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RESEARCH ARTICLE

INTEGRATED TRANSCRIPTOMIC PROFILING OF THE *Candida albicans* RESPONSE TO THE SYNERGISTIC INTERACTION BETWEEN ATOMOXETINE AND FLUCONAZOLE

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Abstract

Candida species are notorious opportunistic pathogens that can cause severe infections, especially in immunocompromised and medically vulnerable patients. Despite the availability of various antifungal drugs, their efficacy is often limited by drug toxicity and antifungal resistance. Therefore, repurposing existing clinically approved drugs is a promising approach to addressing this challenge. In this study, we investigated the potential synergistic effects of combining the antidepressant atomoxetine (ATMX) with the antifungal agent fluconazole (FLC) against *Candida albicans*. Our results demonstrated that ATMX (31.25 µg/mL) and FLC (1.95 µg/mL) exhibited a trend toward synergistic activity *C. albicans*. We performed RNA-Seq analysis to investigate the transcriptional response of *C. albicans* treated with ATMX-FLC combination. Our analysis indicated that the combination has a significant impact on ribosome biogenesis, secondary metabolite biosynthesis, and metabolic pathways. Overall, our findings highlight the potential of repurposing clinically approved drugs as a novel therapeutic strategy for the treatment of *Candida* infections, which continue to pose a significant global public health challenge.

Keywords

Candida albicans,
Checkerboard assay,
Fluconazole,
Gene expression

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1. INTRODUCTION

Candida species are well-known pathogen microorganisms that can cause severe infections, particularly in individuals with compromised immune systems (HIV, AIDS) and chronic health conditions [1]. Among these species, *C. albicans* is the most frequently implicated causative agent of candidiasis and can lead to fatal outcomes under certain conditions. The treatment of candidiasis is primarily limited to azole and non-azole antifungal agents, including polyenes and echinocandins. Although these therapeutic options are generally effective, their clinical use is constrained by several limitations, such as low bioavailability, poor gastrointestinal absorption, drug–drug interactions, adverse side effects, and the inevitable development of multidrug resistance following long-term use [2].

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For example, amphotericin B, a polyene antifungal, is associated with nephrotoxic and hepatotoxic effects in mammals. Although lipid-based formulations have been developed to reduce toxicity, these formulations are often prohibitively expensive. Due to its relatively low toxicity and favorable absorption profile compared to other antifungals aggents, fluconazole is one of the most commonly prescribed drugs for the treatment of *Candida* infections. Due to its relatively low toxicity and favorable absorption profile compared to other antifungal agents, fluconazole is one of the most commonly prescribed drugs for the treatment of *Candida* infections. However, despite its generally positive clinical efficacy, the fungistatic rather than fungicidal nature of azoles often results in incomplete fungal clearance, leading to recurrent infections and the development of drug resistance. Moreover, since most antifungal agents target a limited number of cellular pathways, similarities in their mechanisms of action facilitate the emergence of resistance in fungal pathogens [3, 4].

It is increasingly necessary to research alternative therapeutic options for treating *Candida* infections, given the limitations of antifungal drugs. Since the development of novel antifungal medications through the classical strategy is time-consuming and cannot compete with the rate of evolution of drug resistance, the repurposing of currently used, clinically authorized pharmaceuticals emerges as a promising method for accomplishing this goal [5]. Since the antimicrobial effects of psychotropic drugs (phenothiazine and thioxanthene groups) have been reported in the 1990s, numerous clinically approved drugs that are used for the treatment of other diseases have been examined against fungal pathogens [6].

Among them, sertraline (a selective serotonin reuptake inhibitor, SSRI) has been extensively studied for its antifungal potential against *Candida spp*. and other fungal pathogens [7]. Although the precise mechanisms of antifungal action for most antidepressants remain unclear, several studies suggest that repurposing these agents, particularly in combination with established antifungal drugs, may offer novel therapeutic approaches [8].

In the present study, we investigated the potential interaction between **fluconazole (FLC)**, a widely used antifungal agent, and **atomoxetine (ATMX)**, a selective norepinephrine reuptake inhibitor (SNRI) with known off-target bioactivities, against *Candida albicans*, which showed a trend toward synergy. The checkerboard microdilution method was employed to assess synergy between FLC and ATMX. Furthermore, RNA-Seq transcriptomic analysis was performed to characterize the global gene expression changes in *C. albicans* exposed to the drug combination. The treatment significantly impacted ribosome biogenesis, secondary metabolite biosynthesis, and core metabolic pathways.

2. MATERIALS AND METHODS

2.1. Strains and Drugs

C. albicans (ATCC 90028) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). ATMX and FLC were kindly provided by Abdi Ibrahim Pharmaceutical Co. Ltd., Turkey and Nobel Pharmaceutical Co. Ltd., Turkey, respectively**.** The stock solution of FLC was prepared by dissolving in sterile dH_2O at 100 mg/mL. A stock solution of ATMX at a concentration of 100 mg/mL was prepared using dimethyl sulfoxide (DMSO) as the solvent. All stock solutions were stored at −20 °C and subsequently subjected to two-fold serial dilutions.

2.2. Antimicrobial Assay

Antifungal activities of ATMX and FLC were evaluated *in vitro* by using the broth microdilution method (MIC) according to the Clinical and Laboratory Standards Institute M27-A2 reference method [9]. Briefly, a serial dilution; (1000 μg /mL - 0.98 μg /mL) of the drugs was prepared in steriled dH_2O and

100 µL of each dilution was transferred to a 96-well plate. A volume of 100 µL of *Candida albicans* suspension was added to each well, achieving a final concentration ranging between 0.5–2.5 × 10^3 CFU/mL. The well containing only the inoculated broth without any drug was designated as the growth control, while the lowest concentration showing no visible microbial growth was defined as the minimum inhibitory concentration (MIC) (µg/mL). Following 48 hours of incubation at 37 °C, growth assessment was performed by adding resazurin at a final concentration of 20 µg/mL to each well. The MIC_{50} value was subsequently determined. Ketoconazole served as the reference antifungal agent.

2.3. Synergy Testing by Checkerboard Microdilution Assay

The combined activity of ATMX and FLC against *C. albicans* was investigated using the standard Checkerboard Microdilution Assay [10]. Briefly, serial 2-fold dilutions of ATMX and FLC were prepared; 50 µL of each drug was added to columns (2-12) and rows (A to G) with a fixed amount of one agent and an increasing amount of the second agent. Column 1 and row H contained only ATMX and FLC, respectively. Drug-free wells containing untreated cells were also used as the growth control. Following the addition of 100 µL of *C. albicans* inoculum to each well, the final concentration was adjusted to 2.5 × 10^3 CFU/mL. Drug concentrations in the wells ranged from 0.062 µg/mL to 250 µg/mL. The microtiter plates were then incubated at 37 °C for 48 hours. To assess antifungal activity, MICs for each agent, both individually and in combination, were determined by introducing resazurin at 20 µg/mL to each well. The fractional inhibitory concentration (FIC) was calculated for each well.

For ATMX and FLC, the FIC of the combination is calculated as follows;

$(\sum FIC) = FICA + FICB$
FIC A = MICA combined / MICA alone
FIC B = MICB combined / MICB alone

Drug interactions were categorized based on the calculated $\sum$ FIC values according to the following criteria: synergism if $\sum FIC \leq 0.5$; indifference if $0.5 |< \sum FIC - \leq 4$, and antagonism if $\sum FIC > 4$.

2.4. RNA Seq Analysis

2.4.1. RNA extraction

*Candida albicans (*ATCC 90028) was initially cultivated overnight in Sabouraud Dextrose Broth (SDB) at 37 °C. A subsequent subculture was prepared by diluting the overnight culture to an OD_{600} of 0.1, followed by incubation at the same temperature. Upon reaching the early logarithmic growth phase (OD_{600} = 0.4), cells were exposed to the ATMX/FLC combination. A control group was maintained under identical incubation conditions without drug exposure. Total RNA was extracted from both treated and untreated groups during the exponential phase using the PureLink RNA Mini Kit (Ambion, USA), in accordance with the manufacturer's guidelines. Post-extraction, RNA samples underwent DNase treatment and purification using the RNeasy Kit (Qiagen, Hilden, Germany). RNA quality was verified via NanoPhotometer® spectrophotometry (IMPLEN, USA), while integrity was analyzed using the RNA Nano 6000 Assay Kit on a Bioanalyzer 2100 platform (Agilent Technologies, USA). Quantification of RNA was performed with the Qubit® RNA Assay Kit using the Qubit® 2.0 Fluorometer (Life Technologies, USA.

2.4.2. Library preparation for transcriptome analysis

For each sample, 3 µg of total RNA was used as the input material to construct sequencing libraries using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA), following the manufacturer's protocol. Unique barcode indices were assigned to distinguish individual samples. The

quality and integrity of the libraries were assessed using the Bioanalyzer 2100 System (Agilent Technologies, CA, USA).

2.4.3. Cluster generation and high-throughput sequencing

Indexed libraries were clustered using the cBot Cluster Generation System along with the HiSeq PE Cluster Kit cBot-HS (Illumina), in accordance with the manufacturer's instructions. Sequencing was then conducted on the Illumina HiSeq platform to generate 125 or 150 base pair paired-end reads.

2.4.4. Bioinformatic analysis

Raw sequencing reads in FASTQ format were initially quality-filtered using in-house Perl scripts to remove low-quality bases and adaptor contamination. The resulting high-quality reads were then aligned to the *Candida albicans* WO-1 reference genome using **TopHat v2.0.12**, following index construction with **Bowtie v2.2.3**. The reference genome and corresponding annotation files were obtained from the NCBI Genome database.

Quantification of gene-level expression was performed using HTSeq v0.6.1, producing FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values after normalization for gene length and sequencing depth. An FPKM threshold of either 0.1 or 1 was considered as the baseline for gene expression.

Differential gene expression analysis was performed with the DEGSeq R package (v1.20.0), using three biological replicates for both treatment and control groups. P-values were corrected via the Benjamini-Hochberg method to minimize false discovery rate (FDR). Genes with adjusted P-values below 0.05 were considered significantly differentially expressed.

Gene Ontology (GO) enrichment analysis for DEGs was performed using GOseq, which accounts for bias due to gene length. Significantly enriched GO terms (adjusted $p < 0.05$) are presented in the supplementary materials. Additionally, pathway enrichment was examined using the KEGG database [11], and statistical significance of pathway involvement was evaluated via KOBAS software.

2.5. Statistical Evaluation

All experiments were executed in triplicate (n = 3). Data are expressed as means ± standard deviation (SD). Comparative analysis between experimental and control groups was carried out using Student's t-test, with significance defined at $p < 0.05$.

3. RESULTS

3.1. Antimicrobial Activity

The checkerboard microdilution assay showed that ATMX and FLC exhibited antifungal activity against *C. albicans*, with MIC_{50} values of 62.5 µg/mL and 31.25 µg/mL, respectively. A trend towards synergy was observed, corresponding to an FIC index of ≤ 0.5 when 31.25 µg/mL of ATMX was combined with 1.95 µg/mL of FLC. Although the FIC value approached the synergy threshold, the difference did not reach statistical significance. Nevertheless, the ability of ATMX to reduce the effective concentration of a clinically used antifungal underscores the importance of elucidating its mechanism of action.

3.2. Transcriptional Characteristics of *C. albicans* to ATMX/FLC Combination

To elucidate the molecular mechanisms underlying the inhibitory or cytotoxic effects of the ATMX/FLC combination on *Candida albicans*, transcriptome profiling was performed using RNA sequencing (RNA-Seq). Differential mRNA expression levels between treated and untreated samples were analyzed. Read mapping statistics confirmed high-quality alignment to the *C. albicans* reference genome, with total mapped reads (TMR) exceeding 70% and multiple mapped reads (MMR) remaining below the 10% threshold—both in line with accepted quality standards (**Table 1**).

Table 1. Overview of mapping Status.

Sample name	Treated	Untreated
Total reads	106229792	129187696
Total mapped	82893931 (78.03%)	100326626 (77.66%)
Mapped to multiple sites in the reference genome	2241870 (2.11%)	2811801 (2.18%)
Uniquely mapped to the reference genome	80652061 (75.92%)	97514825 (75.48%)
Read-1	40444475 (38.07%)	48901381 (37.85%)
Read-2	40207586 (37.85%)	48613444 (37.63%)
Non-splice reads	79958873 (75.27%)	96820215 (74.95%)
Splice reads	693188 (0.65%)	694610 (0.54%)

Gene expression analysis using HTSeq revealed both the distribution of transcript abundance across defined expression intervals and individual gene-level expression profiles (**Table 2**). In general, transcripts with FPKM values ≥ 0.1—with a cutoff 1 typically used for active expression—were considered actively expressed.

Table 2. Classification of Gene Expression Profiles Across Defined Ranges

FPKM Interval	Treated	Untreated
0-1	157(2.57%)	2421(39.68%)
1-3	182(2.98%)	184(3.02%)
3-15	1093(17.92%)	222(3.64%)
15-60	2248(36.85%)	1147(18.80%)
>60	2421(39.68%)	2133(34.96%)

To assess gene expression variation under different treatment conditions, FPKM distribution charts and violin plots were generated. For each biological replicate, the mean FPKM value was calculated and used as the representative expression level (**Figure 1A**). Comparative analysis between ATMX/FLC-treated and untreated *C. albicans* samples revealed differential gene expression patterns. A total of 5944 genes were expressed in the treated group, while 5917 were detected in controls. Among these, 5895 genes were commonly expressed across all samples, with 49 and 22 genes uniquely identified in the treated and control groups, respectively (**Figure 1B**). Detailed information on differentially expressed genes (DEGs) is provided in the **Supplementary Table.**

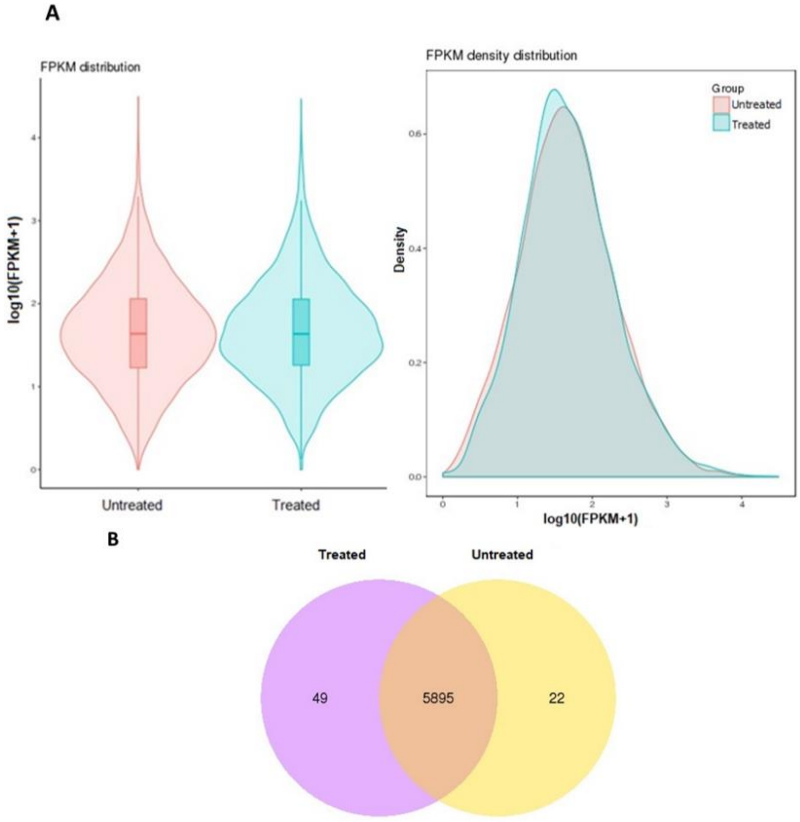

Figure 1. Global transcriptional response of *Candida albicans* to ATMX-FLC treatment. **A:** FPKM distribution diagram and violin plot that compare gene expression levels between ATMX/FLC drug treated and untreated samples. **B:** Venn Diagram of treated and un-treated samples with ATMX-FLC Genes with an adjusted P-value <0.05 found by DESeq were assigned as differentially expressed.

Among the identified genes, 874 were determined to be differentially expressed. Specifically, 535 genes were upregulated and 339 were downregulated in response to ATMX/FLC treatment compared to untreated controls, as illustrated in the volcano plot depicting global gene expression changes (**Figure 2**). The top 10 upregulated and top 10 downregulated genes were selected based on a stringent cutoff of $p < 0.001$. Genes were ranked by their $\log_2$ fold-change values from highest to lowest, obviouusl, hypothetical proteins were excluded from the final list (**Table 3**).

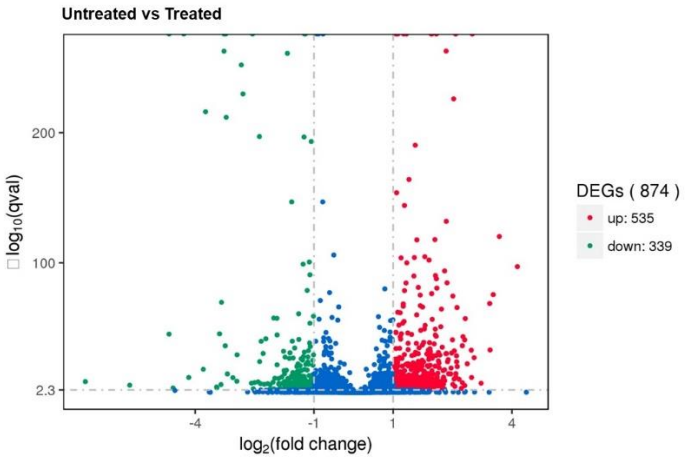

Figure 2. Volcano Plot Depicting Differential Gene Expression Following ATMX-FLC Treatment

Table 3. Top 10 Most Upregulated and Downregulated Genes in Response to ATMX-FLC Exposure

The Top 10 Up-regulated Genes					
Gene_id	Control (untreated)	Treated with ATMX/FLC drug	log2.Fold-change	p-value	Gene description
CAWG_02649 (UGA2)	36.04873	390.9006	3.4388	4.71E-71	succinate-semialdehyde dehydrogenase
CAWG_02912 (NOG1)	27.25517	214.1853	2.9743	8.2572E-35	nucleolar GTP-binding protein 1
CAWG_05006 (NOG2)	24.60739	151.2497	2.6198	5.42E-22	nucleolar GTP-binding protein 2
CAWG_03639 (PWP1)	29.70862	180.5211	2.6032	9.36E-26	periodic tryptophan protein 1
CAWG_02836 (CDR1)	306.6085	1769.154	2.5286	8.98E-229	suppressor of toxicity of sporidesmin
CAWG_01233 (ABC1)	103.5065	589.1671	2.509	9.31E-77	protein ABC1% 2C mitochondrial precursor
CAWG_01875 (NOC2)	21.4009	117.2376	2.4537	3.39E-16	nucleolar complex protein 2
CAWG_00294 (KRR1)	23.10131	121.9131	2.3998	2.30E-16	ribosomal RNA assembly protein mis3
CAWG_03479 (IPK1)	38.98801	202.1157	2.3741	9.24E-26	inositol-pentakisphosphate 2-kinase
CAWG_04382 (IMH3)	224.1143	1136.754	2.3426	1.72E-134	inosine-5'-monophosphate dehydrogenase IMD2
The Top 10 Down-regulated Genes					
Gene_id	Control (untreated)	Treated with ATMX/FLC drug	log2.Fold-change	p-value	Gene description
CAWG_01980 (HHT2)	1027.146	76.98404	-3.7379	7.15E-219	histone H3
CAWG_00975 (HTB2)	1375.074	142.29	-3.2726	1.70E-265	histone H2B.1
CAWG_00976 (HTA2)	1125.017	121.0215	-3.2166	1.40E-214	histone H2A.2
CAWG_01979 (HHF22)	1384.111	199.6148	-2.7937	1.36E-232	histone H4
CAWG_01645 (MLC1)	18.72882	3.240289	-2.5311	0.00031351	myosin regulatory light chain cdc4
CAWG_03681 (SMF11)	47.7087	9.981829	-2.2569	6.52E-08	transporter protein SMF1/ESP1
CAWG_02521 (PXP2)	495.5972	122.5438	-2.0159	8.87E-60	acyl-coenzyme A oxidase 2
CAWG_05917 (TIM22)	174.1465	51.60541	-1.7547	7.57E-19	mitochondrial import inner membrane translocase subunit TIM22
CAWG_04994 (HGT10)	184.8833	57.06388	-1.696	4.00E-19	sugar transporter STL1
CAWG_02742 (POL1)	51.59535	15.96223	-1.6926	2.42E-06	DNA polymerase alpha catalytic subunit

3.2.1. Functional analysis via GO and KEGG enrichment analyses

To gain deeper insights into the functional roles of differentially expressed genes (DEGs) and evaluate the broader biological impact of ATMX/FLC treatment, Gene Ontology (GO) enrichment analysis was performed. A total of 874 DEGs were successfully annotated, covering categories related to biological processes, cellular components, and molecular functions. Among the top 30 significantly enriched GO terms, dominant molecular functions included binding to organic cyclic compounds, heterocyclic compounds, and nucleic acids. Within the biological process category, transmembrane transporter activity—particularly related to metal ion transport—was prominently enriched. In terms of cellular components, the plasma membrane protein complex emerged as the most significantly enriched category (**Figure 3**).

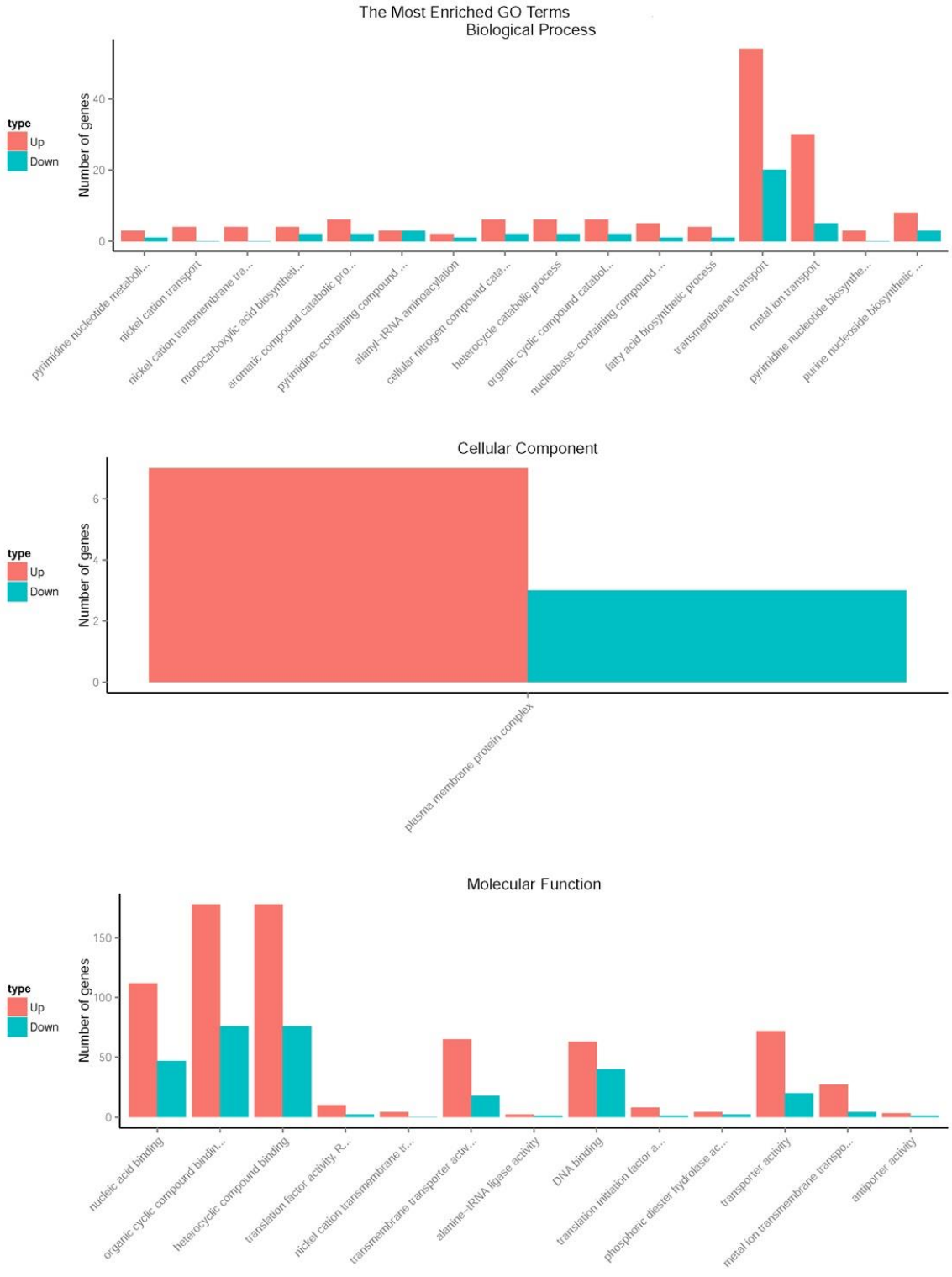

Figure 3. Bar graph of the most enriched GO terms of identified DEGs

Analysis of the most significantly upregulated and downregulated genes in response to ATMX/FLC treatment revealed a substantial number of hypothetical or uncharacterized genes, identified through open reading frame (ORF) annotations. Genes upregulated under drug exposure were predominantly associated with molecular functions such as organic cyclic compound binding, heterocyclic compound

binding, and nucleic acid binding (Figure 4A). In contrast, downregulated genes were notably enriched in functional categories related to DNA binding and hydrolase activity (**Figure 4B**).

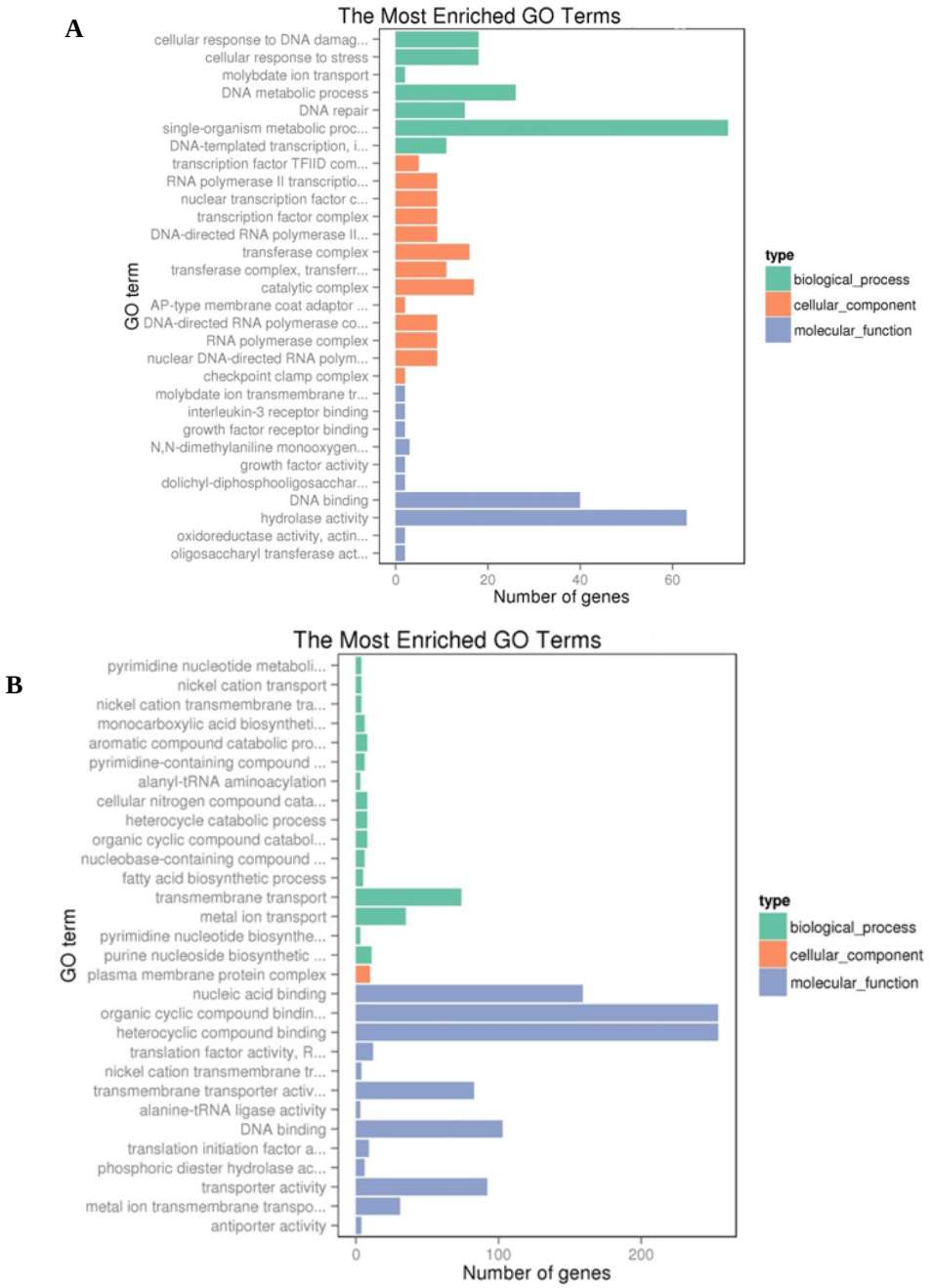

Figure 4. GO term enrichment and KEGG enrichment analyses were conducted using the RNA-seq data. **A:** GO term enrichment analysis of the upregulated genes. The vertical coordinates are the enriched GO terms, and the horizontal coordinates are the numbers of the upregulated genes in these GO terms. **B:** GO term enrichment analysis of the downregulated genes.

To identify the metabolic pathways affected by the ATMX/FLC combination, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed. The analysis revealed 20 pathways significantly upregulated and another 20 significantly downregulated upon treatment. The most enriched pathways among the DEGs included ribosome biogenesis, secondary metabolite biosynthesis, and various metabolic processes, with metabolism-related pathways being particularly dominant (**Figure 5A–B**). Consistent with the findings of Romo et al. [2019], endocytosis-related genes are known to be critical for *C.*

albicans virulence and adaptation to host environments [2]. In agreement with this, our data showed no notable downregulation of genes involved in endocytosis (**Figure 5B**).

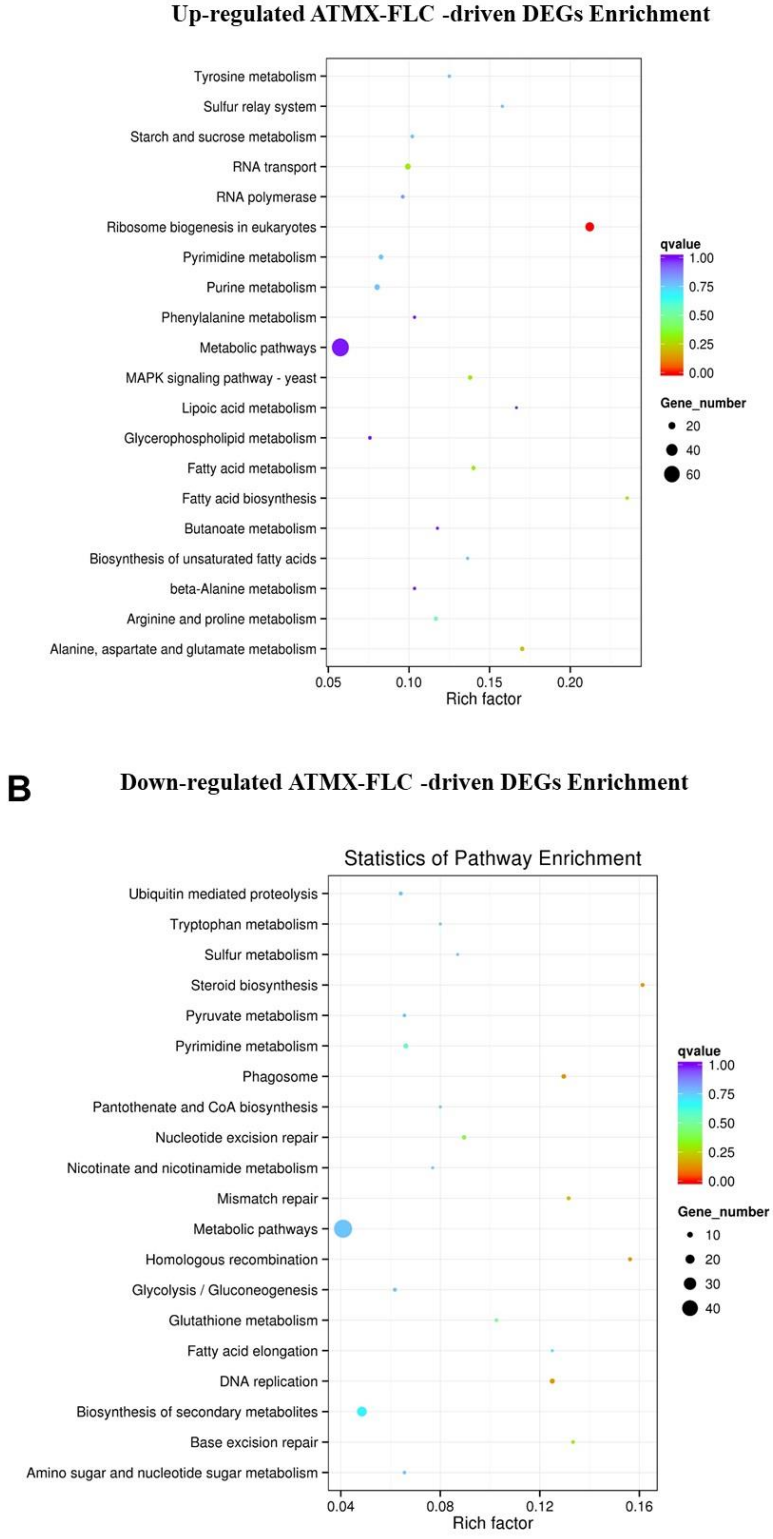

Figure 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment scatter plot of DEGs. The y-axis represents the name of the pathway, and the x-axis represents the Rich factor. Dot size represents the number of different genes and the color indicates the q-value. **A**: Up-regulated ATMX-FLC-driven DEGs Enrichment analysis results. **B**: Down-regulated ATMX-FLC-driven DEGs Enrichment analysis results.

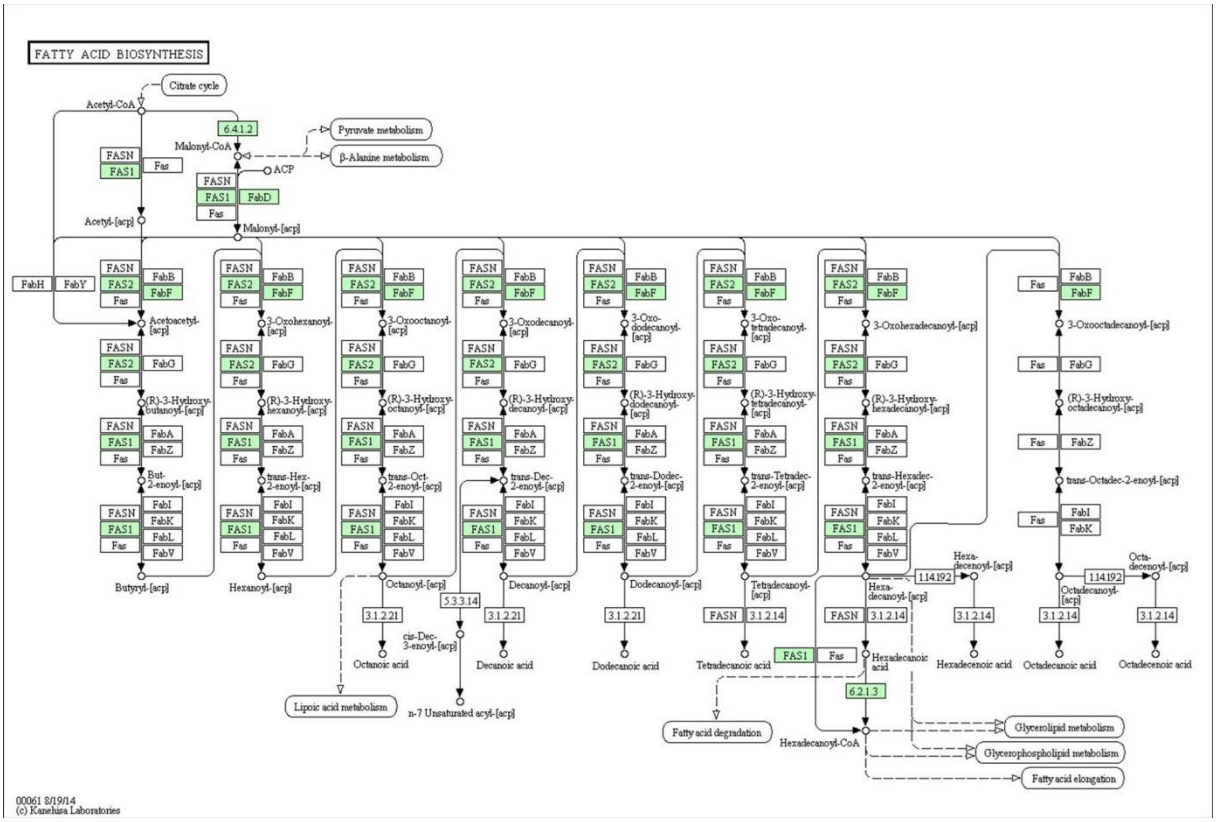

Figure 6: Fatty acid biosynthesis pathway result from the KEGG analysis results for the significantly upregulated genes after the treatment with our drug combination.

4. DISCUSSION

C. *albicans* is a major opportunistic fungal pathogen responsible for severe infections, particularly in immunocompromised individuals. Although antifungal agents such as azoles, echinocandins, and polyenes are routinely used in clinical practice, their prolonged and widespread application has contributed to the emergence of drug-resistant strains. Drug repurposing offers a promising alternative by leveraging existing, approved compounds for novel antimicrobial purposes [12]. Among the repurposed candidates, antidepressants—especially selective serotonin reuptake inhibitors (SSRIs) such as sertraline and fluoxetine—have demonstrated notable antifungal activity, both individually and in combination with azole-class drugs [13, 14]. However, most of these studies have primarily focused on specific mechanisms, such as biofilm inhibition, without examining broader transcriptomic responses [15].

Candida albicans is a prominent opportunistic fungal pathogen associated with severe infections, particularly in immunocompromised individuals. Although antifungal agents such as azoles, echinocandins, and polyenes are widely used in clinical practice, their prolonged and extensive use has contributed to the emergence of drug-resistant strains. In this context, drug repurposing has emerged as a promising strategy, enabling the reuse of approved compounds for novel antimicrobial applications [12].

Notably, *UGA2*, a gene involved in oxidative stress response and γ-aminobutyric acid (GABA) degradation, was among the most significantly upregulated transcripts following ATMX/FLC treatment (**Table 3**). The GABA degradation pathway has been well characterized in *Saccharomyces cerevisiae* and is linked to nitrogen assimilation and cellular stress adaptation [16].

The coordinated upregulation of *UGA1* and *UGA2* in response to GABA exposure facilitates its catabolism into succinate, potentially supporting fungal survival under stress conditions [17]. Additional upregulated genes—such as *NOG1*, *NOG2*, *PWP1*, *NOC2*, *KRR*, and *IMH3*—are associated with ribosomal RNA (rRNA) processing and cellular responses to drug-induced stress. The overexpression of the ABC transporter gene *CDR1*, the mitochondrial electron transport gene *ABC1*, and *IPK1*, a kinase involved in mRNA decay, further indicates a multifactorial stress response mechanism activated by the drug combination [18].

In contrast, histone-encoding genes such as *HHT2*, *HTA2*, *HTB2*, and *HHF22* were markedly downregulated following ATMX/FLC treatment, suggesting potential alterations in chromatin structure and transcriptional regulation. Furthermore, the repression of the DNA replication and repair gene *POL1*, the cytoskeletal regulator *MLC1*, and the membrane transporters *TIM22* and *HGT10* points to widespread cellular disruption. The downregulation of *PXP2*, a gene involved in fatty acid β-oxidation, further indicates a disturbance in energy homeostasis [19].

Fluconazole primarily targets lanosterol 14α-demethylase (*ERG11*), thereby inhibiting ergosterol synthesis and disrupting membrane integrity. In response, fungal cells may activate compensatory lipid biosynthesis pathways. Our observation of upregulated *FAS1*, *FAS2*, *FABF*, and *FABD*—key genes involved in fatty acid biosynthesis—supports the existence of such a compensatory mechanism [20]. *FAS1* and *FAS2*, encoding the α- and β-subunits of fatty acid synthase, are known to be responsive to membrane stress. Similar upregulation has been reported in *Sclerotinia sclerotiorum* under cyclic lipopeptide exposure, reinforcing their role in membrane repair and stress adaptation [21, 22].

Fatty acids, as essential constituents of phospholipids and sphingolipids, play critical roles in maintaining membrane fluidity, mediating signal transduction, and supporting energy metabolism. Remodeling of the fungal cell envelope via lipid biosynthesis is a well-established adaptive strategy to counteract antifungal-induced stress.

Combination therapy remains a pivotal strategy in antifungal drug development, aiming to delay resistance, enhance therapeutic efficacy, and expand the antifungal spectrum. Several studies have demonstrated the synergistic interaction between antidepressants and azole-class agents. For instance, Oliveira et al. [2015] reported enhanced antifungal activity of SSRIs when co-administered with fluconazole against various *Candida* strains [23, 24]. Fluoxetine and sertraline have also been shown to inhibit biofilm formation and suppress fungal metabolic activity. Fuurthermore, sertraline exhibits broad-spectrum antifungal effects against other clinical species such as *Aspergillus* and *Cryptococcus* [11, 13].

Our findings confirm the suggestive synergistic effect of the ATMX/FLC combination against *C. albicans*, accompanied by marked transcriptomic changes across multiple biological systems. Gene Ontology and KEGG enrichment analyses revealed disrupted pathways related to ribosome biogenesis, secondary metabolite biosynthesis, and core metabolism. These perturbations suggest interference with protein synthesis and virulence regulation, potentially via translational inhibition mechanisms [11].

Notably, the downregulation of ribosomal genes alongside upregulation of fatty acid biosynthesis pathways indicates that the ATMX/FLC combination may concurrently target both structural and metabolic components of fungal survival. This dual-action mechanism could enhance antifungal potency while mitigating the emergence of resistance.

In conclusion, our transcriptomic analysis provides compelling evidence that atomoxetine potentiates fluconazole's antifungal activity through multifaceted gene regulation. The observed alterations in genes governing membrane dynamics, transcriptional processes, and ribosome assembly underscore the therapeutic potential of this drug combination. Further in vivo studies focusing on pharmacokinetics, efficacy, and resistance evolution are warranted to support clinical translation.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest

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CRediT AUTHOR STATEMENT

Emilia Qomi Ekenel: Data curation, Analysis; **Benjamin Kreka:** Investigation, methodology**; Handan Açelya Kapkaç:** Investigation, Resources, Writing – review & editing**; Hulya Karaca:** Investigation, Resources, Writing – review & editing.

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