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Genetic Variants Associated with Gefitinib Adverse Events in Non-Small Cell Lung Cancer: A Systematic Review Integrated with Protein-Protein Interaction Network and Structural Modelling

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Abstract

***Introduction**: Lung cancer remains the leading cause of cancer-related death worldwide, with non–small cell lung cancer (NSCLC) accounting for approximately 85% of cases. Gefitinib is a tyrosine kinase inhibitor frequently used in NSCLC with favorable outcome. However, many patients develop severe adverse effects which might be influenced by genetic variability. Therefore, we aim to systematically review the gene variants and its association with gefitinib-related adverse effects in NSCLC patients, as well as investigate the biological process involved. **Methods**: A systematic search was conducted according to PRISMA guidelines across PubMed, Scopus, and Cochrane. Studies investigating the association between genetic variations with gefitinib-related adverse effects in NSCLC were included. Risk of bias was assessed using the Cochrane RoB-E. Extracted data encompassed study and patient characteristics, adverse effects, and gene variations. Significant genes identified from included studies were analyzed through PPI network analysis, and the hub proteins found were visualized through Chimera. **Results**: Sixteen studies involving 1,176 patients were included, with Japanese populations being the most studied. Gene variants of CYP2D6, CYP3A4, ABCB1, ABCG2, EGFR, FOXO3, IKBKB, and AKT1 were found to be associated with adverse effects such as hepatotoxicity, skin rash, and diarrhea among NSCLC patients. Metabolism and inflammatory pathways might be involved in gefitinib-related adverse effects. **Conclusion**: Genetic variations in CYP2D6, CYP3A4, ABCB1, ABCG2, EGFR, FOXO3, IKBKB, and AKT1 may influence gefitinib-associated adverse effects, highlighting the need of pharmacogenomic testing to guide personalized treatment and improved patient safety.*

***Keywords**: Genetic Variants, Gefitinib, Non-Small Cell Lung Cancer, Adverse Effects, Protein-protein interaction*

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1. INTRODUCTION

Lung cancer remains the leading cause of cancer-related deaths around the world. Non-small cell lung cancer (NSCLC) makes up nearly 85% of all cases.[1] Without targeted therapy, survival rates are low, especially for patients diagnosed at advanced stages. The discovery of activating EGFR mutations changed how we manage NSCLC. This is particularly true in Asian populations, where EGFR mutations occur in 40-60% of cases, which is three to four times higher than in Western groups.[2] Gefitinib, a first-generation EGFR tyrosine kinase inhibitor (EGFR-TKI), became the first-line standard due to its ability to block faulty EGFR signaling and significantly improve survival for patients with mutations.[3]

Gefitinib provides significant clinical benefits, but its use is often limited by treatment-related adverse effects. Skin reactions are the most common; they can range from mild acne-like eruptions to severe inflammatory lesions that may need changes to treatment.[4,5] Gastrointestinal issues and increases in liver enzymes are also well-known and greatly add to the overall burden of adverse events.[4] Although they are less common, interstitial lung disease is the most serious complication, with reported rates of about 5 to 6% in Japanese populations and a high risk of mortality.[6] These adverse effects often result in dose adjustments, supportive care, or stopping therapy early.

The severity of adverse effects varies greatly among patients, indicating that toxicity is znot random but is determined by biological factors. Roden et al. point out that people do not respond the same way to medications. DNA variants can influence protein function and subsequent drug activity. This explains why some patients face severe adverse effects while others do not.[7] With the rise of pharmacogenomic testing, it is clear that genetic differences among individuals can raise the risk of reactions to certain drugs.[8] Therefore, variations in genes involved in the metabolism and transportation of gefitinib, such as CYP3A5, CYP2D6, ABCB1, and ABCG2, may clarify why some people have severe adverse effects while others tolerate the treatment well.[9–13]

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has created guidelines to assist in choosing and dosing certain drugs based on genetic differences to reduce the risk of adverse effects, particularly in statin therapy.[14] However, current pharmacogenomic studies on gefitinib are limited. Sample sizes

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are small, genetic markers vary across populations, and results often lack consistency. Therefore, incorporating a pharmacogenetic approach into clinical practice is crucial. This helps predict how effective a treatment will be, its tolerability, potential adverse effects, and guides dose adjustments. This study will systematically review the current evidence on the link between genetic variants and adverse effects of gefitinib in NSCLC patients. We will explore the main pathways involved using a protein-protein interaction (PPI) network approach and visualize key protein structures to show the locations of important variants.

2. METHODS

2.1 Literature Searching Strategies

This systematic review was conducted in accordance with PRISMA guidelines.[15] Pubmed, Scopus, and Cochrane databases were searched up to 2 November 2025 using the Boolean combination of following keywords: (gefitinib) AND (non-small-cell lung cancer OR NSCLC) AND (pharmacogenomic OR pharmacogenetic OR genetic polymorphism OR single nucleotide polymorphism OR gene variant) AND (toxicity OR adverse event OR adverse reaction OR adverse effect OR hypersensitivity). All studies gathered in the initial screening were reviewed in full to evaluate their eligibility for inclusion. Three reviewers (MS, AM, RR) independently assessed each study. Any discrepancies in this step were resolved through consensus.

2.2 Inclusion and Exclusion Criteria

Eligible studies were required to meet following inclusion criteria: (a) studies involving human, (b) studies evaluating the association of genetic variants with adverse events from gefitinib in NSCLC patients, (c) full text paper available, (d) written in English language. Studies were excluded if they meet following exclusion criteria: (a) non-human studies (in silico/in vitro/animal studies), (b) case report/review articles/editorials/abstract, (c) non-NSCLC patients, (d) intervention other than gefitinib, (e) not evaluating the association of adverse effects and gene variants, (f) non-English articles, (g) duplication.

2.3 Data Extraction

For each study included in the analysis, key information was extracted in a descriptive manner. Collected data included: authors, publication year, drug and dosage, study design, patient characteristics (number of patients, age, ethnicity, disease stage), adverse effects, methods for gene identification, instruments

for assessing adverse effects, and details of gene variants (gene, genotype, and polymorphism). Adverse effects analyzed included hepatotoxicity, skin rash, and diarrhea, as the most frequently reported adverse effects of gefitinib in the literature.[16,17]

2.4 Quality assessment

The quality of each study was evaluated independently by three reviewers (MSAA, AMRP and SR) according to Cochrane Risk Of Bias In Non-randomized Studies-of Exposures (ROBINS-E).[18] Any disagreements on the assessment were resolved through consensus.

2.5 Protein-Protein Interaction Analysis

To explore the functional interactions among genes associated significantly with adverse effects of gefitinib, we performed a protein-protein interaction (PPI) network analysis using STRING platform version 11.0.[19] Only gene products with a statistically significant association ($p<0.05$) with gefitinib-related adverse effects were included. The network was generated using a high-confidence minimum interaction score of 0.7 across seven prediction channels (text-mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence) for *Homo sapiens*. Interactors were clustered using the STRING k-means algorithm. The number of clusters was set to two, based on the rule of thumb $k = \sqrt{(n/2)}$, where n represents the number of nodes (protein interactors) in the network.

Hub proteins were identified based on their position within each cluster and the number of visible interactions (edges) which connect them to other proteins. Proteins located in the center of the network and with the highest number of connecting edges were considered as hub candidates.

2.6 Structural Visualization of Key Proteins

To visualize the structural context of key proteins identified through PPI analysis, we conducted an in-silico study involving structural analysis using UCSF Chimera. Only proteins that were considered as hubs from PPI analysis were visualized in this context. As an additional analysis, we also visualized EGFR as the main target of the EGFR tyrosine kinase domain. Protein structures were obtained from the Protein Data Bank and pre-processed in UCSF Chimera by removing solvent molecules and irrelevant heteroatoms, adding hydrogens, and correcting residue geometry to ensure proper structural representation. Ribbon representations were used to highlight the overall domain organization, while key residues located within catalytic or

predicted interaction sites were displayed in stick format. For EGFR, the structure was specifically oriented to reveal the ATP-binding cleft and the ligand-binding pocket of the tyrosine kinase domain. Hub proteins were processed using the same visualization workflow to allow consistent comparison of their structural features.

3. Result

3.1 Characteristics of studies

The search criteria on Pubmed, Scopus, and Cochrane results in 71 hits of which 27 were duplicates. Following initial screening, 15 studies were excluded as seen in **Figure 1.** After full-text evaluation, 16 studies met the inclusion criteria and were included in this review. The identified studies were published between 2006 and 2022, with a total of 1,176 participants included (mean= 114.4 ± 64.5) ranging from 25-91 years old. Most of the studies involved Japanese (n=6) and Chinese ethnicity (n=5), while other studies involved Caucasian (n=4), and Taiwanese.[12] The stage of NSCLC ranged from Ia to IV, with most studies including only IIIb-IV stage (n=8). All studies were reported to use gefitinib (250 mg, once daily) according to guidelines for NSCLC. Regarding the instrument to assess adverse effects, the most frequently used was Common Terminology Criteria for Adverse Events (CTCAE) (n=10), followed by common toxicity criteria manual version 3.0 (n=1), and National Cancer Institute Common toxicity criteria manual version 3.0.9 (n=1), while the rest of the studies did not specify the instrument.

Among the 16 studies identified, 5 studies investigated polymorphisms in CYP2D6.[11,16,20–22] Three studies evaluated CYP3A4 variants.[16,20,22] One study examined FOXO3.[8] Variants in ABCB1 were assessed in 3 studies[9,10,20], and 5 studies looked at ABCG2 polymorphisms.[9,13,20,23,24] Four studies analyzed EGFR variants [9,12,25,26]

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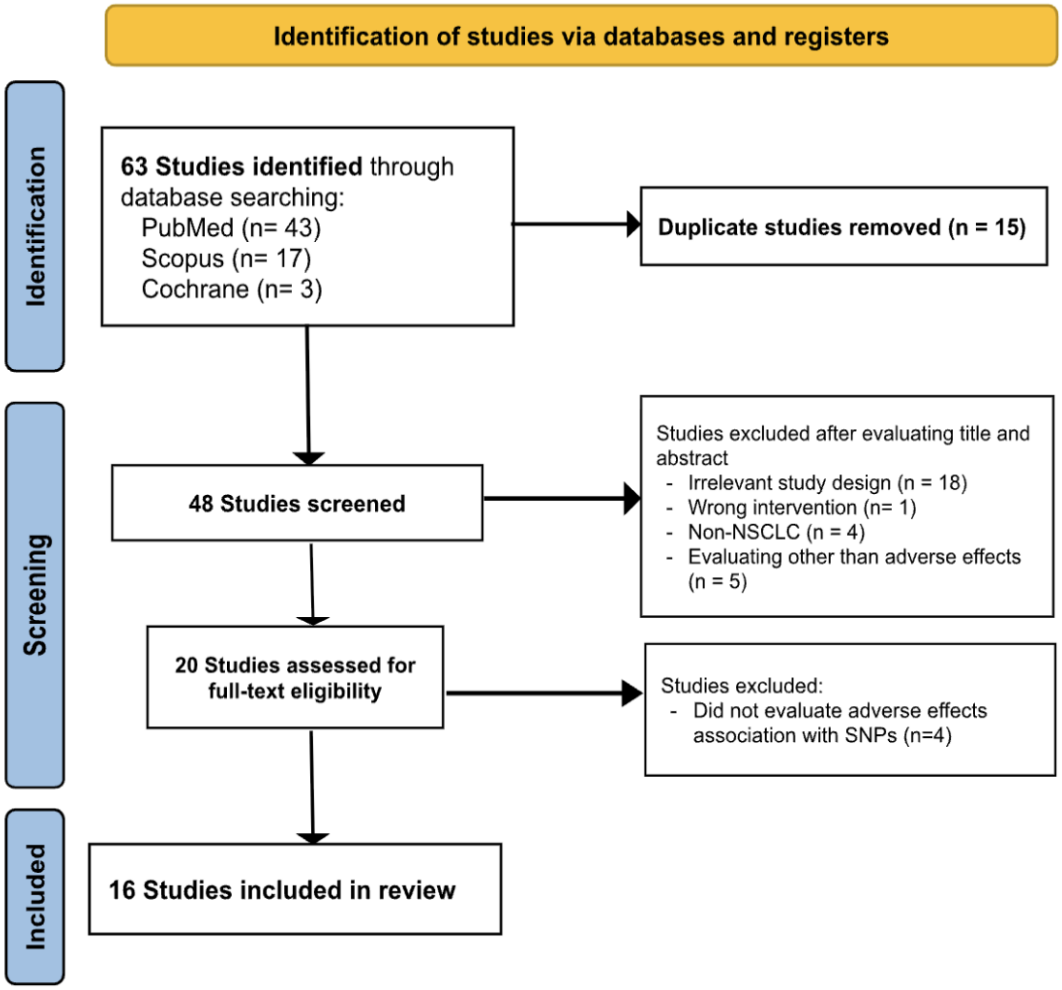

Figure 1. Flowchart of literature screening process following PRISMA guideline.

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Table 1. Characteristics of the included studies

No.	Author & Year	Drug (dose)	Study Design	Patient characteristics				Adverse effects	Methods of Gene Identification	Adverse Effect Assessment Instrument
				Number of patients	Age (mean)	Ethnicity	Disease Stage			
1	Kwok WC, et al. 2022	Gefitinib (250 mg/day)	Retrospective cohort study	152	66.7± 11.5	Chinese	N/A	- Hepatotoxicity (34.2%) - Skin rash (74.3%) - Diarrhea (34.9%)	Multiplex SNP microarray	CTCAE
2	Guan S, et al. 2022	Gefitinib (250 mg/day)	Prospective observational study	180	57 (28-88)	Chinese	IIIb - IV	Hepatotoxicity	MassARRAY system for 194 tag SNPs	CTCAE v4.0
3	Ma et al., 2017	Gefitinib (250 mg/day)	Prospective observational study	59	56 (31-77)	Chinese	IIIb – IV	- Hepatotoxicity - Skin rash - Diarrhea - ILD	Sequenom MassARRAY genotyping	CTCAE v4.0
4	Kobayashi, et al 2015	Gefitinib (250 mg/day)	Prospective observational study	31	68 ± 8.6 (51-81)	Japanese	III b – IV	- Hepatotoxicity - Skin rash - Diarrhea	PCR-RFLP	CTCAE v4.0
5	Suzumura T et al., 2012	Gefitinib 250 mg/day	Retrospective cohort study	232	67 (24-90)	Japanese	III – IV	- Hepatotoxicity (48.3%) - Skin rash (66.8%) - Diarrhea (25.9%)	Real-time PCR genotyping and TaqMan® SNP Genotyping	CTCAE v4.0
6	Tamura, et al. 2012	Gefitinib 250 mg/day	Prospective observational study	83	65 (36-86)	Japanese	Ia – IV	- Skin rash (27.7%) - Hepatotoxicity (18%) - Diarrhea (4.8%) - ILD (6%)	PCR and TaqMan® SNP Genotyping	CTCAE v4.0
7	Lemos, et al., 2011	Gefitinib 250 mg/day (oral)	Retrospective pharmacogenetic study	94	63.5 (40–85)	Caucasian (Italian, Dutch)	IIIb – IV	- Skin rash (25.3%) - Diarrhea (38.8%)	TaqMan® SNP Genotyping Assay + Tissue IHC for ABCG2 protein	Common toxicity criteria manual version 3.0

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8	Giovannetti, et al. 2010	Gefitinib 250 mg/day	Prospective observational study	96	64	Caucasian	IIIb - IV	- Skin rash - Diarrhea	PCR-based direct sequencing of AKT1 and EGFR SNPs	National Cancer Institute Common toxicity criteria manual version 3.0.9
9	Huang et al., 2009	Gefitinib (250 mg/day)	Prospective pharmacogenetic study Phase II	52	66 (39-86)	Taiwanese	IIIb – IV	- Skin Rash (63%)	PCR, direct sequencing	CTCAE v3.0
10	Cusatis G, et al. 2006	Gefitinib 250 mg/day	Retrospective pharmacogenetic cohort	173	67 (25-91)	Italy	III – IV	- Diarrhea (16%)	PCR-based genotyping and direct sequencing	N/A
11	Xin S. et al., 2020	Dose not stated	Retrospective genetic association study	109	N/A	Chinese	N/A	- Hepatotoxicity - Skin rash - Diarrhea	Sequenom MassARRAY genotyping	N/A
12	Liu et al., 2008	Gefitinib (250 mg, once daily)	Prospective observational study	92	N/A	Predominantly Caucasian	IIIb - IV	- Skin rash - Diarrhea	PCR and sequencing	N/A
13	Takimoto et al., 2013	Gefitinib (250 mg, once daily)	Prospective observational study	55	73 (38-89)	Japanese	N/A	Hepatotoxicity	PCR-RFLP	CTCAE v3.0
14	Ruan et al., 2015	Gefitinib (250 mg, once daily)	Prospective observational study	226	60.75 (44-80)	Chinese	IIIa - IVb	- Skin rash - Diarrhea	PCR and MassARRAY genotyping	N/A
15	Sugiyama et al., 2015	Gefitinib (250 mg, once daily)	Prospective observational study	239	63 (24-74)	Japanese	IV	Hepatotoxicity (31.6%)	DNA microarray	CTCAE v4.0
16	Akasaka K, et al., 2009	Gefitinib (250 mg/day)	Prospective observational study	75	62 (36-80)	Japanese	IIIb – IV	- Hepatotoxicity - Skin rash - Diarrhea	PCR and sequencing	CTCAE v3.0

Abbreviations: CTCA, Common Terminology Criteria for Adverse Events; CYP, cytochrome P450; ILD, interstitial lung disease; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism;

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IKBKB and RELA polymorphisms were each examined in one study.[27] Additionally, CYP3A5[20], AKT1[25], and CYP1A1 were evaluated in one study each.[28] Notably, 8 genes showed a significant link to adverse effects: CYP3A4, CYP2D6, ABCG2, ABCB1, EGFR, IKBKB, AKT1, and FOXO3. For gene identification methods, the most commonly used was PCR (n=11), followed by MassARRAY sequencing, SNP microarray, and DNA microarray. The full list of genes and polymorphisms investigated across the included studies is summarized in **Table 1**.

3.2 Quality of studies

To evaluate the quality of the included study, we utilized the Risk Of Bias In Non-randomized Studies-of Exposures (ROBINS-E) to evaluate bias due to confounding, measurement of exposure, participant selection, post-exposure intervention, missing data, outcome measurement, and selective reporting.[18] Nine studies included are deemed to have an overall low risk of bias, while 5 of them have some concerns of bias, and two have high risk of bias **(Figure 2)**.

Most risk of bias arise from confounding (**Figure 3)**, where several studies did not perform adjustment on confounders. [11,21,28] One study had imbalanced baseline characteristics such as gender, age, smoking status, and cancer stage[28] and some patients in Takimoto, et al.

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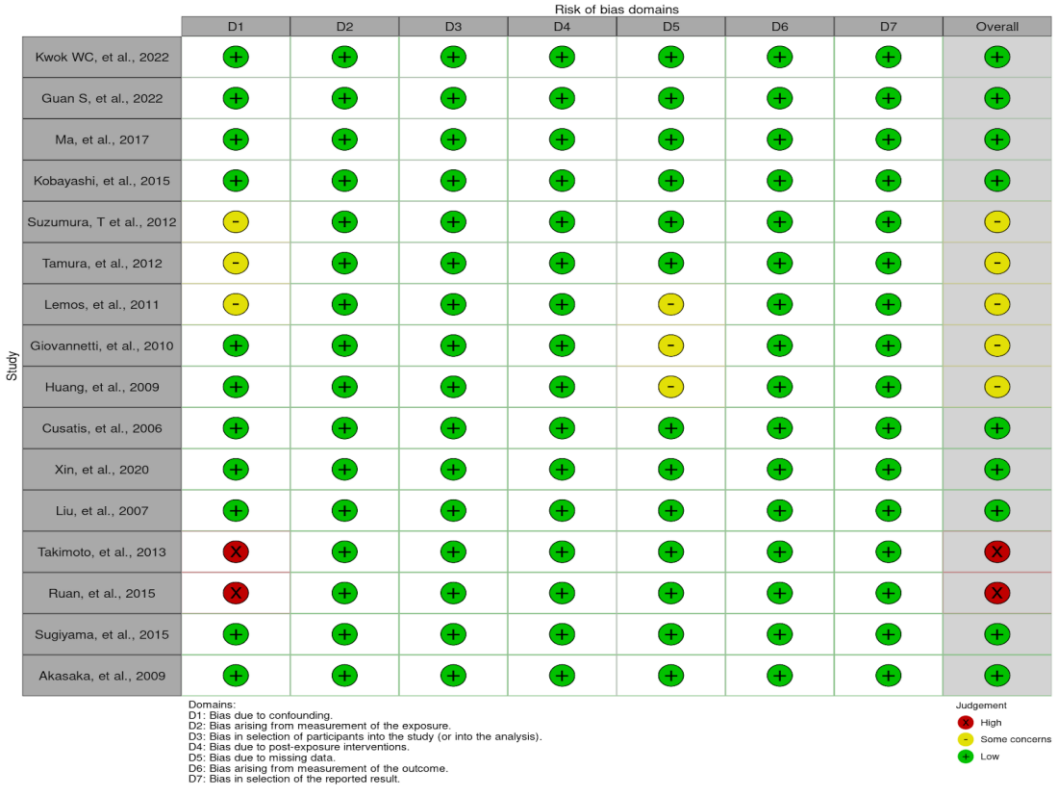

Figure 2. Risk of bias analysis through the Risk of Bias in Non-randomized Studies-of Exposures (ROBINS-E) tools across seven domains.

also took medications other than gefitinib, which might interfere with the result.[21] Three studies had some drop out participants due to either death or worsening conditions, which might cause bias due to missing data.[12,13,25]

3.3 Adverse Effects Related to Gene Variations

3.3.1 Hepatotoxicity

Ten cohort studies reported genetic variations related to gefitinib-induced hepatotoxicity. CYP2D6 rs28371725 was the most consistent variant, showing significant associations across three studies: Kwok et al. (OR 3.773; 95% CI 1.046–13.610; p=0.043), Sugiyama et al. (OR 14.5; 95% CI 1.56–346.5; p=0.04), and Takimoto et al. (p=0.024)[16,21,22] **(Table 2)**. Two studies that looked at CYP3A4 rs2242480 also demonstrated increased hepatotoxicity risk, as reported by Kwok et al. (OR 20.0; 95% CI 2.381–167.965; p=0.006) and Sugiyama et al. (OR 6.84; 95% CI 1.87–33.15; p=0.0069).[16,22] Associations from single studies were also noted for FOXO3 rs4946935 (OR 18.02; 95% CI 2.473–459.1784; p=0.018)[8], ABCB1 rs1128503 (OR 15.78; 95% CI 2.01–124.1; p=0.0087)[10], and RELA rs11227247 (GG) (OR 0.212; 95% CI 0.062–0.726;

p=0.013).[27] Additionally, EGFR rs712829 (−216 G/T) was found to be significant in one study by Liu et al. (p=0.004).[26]

Several variants showed no significant link to hepatotoxicity, even though hepatotoxicity was reported in the group. These included CYP2D6 rs28371725 in Kwok et al.[16], ABCG2 rs2231137[23], ABCG2 rs2231142[20], ABCG2 rs72552713[24], CYP3A4 rs2242480[20], CYP3A5 rs776746/rs776746[20], and the ABCB1 rs1128503/rs2032582/rs1045642 haplotype.[20] All of these consistently had p>0.05. Overall, the main factors contributing to hepatotoxicity risk were CYP2D6 rs28371725, CYP3A4 rs2242480, FOXO3 rs4946935, ABCB1 rs1128503, RELA rs11227247, and EGFR rs712829, while other gene variants showed no consistent association.

3.3.2 Skin Rash

Ten cohort studies reported gefitinib-induced skin rash. Six of these studies identified important pharmacogenomic association. Kobayashi et al. showed that the ABCB1 rs2032582 (2677G>T/A)

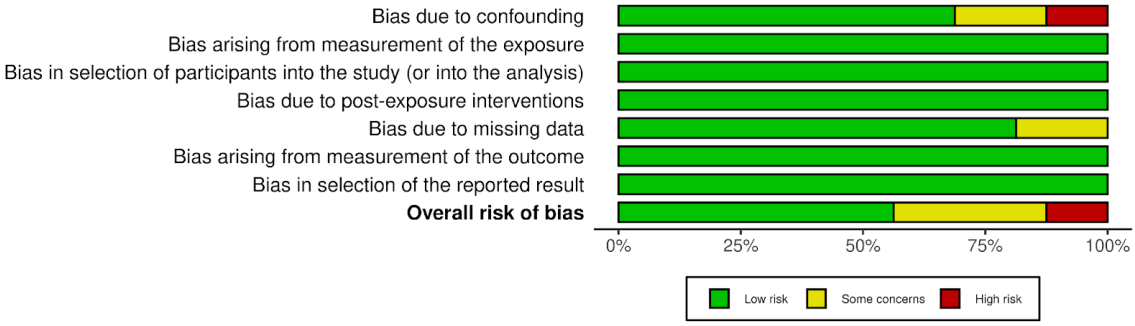

Figure 3. Summary of risk of bias across domains analysed using ROBINS-E tools.

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Table 2. Genetic variations observed in the included studies and its association with adverse effects of gefitinib in NSCLC patient

No.	Author & Year	Gene	Genotype	Polymorphism	Adverse Effects								
					Hepatotoxicity			Skin rash			Diarrhea		
					p- value	OR	95% CI	p- value	OR	95% CI	p- value	OR	95% CI
1	Kwok WC, et al. 2022	CYP2D6	CT	*rs28371725*	**0.043**	3.773	1.046–13.610	N/A	N/A	N/A	N/A	N/A	N/A
		CYP2D6	AA	*rs1065852*	0.05	3.368	1.000–11.345	N/A	N/A	N/A	N/A	N/A	N/A
		CYP3A4	TT	*rs2242480*	**0.006**	20.0	2.381–167.96	N/A	N/A	N/A	N/A	N/A	N/A
2	Guan S, et al. 2022	FOXO3	AA	*rs4946935*	**0.018**	18.02	2.473 - 459.1784	N/A	N/A	N/A	N/A	N/A	N/A
3	Ma et al., 2017	ABCB1	TT	*rs1128503*	**0.0087**	15.78	2.01–124.1	N/A	N/A	N/A	N/A	N/A	N/A
4	Kobayashi, et al 2015	CYP3A4	TT	*rs2242480*	0.993	N/A	N/A	0.196	N/A	N/A	0.703	N/A	N/A
		CYP3A5	G/G or A/G	*rs776746* or *rs776746*	0.697	N/A	N/A	0.324	N/A	N/A	0.062	N/A	N/A
		CYP2D6	CT	*rs28371725*	0.685	N/A	N/A	0.132	N/A	N/A	0.885	N/A	N/A
		ABCG2 421	CA/AA	*rs2231142*	0.851	N/A	N/A	0.208	N/A	N/A	0.366	N/A	N/A
		ABCB1 1236	C/C or C/T or T/T	*rs1128503*	0.250	N/A	N/A	0.174	N/A	N/A	0.238	N/A	N/A
		ABCB1 2677G	G/G or G/T or A/A	*rs2032582*	0.791	N/A	N/A	**0.032**	N/A	N/A	0.251	N/A	N/A
		ABCB1 3435	C/C or C/T or T/T	*rs1045642*	0.066	N/A	N/A	0.507	N/A	N/A	0.769	N/A	N/A
5	Suzumura T et al., 2012	CYP2D6	AA	*rs1065852*	N/A	N/A	N/A	**0.03**	0.44	0.21–0.94	N/A	N/A	N/A
6	Tamura, et al. 2012	ABCG2 34	G/A	*rs2231137*	0.542	N/A	N/A	**0.046**	N/A	N/A	1	N/A	N/A
7	Lemos, et al., 2011	ABCG2 −15622	C/T	*rs7699188*	N/A	N/A	N/A	0.57	N/A	N/A	**0.01**	N/A	N/A
8	Giovannetti, et al. 2010	EGFR −191	C/A	*rs712830*	N/A	N/A	N/A	**0.031**	N/A	N/A	**0.001**	N/A	N/A
		EGFR −216	G/T	*rs712829*	N/A	N/A	N/A	0.31	N/A	N/A	**0.01**	N/A	N/A
9	Huang et al., 2009	EGFR [CA]n			N/A	N/A	N/A	**0.031**	N/A	N/A	N/A	N/A	N/A
		EGFR R521K	G/A	*rs2227983*	N/A	N/A	N/A	0.720	N/A	N/A	N/A	N/A	N/A
		EGFR –216	G/G	*rs712829*	N/A	N/A	N/A	0.104	N/A	N/A	N/A	N/A	N/A
10	Cusatis G, et al. 2006	ABCG2 421	CA/AA	*rs2231142*	N/A	N/A	N/A	0.99	N/A	N/A	**0.0046**	N/A	N/A
		ABCB 3435	CT/TT	*rs1045642*	N/A	N/A	N/A	0.67	N/A	N/A	0.85	N/A	N/A
		EGFR − 216	G/T	*rs712829*	N/A	N/A	N/A	0.99	N/A	N/A	0.33	N/A	N/A
		EGFR −191	C/A	*rs712830*	N/A	N/A	N/A	0.65	N/A	N/A	0.56	N/A	N/A
		EGFR (CA) n			N/A	N/A	N/A	0.26	N/A	N/A	0.99	N/A	N/A
11	Xin S. et al., 2020	IKBKB	CC	*rs2272733*	N/A	N/A	N/A	**0.013**	3.91	0.087-0.753	N/A	N/A	N/A
			CC	*rs12142086*	N/A	N/A	N/A	**0.013**	3.640	1.320-10.039	N/A	N/A	N/A
			TT	*rs2151222*	N/A	N/A	N/A	N/A	N/A	N/A	**0.023**	6.17	0.034-0.775
		RELA	GG	*rs11227247*	**0.013**	0.212	0.062-0.726	N/A	N/A	N/A	N/A	N/A	N/A
12	Liu et al., 2008	EGFR − 216	G/T	*rs712829*	**0.004**	N/A	N/A	N/A	N/A	N/A	**0.004**	N/A	N/A
13	Takimoto et al., 2013	CYP2D6	CT	*rs28371725*	**0.024**	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
14	Ruan et al., 2015	CYP1A1		*rs1048943*	N/A	N/A	N/A	0.1076	N/A	N/A	N/A	N/A	N/A
15	Sugiyama et al., 2015	CYP2D6	CT	*rs28371725*	**0.04**	14.5	1.56-346.5	N/A	N/A	N/A	N/A	N/A	N/A
		CYP3A4	TT	*rs2242480*	**0.0069**	6.84	1.87-33.15	N/A	N/A	N/A	N/A	N/A	N/A
16	Akasaka, et al., 2009	ABCG2	c.376C >T	*rs 72552713*	0.557	N/A	N/A	0.791	N/A	N/A	0.709	N/A	N/A

polymorphism was significantly associated with rash. This was especially true for individuals with the G/G, G/T, T/T, T/A, or A/A genotypes (p = 0.032).[20] Suzumura et al. noted a protective effect from CYP2D6 rs1065852 (AA), which lowered the risk of rash (OR 0.44; 95% CI 0.21-0.94; p = 0.03).[11] Further support for transporter involvement came from Tamura et al. They found a significant association between ABCG2 rs2231137 (GA/AA) and an increased incidence of skin rash (p = 0.046).[23] In the EGFR pathway, Giovannetti et al. identified EGFR rs712830 (−191 C/A) as significantly associated with rash (p = 0.031).[25] Huang et al. reported a similar link for the EGFR (CA)n repeat (p = 0.031).[12] Additionally, Xin et al. discovered two variants in the inflammatory pathway, IKBKB rs2272733 (CC) (OR 3.91; 95% CI 0.087-0.753; p = 0.013) and IKBKB rs12142086 (CC) (OR 3.640; 95% CI 1.320-10.039; p = 0.013).[27] Both showed significant connections with skin rash occurrence.

3.3.3 Diarrhea

Eight cohort studies reported gefitinib-induced diarrhea, with five identifying significant pharmacogenomic associations. Lemos et al. showed a significant association for ABCG2 rs7699188 (C/T) with increased diarrhea risk (p = 0.01).[13] EGFR variants including rs712830 (−191 C/A), rs712829 (−216 G/T), and R497K (rs11543848) were all significantly associated with diarrhea (p=0.001,p=0.01, p=0.02)[9], findings further supported by Liu et al., who also reported EGFR rs712829 as significant (p = 0.004).[26] Moreover, ABCG2 rs2231142 (421 C>A) and IKBKB rs2151222 (TT) were significantly correlated with diarrhea occurrence (p=0.0046 and p=0.023, respectively).[9,27] In contrast, other evaluated variants including CYP2D6 rs28371725[5], CYP3A4 rs2242480/[20], CYP3A5 rs776746/rs776746[20], ABCB1 rs1128503/rs2032582/rs1045642 haplotypes[9,20] and several non-significant EGFR/[CA]n[25] rs712829/rs712830[9], AKT1 rs1130233/rs2494732[25] and ABCG2 rs2231142/rs2231137/rs72552713[9,20,23] alleles exhibited no meaningful association across the remaining cohorts. Overall, the strongest predictors of gefitinib induced diarrhea were found in ABCG2, EGFR, and IKBKB polymorphisms, while metabolic gene variants did not demonstrate consistent effects.

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3.4 Protein-protein Network Analysis

Protein-protein interaction network was constructed using the protein products of genes that demonstrated significant associations with adverse effects ($p<0.05$) **(Figure 4)**. The network, consisting of 8 proteins (nodes), 7 interaction links (edges), an average node degree of 1.75, and 2 clusters (clustering coefficient of 0.708), illustrates the interactions between genes involved in the development of adverse effects. The first cluster, composed of CYP2D6, CYP3A4, ABCB1, and ABCG2, represents a xenobiotic metabolism and transport module, with EGFR connected indirectly. CYP2D6 and CYP3A4 are central nodes in this cluster, showing multiple thick lines, indicating high-confidence interactions. This might suggest their central role in gefitinib metabolism. While the second cluster, consisting of FOXO3, IKBKB, and RELA forms an inflammatory module.

Based on the KEGG Pathways, the main biological pathways include response to xenobiotic stimulus and drug, alkaloid catabolic process, as well as response to cobalamin and chemicals, which are related with the genes involved in the network. Furthermore, the gene ontology analysis demonstrated that response to stimulus and drug, and alkaloid catabolic process were the most significant processes for genes in the network. For molecular function, the genes associated with xenobiotic transmembrane transporting ATPase activity and binding of drug, heterocyclic compound, organic cyclic compound, and protein kinase. The genes in the network were present in the receptor complex and apical plasma membrane based on the cellular components of gene ontology analysis.

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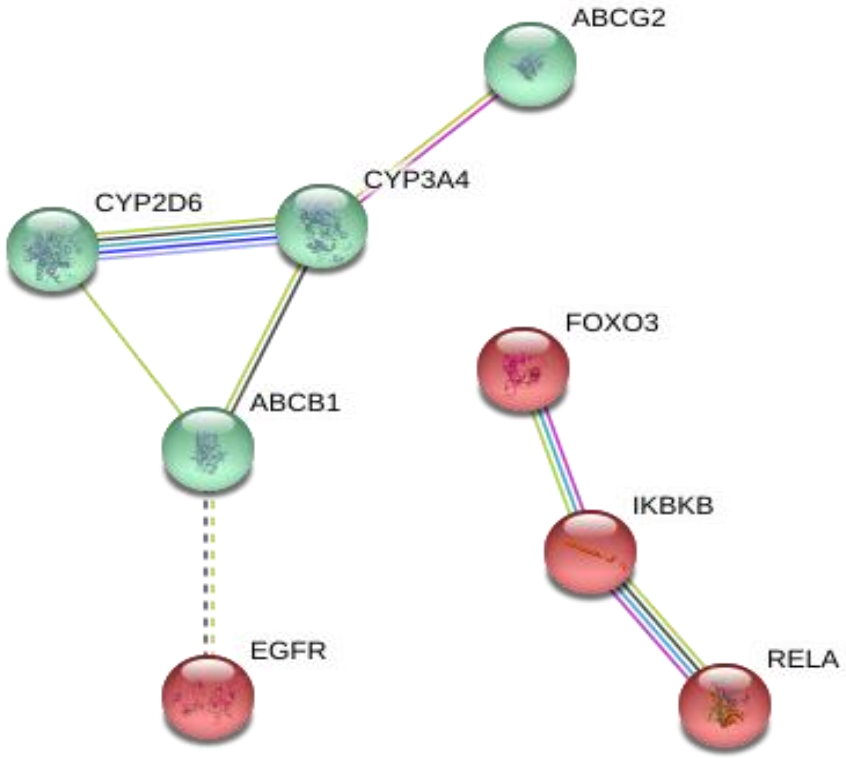

Figure 4. Protein–protein interaction (PPI) network of the 8 significant genes generated using STRING v.11.0. Two major functional clusters are observed: a metabolic–transport and signaling cluster consisting of CYP2D6, CYP3A4, ABCB1, ABCG2, and EGFR and an inflammatory cluster consisting of FOXO3, IKBKB, and RELA. Line thickness indicates the strength of data supporting the protein interactions.

3.5 Tyrosine Kinase Domain Structure and Gefitinib Binding Site Visualization

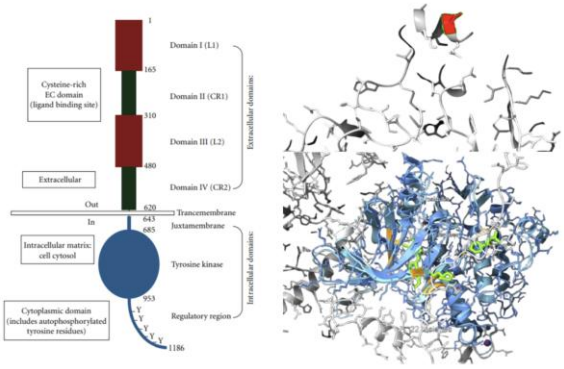

Figure 5. *(a)* Schematic illustration of EGFR showing the extracellular domains (I–IV), the transmembrane region, and intracellular segments including the tyrosine kinase and regulatory domains with autophosphorylation sites. The R497K variant is located in the extracellular region.[29] *(b–c)* Three-dimensional visualization of EGFR generated with UCSF Chimera, highlighting the tyrosine kinase domain (blue) and the binding of gefitinib (yellow) within the ATP-binding pocket. Key surrounding residues involved in ligand interaction are shown in orange. Visualization aimed to characterize gefitinib's binding pocket within EGFR, revealing that the drug occupies the ATP-binding cleft and interacts with key residues (Met793, Thr790), supporting its inhibitory mechanism and potential variant-dependent effects.

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4.Discussion

Gefitinib is widely recognized as one of the main targeted treatments for patients with NSCLC. However, many patients face substantial adverse effects despite the clinical benefits. Current evidence indicates that genetic variations among individuals significantly contribute to the varying levels of drug-related adverse effects. However, pharmacogenomic studies have shown mixed results, some link genetic factors to adverse effects, while others do not. To clarify this issue, we carried out an exploratory systematic review, identifying eight genes (CYP2D6, CYP3A4, ABCB1, ABCG2, FOXO3, EGFR, IKBKB, and RELA) that are associated with adverse effects from gefitinib. We also performed a PPI network analysis to examine the molecular and signaling pathways of these significantly associated genes, which might be candidates for further clinical studies.

Gefitinib is largely metabolized by CYP450 enzymes, especially CYP3A4 and CYP2D6.[10] After metabolism, gefitinib is transported systematically by active efflux pumps (ABCB1 and ABCG2).[30] Genetic variations in these pathways could make individuals more susceptible to gefitinib-related adverse effects.[8]

Hepatotoxicity, a common adverse effect of gefitinib, may result from direct toxicity of xenobiotics or immune responses.[8] Reduced-function variants in CYP2D6 (rs28371725) and CYP3A4 (rs2242480) likely hinder gefitinib metabolism, leading to reduced clearance and potentially increasing systemic exposure and the risk of hepatotoxicity.[16,21,22] Similarly, loss-of-function variants in ABCB1 (rs1128503) and ABCG2 (rs2231142) may reduce the drug's hepatic efflux, showing similarities to transporter-related toxicity found with NSAIDs and psychiatric drugs.[31,32] FOXO3, which regulates autophagy and protects mitochondria, shows cytoprotective effects against xenobiotics.[8] Modifications in FOXO3 could disrupt autophagy and lead to a buildup of reactive metabolites, causing liver cell damage.[8]

Several genetic variations also affect skin and gastrointestinal adverse effects. Reduced-function CYP2D6 (rs28371725) variants might heighten the systemic and skin exposure to gefitinib, intensifying EGFR inhibition in skin cells.[10,32] This effect may be worsened by ABCB1 (rs1128503) loss-of-function variants that limit drug transport across the skin barrier, leading to increased local drug concentrations.[30] These mechanisms can increase EGFR inhibition in skin cells, disrupting

skin turnover and function, which may result in inflammatory acne-like eruptions.[33] In the gastrointestinal system, ABCG2 (rs2231142) and ABCB1 (rs1128503) loss-of-function variants could raise gefitinib levels in intestinal cells, disrupting epithelial integrity and ion transport, thus leading to secretory diarrhea.[9] Since EGFR is essential for keeping the intestinal lining intact and helping with mucosal repair, stronger inhibition to EGFR due to genetic variations can further hinder intestinal healing and make it more vulnerable to injury. This can lead to diarrhea linked to gefitinib.[34]

Our PPI analysis identified two main clusters that align well with the biological mechanisms driving gefitinib-related adverse effects. The first cluster includes CYP2D6, CYP3A4, ABCB1, ABCG2, and EGFR, representing the pharmacokinetic pathway that governs gefitinib transport and metabolism. Variations in this cluster could weaken drug metabolism or efflux capacity, leading to higher systemic or intracellular gefitinib levels and increasing the risk of liver toxicity, skin rashes, or gastrointestinal issues. In this analysis, EGFR had an indirect link with ABCB1 as a drug transporter, suggesting a potential connection between transporter gene variants and gefitinib exposure and toxicity. The strong interaction between CYP2D6 and CYP3A4 further reinforces their potential role as key genes influencing toxicity through impaired metabolism. The second cluster included FOXO3, IKBKB, and RELA, which are involved in inflammation, cell death, and maintaining epithelial integrity. In this group, IKBKB interacts with RELA, an important part of the NF-κB transcription factor, while FOXO3 regulates cell survival and autophagy. These findings suggest that genes linked to gefitinib adverse effects extend beyond metabolism to include inflammatory and cellular stress-response pathways.

Functional interpretation of each gene further supports these findings: reduced-function variants of CYP2D6 (rs28371725) or CYP3A4 (rs2242480) may decrease metabolic clearance, while impaired ABCB1 (rs1128503) or ABCG2 (rs2231142) activity may enhance drug accumulation. Variants in FOXO3, IKBKB, and RELA may influence vulnerability to inflammation and tissue injury, while EGFR connects both metabolic and inflammatory factors as the main drug target. Collectively, the PPI clustering and structural in silico results demonstrate that gefitinib toxicity emerges from the convergence of two biological modules: a metabolic transport pathway that

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determines drug exposure, and a signaling inflammatory pathway that modulates tissue sensitivity. Integrating these pathways clarifies why multiple genetic variants contribute to adverse effects.

Although genetic variation appears to play an important role in shaping individual susceptibility to gefitinib-related adverse effects, these toxicities are not solely determined by genetics. Other factors, such as age, liver function, comorbidities, concomitant medications, and overall drug doses, may also influence the severity of these adverse effects.[35–39] Another notable gap is the predominance of research originating from East Asian populations, especially Japan and China, which limit our ability to generalize the findings. This pattern is likely driven by the higher prevalence of EGFR mutations in Asian patients[16], as well as variations in CYP2D6[40] and ABCG2.[41] Future studies involving more diverse, multiethnic cohorts are needed to fully capture the gefitinib-related adverse effects in the real world.

Based on these findings, we recommend early genetic screening for key variants in genes like CYP2D6, CYP3A4, ABCB1, ABCG2, EGFR, and FOXO3 prior to gefitinib treatment to guide personalization. This might help clinicians to optimize dosages, identify high-risk patients, and choose alternative therapies when needed. Furthermore, functional studies are also needed to explore the biological effects of gene variations and better understand how they work together to cause hepatotoxicity, skin rashes, and diarrhea.

Looking ahead, integrative methods such as multi-omics analysis, machine learning based predictive modeling, and experimental validation of PPI and structural predictions could deepen our understanding of the complex interactions between drug metabolism, tissue sensitivity, and inflammatory pathways. These approaches may lead to more accurate and personalized management strategies for NSCLC patients receiving gefitinib.

5.Conclusion

Collectively, the findings of this review show that genetic variation significantly influences individual susceptibility to gefitinib-related adverse effects. Across the included studies, eight genes CYP2D6, CYP3A4, ABCB1, ABCG2, EGFR, FOXO3, IKBKB, and RELA consistently emerged as important factors contributing to adverse effects through their roles in drug metabolism, transporter activity, epithelial integrity, and inflammatory signaling.

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