



Brief Report

FunResDB—A web resource for genotypic susceptibility testing of *Aspergillus fumigatus*

Michael Weber^{1,*}, Jonas Schaer¹, Grit Walther¹, Kerstin Kaerger¹, Jörg Steinmann², Peter-Michael Rath², Birgit Spiess³, Dieter Buchheidt³, Axel Hamprecht⁴ and Oliver Kurzai^{1,5}

¹German National Reference Center for Invasive Fungal Infections – NRZMyk, Septomics Research Centre, Leibniz-Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute, 07745 Jena, Germany, ²Institute of Medical Microbiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany, ³Department of Hematology and Oncology, Mannheim University Hospital, University of Heidelberg, Mannheim, Germany, ⁴Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany German Centre for Infection Research (DZIF), Partner Site Bonn-Cologne, Cologne, Germany and ⁵Center for Sepsis Control and Care, University Hospital Jena, Jena, Germany

*To whom correspondence should be addressed. Michael Weber, Leibniz Institute for Natural Product Research and Infection Biology, Beutenbergstraße 11a, 07745 Jena, Germany. Tel: +49 3641 532-1332; E-mail: michael.weber@leibniz-hki.de

Received 5 September 2016; Revised 16 December 2016; Accepted 24 February 2017; Editorial Decision 4 January 2017

Abstract

Therapy of invasive aspergillosis is becoming more difficult due to the emergence of azole resistance in *Aspergillus fumigatus*. A majority of resistant strains carries mutations in the *CYP51A* gene. Due to a lack of sensitivity of culture-based methods, molecular detection of *A. fumigatus* has become an important diagnostic tool. We set up the database FunResDB (www.nrz-myk.de/funresdb) to gather all available information about *CYP51A*-dependent azole resistance from published literature. In summary, the screening resulted in 79 *CYP51A* variants, which are linked to 59 nonsynonymous mutations. A tailor-made online sequence analysis tool allows for genotypic susceptibility testing of *A. fumigatus*.

Key words: fungal infection, *Aspergillus fumigatus*, triazoles, drug susceptibility, database.

Therapy of invasive aspergillosis is becoming more difficult due to the emergence of azole resistance in *Aspergillus fumigatus*. A majority of resistant strains carries mutations in the *CYP51A* gene. Due to a lack of sensitivity of culture-based methods, molecular detection of *A. fumigatus* has become an important diagnostic tool. We set up the database FunResDB (www.nrz-myk.de/funresdb) to gather all available information about *CYP51A*-dependent azole resistance from published literature. In summary, the screening resulted in 79 *CYP51A* variants, which are linked

to 59 nonsynonymous mutations. A tailor-made online sequence analysis tool allows for genotypic susceptibility testing of *A. fumigatus*.

The worldwide emergence of azole resistance in *Aspergillus fumigatus* has become a major concern for the clinical management of invasive aspergillosis.^{1,2} Mutations in the target protein lanosterol 14 alpha-demethylase (*CYP51A*) can lead to partial or complete azole resistance, which in turn may increase the risk of therapeutic failure.^{3,4} The most frequently occurring mutation conferring

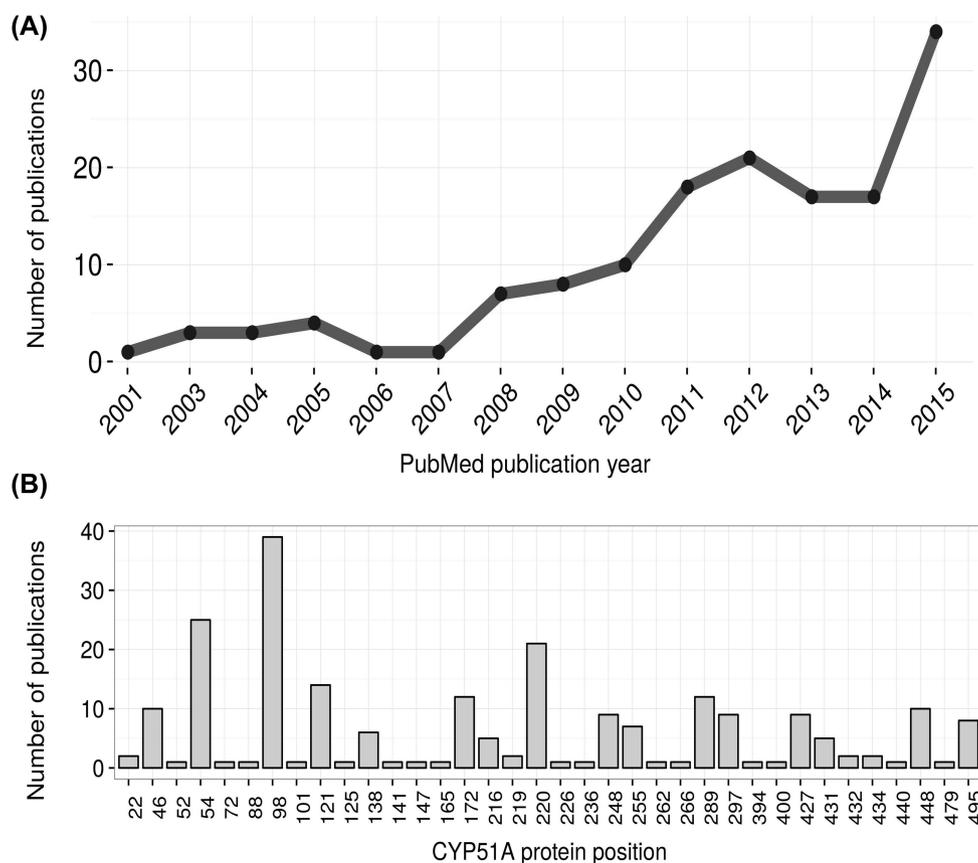


Figure 1. (A) Number of publications found in PubMed for search query 'Aspergillus fumigatus CYP51A azole resistance' grouped by year of publication. (B) Comparison of the frequency of mutated CYP51A protein positions on the basis of reported azole-resistant genotypes in curated publications.

pan-azole resistance leads to replacement of leucine by histidine at position 98 in combination with a promoter tandem repeat (TR₃₄/L98H).⁵ However, several mutations at other positions also confer resistance and the number of publications describing such mutations has risen exponentially (Fig. 1).⁶ On the other hand, there are point mutations in *CYP51A* that do not result in azole-resistance. As culture remains negative in more than 50% of invasive aspergillosis cases and the reference methodology for phenotypic susceptibility testing is not routinely used in most diagnostic laboratories, molecular tools for detection of mutations mediating resistance have become a major focus of interest. This situation is comparable to viral susceptibility testing, which is routinely performed by genotyping and is used to predict drug resistance, analyze treatment failure, and help in guiding second-line and salvage therapies.⁷ In human immunodeficiency virus (HIV), genotypic resistance testing is evaluated using online databases that include known genotype-phenotype correlations. These databases are essential tools for individual susceptibility testing but also for surveillance of drug resistance.⁸ Here, we set up FunResDB, a database which collects and stores all available

information about *CYP51A*-dependent *A. fumigatus* drug resistance. A web application is available on the web server of the German National Reference Center for Invasive Fungal Infections (<http://www.nrz-myk.de/funresdb>). FunResDB includes an online bioinformatics tool that enables automated sequence analysis and genotyping for clinical sequence data.

The database stores all relevant data including the essential information about azole drug susceptibility, amino acid substitutions, and literature references. All content was obtained by comprehensive manual screening of biomedical publications. The web application is structured in the main sections 'Search' and 'Explore.' On the search page, users first select a reference gene (*CYP51A* by default) and then either enter (i) corresponding isolated genetic sequences or (ii) mutation names (e.g., L98H) to search the database. In the first case, a custom sequence analysis tool is initiated, which is described below, in order to determine mutations from input-reference gene alignment. After submitting the search query, the resulting table presents matching fungal strains including their individual mutations, drug susceptibility levels, and publication references. For *A. fumigatus*

CYP51A, we obtained the reference sequence from Genbank (AF338659). Nucleotide triplets of aligned regions are translated into amino acids using the reading frame of the reference sequence. In the last step, input and reference protein sequence are compared to identify amino acid alterations in the input. In case of an unexpected high number of amino acid substitutions in the input sequence our tool issues a warning to suggest double-checking of quality and origin of the input sequences. If a 5' upstream region is provided, this subsequence is checked for tandem repeats using the etandem tool from Emboss toolbox. As a default setting, tandem repeats with a length of at least 20 bp will be reported on the result page.

Database content was compiled by manual curation of biomedical publications obtained from PubMed database. We retrieved relevant publications by querying the search terms 'Aspergillus fumigatus AND (CYP51 or CYP51A) AND azole AND resistance' and manually screened all resulting 190 publications to extract information about isolates, drug resistance and nonsynonymous mutations in the CYP51A protein sequence (for complete literature list see supplemental material). Overall, the current version of the database contains data for 79 distinct *A. fumigatus* CYP51A genotypes, which are linked to 59 nonsynonymous mutations. Importantly, the database also contains information on known mutations that are not linked to resistance, to inform users on phenotypically silent mutations. Furthermore, three tandem repeats of the upstream intergenic region denoted as TR34, TR46, TR53 are reported, each of them is known to contribute to the resistance phenotype.¹ In the web interface, all mutations can be searched and FunResDB automatically retrieves all published literature for any mutation.

In order to facilitate mutation detection, FunResDB provides a mutation search tool which analyzes input DNA sequences (FASTA or plain text format) and returns detected amino acid alterations in a straightforward manner. Currently, any subsequence of *A. fumigatus* CYP51A with a minimal length of 50 bp is allowed as an input. After submission, sequences are aligned and translated. The result page contains a list of altered protein positions and a corresponding resistance data table summarizing all available literature data in a compact format. It also includes additional information to guide the interpretation of the susceptibility data, especially for ambiguous phenotypes. Furthermore, our tool performs a tandem repeat search in the 5' proximal upstream region of the input sequence. Multiple copies of subsequences in the promoter region of *A. fumigatus* CYP51A have been associated with azole resistance.

For database evaluation we conducted a batch analysis of all *A. fumigatus* CYP51A sequences available on NCBI Genbank database (n = 150).⁹ In sum, 18 sequences (12%)

were correctly identified to contain mutations, which are associated with azole resistance in the database. Additionally, the FunResDB tool detected a novel mutation (S496T), which has not yet been described in the literature. The corresponding Genbank entry (KT231984) quotes unpublished data, and the respective mutation will be added to the database once the data are published.

In conclusion, FunResDB is the first publically available web application for the analysis of fungal drug susceptibility based on genetic sequences. It provides both a repository for drug susceptibility data as well as essential analysis tools. The database constitutes a curated collection of data that are widely scattered across the literature. With regular updates, that are facilitated by an automated PubMed query tool, the database also serves as a monitor for an anticipated increase of resistant clinical isolates in the future and would allow detection of rare or new mutations conferring resistance. It should, however, be noted that there are limitations: a variable percentage of azole resistance is not linked to mutations in CYP51A.² Therefore, report of a wild-type CYP51A sequence does not infer susceptibility in all cases. Furthermore, the database just reports on published associations between genotype and phenotype. In most cases, functional confirmation that a given mutation confers resistance is missing. Despite this, the FunResDB sequence analysis is a powerful tool that facilitates the search and annotation of newly sequenced isolates and enables comparison with a large amount of available published isolates. This way, genotypic susceptibility testing can be performed as a routine task using a single analysis tool. Furthermore, the flexible database setup enables future incorporation of data for other fungal species and other types of resistance. As an example, echinocandin resistance in *Candida spp.* is emerging and in most cases mediated by point mutations in the target genes (FKS1/2). Thus, FunResDB is ideally suited to also incorporate FKS mutations and sequence analyses.

Work in the NRZMyk is supported by the Robert-Koch Institute from funds provided by the German Ministry of Health (grant no. 1369-240 to O.K.). This project was funded by the German Ministry for Education and Science within the program Unternehmen Region, Zwanzig20 (Project FINAR: grant no. 03ZZ0809A).

Supplementary material

Supplementary data are available at [MMYCOL](http://www.mycology.com) online.

References

1. Verweij PE, Chowdhary A, Melchers WJG et al. Azole resistance in *Aspergillus fumigatus*: can we retain the clinical use of mold-active antifungal azoles? *Clin Infect Dis.* 2016; 62: 362–368.

2. Verweij PE, Ananda-Rajah M, Andes D et al. International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*. *Drug Resist Updat*. 2015; 21–22: 30–40.
3. Howard SJ, Cerar D, Anderson MJ et al. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis*. 2009; 15: 1068–1076.
4. Albarrag AM, Anderson MJ, Howard SJ et al. Interrogation of related clinical pan-azole-resistant *Aspergillus fumigatus* strains: G138C, Y431C, and G434C single nucleotide polymorphisms in cyp51A, upregulation of cyp51A, and integration and activation of transposon Atf1 in the cyp51A promoter. *Antimicrob Agents Chemother*. 2011; 55: 5113–5121.
5. Spiess B, Postina P, Reinwald M et al. Incidence of Cyp51 a key mutations in *Aspergillus fumigatus*: a study on primary clinical samples of immunocompromised patients in the period of 1995–2013. *PLoS One*. 2014; 9. doi:10.1371/journal.pone.0103113.
6. Steinmann J, Hamprecht A, Vehreschild MJGT et al. Emergence of azole-resistant invasive aspergillosis in HSCT recipients in Germany. *J Antimicrob Chemother*. 2014; 70: 1522–1526.
7. Tang MW, Shafer RW. HIV-1 antiretroviral resistance: scientific principles and clinical applications. *Drugs*. 2012; 72: 1–25.
8. Shafer RW. Rationale and uses of a public HIV drug-resistance database. *J Infect Dis*. 2006;194 (Suppl): S51–58.
9. Benson DA, Karsch-Mizrachi I, Lipman DJ et al. GenBank. *Nucleic Acids Res*. 2007; 35 (Suppl. 1): 21–25.