therapy did not seem warranted. Moreover, they recommended further research, as the mechanisms underpinning AZA intolerance in children remain obscure (DE RIDDER et al. 2006).

4.3. *NUDT15/MTH2*

An enzyme of the Nudix hydrolase superfamily is coded by the *NUDT15* gene. Members of this superfamily mediate hydrolysis of nucleoside diphosphates, including substrates like 8-oxo-dGTP, produced in the aftermath of oxidative damage, and may cause base mispairing during DNA replication, resulting in transversions. The encoded enzyme is a negative regulator of thiopurine activation and toxicity. NUDT15 converts 6-TGTP, an active thiopurine metabolite, to the monophosphate thioguanosine nucleotide 6-TGMP. These metabolites suppress the immune system, and their excessive concentration can cause myelotoxicity. So, polymorphisms in the *NUDT15* gene modify enzymatic activity, leading to a toxic buildup of 6-TGTP and 6-TdGTP, consequently causing leukopenia. This gene has a multitude of pseudogenes (committee 2023d; Suzuki et al. 2023).

NUDT15 or *MTH2* gene variations linked with severe thiopurine-related hematotoxicity were studied in 107 European patients, and an independent cohort of 689 acute lymphoblastic leukemia patients was used as a control. They found *NUDT15* gene mutations in 14 patients (13%), including a novel variant (p. Met1Ile). Out of 5 missense variants of NUDT15, a few missense variants have previously been associated with thiopurine-related toxicity (rs746071566, rs116855232). Moreover, 13% of subjects, with *NUDT15* variants, experienced early adverse effects within three months of treatment. The study rather boldly claimed that collectively *NUDT15* and *TPMT* genes explain half of severe thiopurine-related hematotoxicity, prescribing preemptive genetic testing in Europeans (Schaeffeler et al. 2019).

Another study illustrated the impact of genetic variations in *TPMT* and *NUDT15* genes on the discontinuation of AZA therapy, due to myelotoxicity, in patients with inflammatory conditions. The study used electronic health records from Vanderbilt University's biobank to find a potential association between metabolizer status and AZA discontinuation, prompted by severe bone marrow suppression. They identified 1,403 patients with various inflammatory diseases who were new users of AZA. Interestingly, most cases of discontinuation due to myelotoxicity occurred in patients with normal *TPMT* and *NUDT15*

metabolizer status. However, missing clinical information, limited sample diversity, and the complexity of determining the impact of other genes on AZA metabolism cast a shadow of doubt on the findings. This risk remained even after considering factors like age, sex, and dose, highlighting the need for further research into genetic and non-genetic factors leading to myelotoxicity (Dickson et al. 2022).

In a similar fashion, (Yang et al. 2014) reported that a non-synonymous SNP in *NUDT15* (R139C, rs116855232) was very strongly associated with thiopurine-induced early leukopenia among Koreans. In this paper, an immunochip-based 2-stage association study was performed by researchers in 978 subjects, from Korea, undergoing thiopurine therapy for CD. The Koreans, carrying this SNP, had a high sensitivity (89.4%) and specificity (93.2%) for early leukopenia.

This association was also confirmed in the Japanese population (Tanaka et al. 2015). In a study, 92 Japanese pediatric acute lymphoblastic leukemia (ALL) patients were genotyped prior to AZA administration. The results indicated that the carriers of the variant T allele of rs116855232 were significantly more prone to leucopenia during maintenance therapy. Moreover, Patients with the TT genotype of rs116855232 required a 6-MP dose reduction, and this was significantly more likely than in CC genotype carriers. The study recommended pre-AZA-therapy genotyping for *NUDT15* for Asians, similar to the *TPMT* testing guideline in populations of European descent.

In China, by (Zhu et al. 2016), 253 Chinese CD patients were enrolled for a study about understanding the influence of *NUDT15* R139C (rs116855232) on thiopurine-induced leukopenia. They found this variation to be strongly linked with the incidence of leukopenia (70.2% mutation vs. 12.8% wild type; P=8.61×10−19; odds ratio, 10.80; 95% CI, 5.89–19.83). Unlike the White population, the *TPMT* genotype was not associated with leukopenia in the Chinese population (P = 0.44).

In India, a pilot study by (Shah et al. 2017) was performed. In this an analysis was performed on 69 Indian patients on thiopurine therapy to establish a connection among *NUDT15* C415T (rs116855232) and *TPMT* (*2 (238G>C, rs1800462), *3A (c.460G>A rs1800460 and c.719A>G rs1142345), *3B (c.460G>A rs1800460), and *3C (719A>G, rs1142345)) polymorphism and leukopenia induction due to thiopurine. The distribution of NUDT15 genotypes was as follows: 86.9% for CC, 11.5% for CT, and 1.5% for TT. Notably, *TPMT* genetic variants were not detected in the study. Among the 60 patients without the NUDT15 variant, non-experienced leukopenia. Conversely, among the nine patients with the NUDT15 variant, six developed leukopenia, demonstrating a highly significant relationship (P-value < 0.0001). Though rarely, this SNP was significantly connected with thiopurine-induced leukopenia in European subjects with IBD.

4.4. **ACKR1**

The protein encoded by this gene is a glycosylated membrane protein and a non-specific receptor for several chemokines. Polymorphisms in this gene are the basis of the Duffy blood group system. Two transcript variants encoding different isoforms have been found for this gene (committee 2023b) . It is noteworthy that *ACKR1* indirectly influences AZA therapeutic outcomes.

A genetic analysis of people, recruited from White and Black races, revealed that the *ACKR1* variant (rs2814778-CC), common in individuals of African descent, is linked to an increased risk of AZA discontinuation due to hematopoietic toxicity and lower thiopurine dosing. Patients with the CC genotype (101 individuals) discontinued AZA at a higher rate than those with the TT or TC genotype (1365 individuals) due to blood-related problems. Even when race was considered, the CC genotype's risk persisted (HR 2.61). The elevated risk linked to race was

eliminated after accounting for genotype. However, lower blood cell counts and dosage were seen in patients with the CC genotype. Another related investigation in a cohort of 94 African children, who were given AZA for leukemia, confirmed this lower dose trend (Dickson and Daniel 2022).

Similarly, through clinical assessment of neutrophil counts following thiopurines, a complication arose by a common variant in *ACKR1.* This variant affected baseline neutrophil counts, a finding referred to as Duffy-null Associated Neutrophil Count, formerly known as constitutional neutropenia or benign ethnic neutropenia (Kelan Tantisira and Scott T Weiss Jun 2023). Moreover, a search conducted through systematic literature (of previous 5 decades) across major electronic databases, like MEDLINE, suggested testing for *ACKR1* (rs2814778) and using it as a pharmacogenetic biomarker to predict drug-induced hematological adverse or side effects(Pattanaik, Jain, and Ahluwalia 2021). While the underlying mechanism remains a mystery, for now, further research is needed.

4.5. MTHFR

To convert homocysteine into methionine, *MTHFR* encodes a protein that aids in the conversion of 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. Any genetic variation in it predisposes one to developing leukemia, colon cancer, vascular disease, and neural tube abnormalities. Additionally, MTHFR deficiency results from these variations (committee 2023g). Hence, genetic variations in folate metabolism, particularly those in the *MTHFR* gene, could influence TPMT activity and subsequently impact the response to thiopurine drug therapy, such as AZA.

In a recent study, involving 102 Serbian patients, suffering from a rare condition called systemic sclerosis, a missense variant C677T (rs1801133) in the *MTHFR* gene was analyzed. The variant allele carriers had a twofold higher risk of developing elevated right ventricular systolic pressure when treated with AZA, making it an unfavorable drug for those patients (Jelovac et al. 2023).

In another study, the patients were genotyped for various genetic variants, including *TPMT**3A (c.460G>A rs1800460 and c.719A>G rs1142345), *3C (719A>G, rs1142345), *2 (238G>C, rs1800462) (variants associated with decreased TPMT activity), *MTHFR* 677C>T (rs1801133), and 1298A>C (rs1801131) variants (variants associated with folate metabolism). While it does not explicitly mention the duration of the study or the number of patients recruited, it suggests that *MTHFR* gene variants, *MTHFR* 677C>T (rs1801133) and 1298A>C (rs1801131), could potentially impact the activity of MTHFR enzyme. They also added that the homozygous *MTHFR* 677TT (rs1801133) genotype also influences TPMT enzyme activity by reducing it, thus decreasing AZA efficacy. However, the precise clinical implications of these interactions are not fully understood and, therefore, require further investigation (Arenas et al. 2005).

However, in another study done at The Royal Infirmary of Edinburgh, 65 liver transplant recipients, none of the variants i.e. *MTHFR* 677TT(rs1801133) genotype, *MTHFR* 677T(rs1801133)/1298C(rs1801131) compound heterozygous genotype or the 1298CC variant genotype could be significantly associated with AZA intolerance (P = 1.000, P = 1.000, and P = 0.6899, respectively) (Breen et al. 2005).

4.6. XDH

Xanthine dehydrogenase/oxidase (XDH/XO) is a critical enzyme in the AZA catabolic pathway. It mediates oxidation of 6-mercaptopurine to 6-thioxanthine and 6-thiouric acid (Rashidi et al. 2007). Although the XDH enzyme is the second major contributor to AZA breakdown, polymorphisms in the *XDH* gene have rarely been studied. However, some studies report that any malfunction in this gene may cause xanthinuria, impacting respiratory stress and increasing flu vulnerability via oxygen-related mechanisms (committee 2023h).

A Polish study was conducted on 156 renal transplant white recipients, whose blood samples were collected post-transplantation from cadaveric donors from 1998 to 2005. The aim was to study the influence of *XDH* genetic variation on AZA catabolism. Interestingly, SNPs *XDH* c.837C>T (rs206797) (P=0.048, OR 0.23, 95% CI 0.05–1.05) demonstrated a weak protective effect against ADRs. However, they did not observe any noticeable influence of *XDH* SNPs on the clinical parameters of AZA-treated patients (Kurzawski et al. 2012).

Moreover, to analyze if an association between four *XDH* variants (rs45523133, rs45547640, rs72549369, and p.Thr910Lys) and thiopurine-induced leukopenia exists, 964 CD patients of Korean origin, treated with thiopurines, were recruited. In the epistasis analysis, only *XDH* (rs45547640) showed a statistically significant association with early leukopenia. However, no firm connection could be established between the other two variants of *XDH* and thiopurine-induced leukopenia (Park et al. 2016).

4.7. ***MOCOS***

The enzyme responsible for activating the molybdenum cofactor by adding sulfur is encoded by the *MOCOS* gene. This step is crucial for XDH and AOX1 enzyme activity. Moreover, variations cause classical xanthinuria type II (committee 2023e). Numerous studies concluded that through an indirect involvement in thiopurine metabolism, *MOCOS* variants induce thiopurine resistance because of their TPMT activity-modifying action (Karas-Kuzelicki, Milek, and Mlinaric-Rascan 2010; Coelho et al. 2016)

Molybdenum cofactor sulfurase enzyme (encoded by *MOCOS*) is essential for the activities of XDH and AOX1 enzymes (Pavelcova, Petru, and Stiburkova 2017). A decade-long study, involving 139 pediatric acute lymphoblastic leukemia patients in Korea, studied rs3744900 SNP in the *MOCOS* gene. The paper concluded that *MOCOS* (rs3744900) is one of the polymorphisms associated with thiopurine-related toxicities like neutropenia, hepatotoxicity, and treatment interruption or discontinuation ($P < 0.05$) (Choi et al. 2019).

A study, involving a total of 406 autism spectrum disorders (ASD) patients, concluded that *MOCOS* gene variations like rs594445 and rs1057251 can impact the encoded protein's function leading to ASD. Notably, these variations were also linked to responses to AZA ADRs and asthma development, respectively, further emphasizing their potential significance in influencing the outcomes of AZA therapy (Taheri et al. 2020).

Interestingly, two SNPs may have a synergistic effect on drug efficacy, as shown in a study (SMITH et al. 2009) done on 208 patients, recruited as part of a prospective multi-center study of AZA for the treatment of IBD, in London. This paper found that a weak protective effect against AZA-related ADRs from SNPs *XDH* c.837C>T rs4407290 (P=0.048, OR 0.23, 95% CI 0.05–1.05) and *MOCOS* c.2107A>C (rs594445), (P=0.058 in recessive model, OR 0.64, 95%CI 0.36–1.15). This effect was stronger when both SNPs were present together (P=0.019).

However, in the same study, excluding non-Caucasian individuals made the connection between these genetic differences and negative drug reactions less clear. The influence of the *XDH* 837T (rs4407290) variant on adverse drug reactions became uncertain (P=0.08, OR 0.26, 95%CI 0.06–1.22), and the impact of the *MOCOS* 2107C (rs594445) variant on ADRs also showed some uncertainty (P=0.056 in a recessive model, OR 0.26, 95%CI 0.36–1.20) (SMITH et al. 2009).

Moreover, an analysis of common SNPs in the coding areas of the *XDH*, *AOX1*, and *MOCOS* genes, conducted in 156 patients, receiving AZA for the first year following surgery, indicated that the effects of *MOCOS* (rs594445) polymorphism are independent of other investigated gene variations and might influence AZA dosage, similar to *TPMT* heterozygosity (Kurzawski et al. 2012). Since loss-of-function mutations within the *MOCOS*

gene have been shown to cause classic xanthinuria type II, a missense (rs594445) polymorphism may lead to altered enzyme activity. This may result in decreased metabolic capacity of XDH and AOX1 enzymes, translating to slower thiopurine metabolism. Further research is needed to prove a positive correlation.

4.8. AOX1

AOX1 produces hydrogen peroxide and, under certain conditions, can catalyze the formation of superoxide. It is claimed to be involved in thiopurine catabolism. This involvement makes *AOX1* a natural suspect in case of aberrant response to AZA therapy (committee 2023a). In the study by (SMITH et al. 2009), conducted on a cohort of 192 AZA-treated IBD patients, SNP *AOX1* c.3404A>G (rs55754655) found a lack of response to the therapy (P=0.035, OR 2.54, 95%CI 1.06–6.13). When combined with TPMT activity, it further helped with accurately predicting a subject's odds of responding to the treatment.

Another study, involving 156 renal transplant white recipients, reported that *AOX1* rs55754655 variant allele carriers received a higher mean AZA dose 3-, 6-, and 12 months post-transplantation ($P < 0.05$). Increasing the AZA dose than normal could prove to be fatal due to its severe impacts on the hematological profile. This warrants further research into the association between this SNP and AZA dosing (Kurzawski et al. 2012).

A comprehensive study, encompassing four genes *TPMT, NUDT15, AOX1,* and *NME1* was done. The researchers divided patients into two cohorts. One was 'the discovery cohort (N = 1201)' which included patients receiving AZA prescriptions for an inflammatory condition, such as CD, ulcerative colitis, systemic lupus erythematosus, or RA. The other called 'validation cohort' included patients who belonged to races other than White (i.e., Black, Asian, other, or unknown) or who were on AZA for non-inflammatory indications (i.e., organ transplant, other, or unknown; N = 517). Through this study, they uncovered a correlation between increased expression of *AOX1* and increased incidences of AZA-induced myelotoxicity (Daniel et al. 2022)

In contrast, a Jordanian study that involved one hundred IBD patients, aged 17-72 years, taking AZA, recruited, and genotyped for *AOX1* 3404 G (rs55754655), *XDH*1936C, and *XDH*2107C polymorphisms, found no statistically significant association (p-value>0.05) between the *AOX1*3404G (rs55754655). Moreover, the study showed an interesting correlation between the age of the patient and the response to AZA (p-value=0.013) (Mahasneh 2020).

5. Discussion

Considering the studies mentioned above, one can correlate genetic polymorphism and AZA therapeutic outcomes. Drug response and genetic polymorphism are not only influenced by pharmacokinetic or pharmacodynamics variations but also racial and ethnic factors play a pivotal part. To comprehend the impact of genetic polymorphisms on AZA, *TMPT* is usually a prime candidate. In the review article, (Sahasranaman, Howard, and Roy 2008) concluded that *TPMT*3A* is a common mutation that reduces enzyme function. There are also other variants like *TPMT**2 (rs1800462), *TPMT**3B (rs1800460), and *TPMT**3C (rs1142345), each impacting TPMT activity in a specific way. Some individuals inherit two copies of these low-activity variants, making them more prone to myelosuppression. The variant usually changes the level of enzymatic activity which either expedites the drug-elimination process or leads to toxic metabolite accumulation (Cattaneo, Baldelli, and Perico 2008).

The next significant genetic polymorphism that controls the fate of AZA treatment, by altering the enzymatic activity, is that of *ITPA.* A study reported that *ITPA* gene polymorphisms may account for the non-TPMT-related adverse effects. A mutation called *ITPA* 94C>A

(rs1127345) can result in decreased ITPase activity. People with reduced ITPase activity, owing to these mutations, might experience ADRs when taking drugs like AZA. These reactions can include flu-like symptoms, rash, and pancreatitis. Interestingly, the *ITPA* 94C>A (rs1127345) mutation is more prevalent in Asian populations compared to Caucasians (Sahasranaman, Howard, and Roy 2008). However, these changes are not always due to straightforward reasons. Some SNPs may influence drug response by indirectly altering a physiological mechanism, leading to AZA discontinuation or ADRs. For instance, *ACKR1*-mediated neutropenia affects patient care by impeding the administration of drugs like AZA and leading to unwarranted bone marrow biopsies. This trend was observed in patients of African origin. This not only complicates the therapeutic plan for disease management but also adds to the treatment cost (Crawford and Volkman 2023). Likewise, a study on six variants of *AOX1*, with allele frequencies greater than 0.01 in 1 or more populations, obtained from the genome aggregation and 1000 Genomes Project databases, was done. They found that T755l (rs35217482)SNP of this gene, prominent in East Asian populations, significantly interferes with the metabolism of drugs like AZA (Ueda et al. 2022).

As previously mentioned, racial factors and cross-global distribution patterns of genetic polymorphism are also critical for determining AZA therapeutic outcomes and dose calibration. For example, an overview study found that the *NUDT15* 'poor metabolizer' phenotype is observed at a frequency of approximately 1 in 50 people with ancestry from countries in East Asia, which is more common than the *TPMT* 'poor metabolizer' phenotype in people of European origins. It is noteworthy that studies make *NUDT15* R139C (rs116855232) one of the critical factors influencing AZA therapeutic outcomes. According to FDA guidelines for AZA, poor metabolizers should consider alternative therapy, while intermediate metabolizers with *NUDT15* or *TPMT* mutations require dose reduction. Those with mutations in both genes may need more significant dose adjustments (Kelan Tantisira and Scott T Weiss Jun 2023). By understanding these patterns and influences we can not only optimize AZA dosage and outcomes but also improve the safety of the drug. Further research into this area can prove to be beneficial in the therapeutic as well as the economic realm.

6. Conclusions

From the aforementioned studies, it is safe to conclude that genetic polymorphism has a deterministic impact on therapeutic responses to drugs like AZA (Mikhaylenko et al. 2020). This means that genetic variations can be used to develop predictive models to assess potential responses to AZA therapy. Genes like *TPMT*, *ITP*, *AOX1*, *MOCOS*, *XDH*, *MTHFR*, *ACKR1*, and *NUDT15* need to be thoroughly researched in larger populations with diverse backgrounds to develop comprehensive thiopurine-prescribing practice guidelines, considering pharmacoeconomic benefits notwithstanding. Severe myelosuppression and susceptibility to severe infectious diseases make establishing these guidelines imperative. After the FDA's step to mandate *TPMT* and *ITPA* screening before prescribing AZA, it is essential for policymakers, researchers, and healthcare professionals to realize that the one-size-fits-all approach is outdated and that precision medicine should become the new standard. Pharmacogenomics stands out as a key tool for the development of personalized medicine. However, despite numerous studies, the result remains inconclusive and inconsistent, which demands toolkit expansion and making substantial investments (Tarnowski et al. 2016). It is important to consider that rational selection of disease-modifying anti-rheumatic drugs offers various potential advantages, including rapid disease control and reduced long-term

disability, as expounded upon by (Zhang et al. 2014). Adopting a balanced approach with well-informed decision-making has the potential to optimize and maximize these benefits.

7. Recommendations

Numerous studies recommend screening the patients for critical SNPs and variants for predicting therapeutic outcomes, dose calibration, and exploring alternative treatment options. On the other hand, some researchers do not recommend prior genetic testing; however, caution must be practiced. In addition to developing predictive models, contingency therapeutic plans must also be designed to effectively improve and sustain the treatment outcomes. Moreover, international collaborative efforts are needed to deepen the investigation into genetic variants, making it inclusive of all races, ethnicities, and genders for pharmacogenetic data mining, and in this case, for pre- and post-AZA therapy. This has the potential to not only regulate drug usage but also tackle idiosyncrasies that might arise during treatment. Additionally, a method needs to be devised for longitudinal data collection because continuous monitoring of patients on AZA treatment will help identify patterns and refine dosing strategies, contributing to the development of more effective personalized treatment plans. It is noteworthy that this practice has numerous economic benefits, adequately backed by research. However, this is merely the first step because the pharmacogenetic testing accessibility needs to be democratic; otherwise, the dream of precision medication will remain elusive. In addition to access, awareness for both patients and healthcare providers is also advisable regarding personalized medicine and how they can chip in to help with its development. While AZA has been a mainstay in immunosuppression, medical research should look for alternative immunomodulatory agents that have fewer side effects and induce targeted immunosuppression as opposed to general immune response attenuation. Lastly, as genetic information becomes more integrated into the medical decision-making process, patient consent and privacy must be protected through rules and regulations. Incorporating these recommendations could lead to a more comprehensive, effective, and patient-centered approach to AZA therapy, leveraging genetic information for optimized treatment outcomes.

Competing Interests

The authors declare no competing interests.

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Ethics Approval

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Consent Forms

Not Applicable

Author Contributions

Both the authors contributed equally to this manuscript, however, the idea was conceptualized by NS, who also wrote the final manuscript.

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