



KatG as Counterselection Marker for Nontuberculous Mycobacteria

Aron Gagliardi,^a Petra Selchow,^a Sakshi Luthra,^a Daniel Schäfle,^a Bettina Schulthess,^{a,b}  Peter Sander^{a,b}

^aInstitute of Medical Microbiology, University of Zurich, Zurich, Switzerland

^bNational Center for Mycobacteria, Zurich, Switzerland

Aron Gagliardi and Petra Selchow contributed equally. Author order was determined both alphabetically and in order of increasing seniority.

KEYWORDS isoniazid, nontuberculous mycobacteria, *Mycobacterium abscessus*

In a recent issue of *Antimicrobial Agents and Chemotherapy*, Reingewertz et al. (1) report on sensitization of slow-growing, nontuberculous mycobacteria (NTM) to the anti-tubercular drug isoniazid (INH) upon expression of *Mycobacterium bovis* KatG.

KatG functions as a catalase-peroxidase (2, 3) and activates INH, which then inhibits InhA, an enzyme involved in mycolic acid synthesis (4). However, a surge in INH-resistant *Mycobacterium tuberculosis* clinical isolates is jeopardizing the role of INH as a first-line drug (5). In general, resistance to INH can be acquired by mutations in *katG* or, less frequently, in the promoter region of *inhA* (6, 7). In the first case, KatG no longer activates INH, while in the second case, a higher tolerance to the drug is conferred by increased InhA expression (8).

The aim of the aforementioned study was to elucidate the differences between KatG-dependent INH activation in mycobacteria and its effect on INH susceptibility, focusing on the opportunistic pathogens *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium marinum* (both NTM and naturally refractory to INH).

NTM are mycobacteria not belonging to the *M. tuberculosis* complex and encompass both slowly and rapidly growing mycobacterial species (SGM and RGM) (9). As shown by our colleagues and other groups (1, 10–12), NTM usually show an innate decreased susceptibility toward INH. Within NTM, RGM have significantly higher INH MICs than SGM (11–13). The reason for this increased resistance is likely the result of several factors, including a failure to activate the prodrug, target-level mutations, differences in the C-terminal domain of KatG (3), the reduction of intracellular concentration (by means of efflux pumps or decreased permeability) (14), and/or the possible nonessentiality of the mycolic acid synthesis pathway in NTM (12). However, essentiality has been proven by identifying pyridomycin as a specific inhibitor of InhA preventing growth of both *M. tuberculosis* (MIC = 0.39 mg/liter) and NTM (*M. marinum* MIC = 3.13 mg/liter) (15).

In the context of NTM, no other mycobacteria have proven to be as resilient as the emerging opportunistic pathogens from the *Mycobacterium abscessus* complex (16). As members of the RGM (9), their intrinsic antibiotic resistance through drug- and target-modifying enzymes (17) has rendered *M. abscessus* complex infections extremely challenging to treat. Lengthy regimens on multiple drugs with severe side effects are the norm (11, 13). KatG phylogeny pinpointed *M. abscessus* complex as being closer to *M. tuberculosis* than *rpoB*-based phylogeny did (1). Alignment of KatG^{Mabs} with KatG^{Mtb} shows that the most common INH resistance-conferring clinical *M. tuberculosis* mutations (18) are absent in *M. abscessus* ATCC 19977 and sequence identity is high (approximately 72%).

Citation Gagliardi A, Selchow P, Luthra S, Schäfle D, Schulthess B, Sander P. 2020. KatG as counterselection marker for nontuberculous mycobacteria. *Antimicrob Agents Chemother* 64:e02508-19. <https://doi.org/10.1128/AAC.02508-19>.

Copyright © 2020 American Society for Microbiology. All Rights Reserved.

Address correspondence to Peter Sander, psander@imm.uzh.ch.

Ed. Note: The authors of the published article did not feel that a response was necessary.

Published 21 April 2020

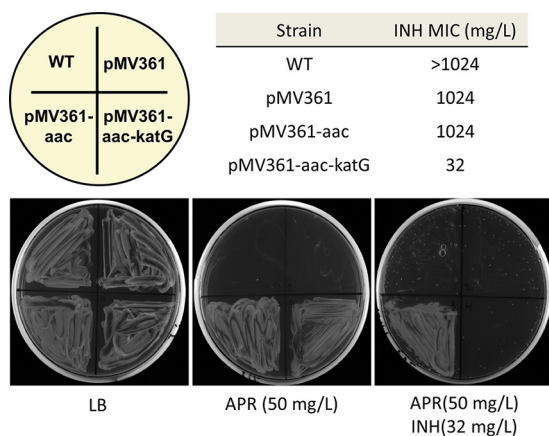


FIG 1 INH susceptibility of *M. abscessus* complex strains upon KatG^{Mtb} expression. MICs (day 7) for the strains used to generate *M. abscessus* complex pMV361-aac-katG (unpublished data) are shown. MICs were measured by broth microdilution assay (25). Luria-Bertani (LB) agar with APR and with APR-INH shows selective growth of *M. abscessus* complex.

Our research group has taken advantage of the resistance of *M. abscessus* complex to INH in a manner similar to that used by Reingewertz and coworkers. In a proof-of-concept study, we heterologously expressed KatG^{Mtb} in *M. abscessus* complex from a pMV361 (19) *attB*-integrative vector containing an apramycin (APR) resistance cassette for selection (*aac*). This allowed us to develop new tools for the genetic manipulation of *M. abscessus* complex and to use INH as an effective counterselection marker for allelic replacements (20–24), even if the MIC was well above achievable therapeutical concentrations (MIC = 32 mg/liter) (Fig. 1). Our observations are in strong agreement with the work from Reingewertz et al., showing a drop in MIC of approximately 30-fold, and are proof that INH susceptibility can be successfully exploited. However, the comparatively high MIC of recombinant *M. abscessus* complex pMV361-aac-katG indicates that besides poor KatG-dependent INH activation, other factors contribute to the high level INH resistance of *M. abscessus*.

ACKNOWLEDGMENTS

This work was supported by the University of Zurich and the Institute of Medical Microbiology. We acknowledge financial support from Lungen Liga Schweiz/Georg and Bertha Schwyzer-Winiker Stiftung (SLA-2018-02) and Foundation for Research in Science and the Humanities at the University of Zurich (STWF-18-011).

REFERENCES

- Reingewertz TH, Meyer T, McIntosh F, Sullivan J, Meir M, Chang Y-F, Behr MA, Barkan D. 2019. Differential sensitivity of mycobacteria to isoniazid is related to differences in KatG-mediated enzymatic activation of the drug. *Antimicrob Agents Chemother* 64:e01899-19. <https://doi.org/10.1128/AAC.01899-19>.
- Zhang Y, Heym B, Allen B, Young D, Cole S. 1992. The catalase–peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* 358: 591–593. <https://doi.org/10.1038/358591a0>.
- Heym B, Zhang Y, Poulet S, Young D, Cole ST. 1993. Characterization of the *katG* gene encoding a catalase–peroxidase required for the isoniazid susceptibility of *Mycobacterium tuberculosis*. *J Bacteriol* 175:4255–4259. <https://doi.org/10.1128/jb.175.13.4255-4259.1993>.
- Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um K, Wilson T, Collins D, de Lisle G, Jacobs W. 1994. *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* 263: 227–230. <https://doi.org/10.1126/science.8284673>.
- Gumbo T, Louie A, Liu W, Ambrose PG, Bhavnani SM, Brown D, Drusano GL. 2007. Isoniazid's bactericidal activity ceases because of the emergence of resistance, not depletion of *Mycobacterium tuberculosis* in the log phase of growth. *J Infect Dis* 195:194–201. <https://doi.org/10.1086/510247>.
- Larsen MH, Vilchèze C, Kremer L, Besra GS, Parsons L, Salfinger M, Heifets L, Hazbon MH, Alland D, Sacchetti JC, Jacobs WR, Jr. 2002. Overexpression of *inhA*, but not *kasA*, confers resistance to isoniazid and ethionamide in *Mycobacterium smegmatis*, *M. bovis* BCG and *M. tuberculosis*. *Mol Microbiol* 46:453–466. <https://doi.org/10.1046/j.1365-2958.2002.03162.x>.
- Coll F, McNerney R, Preston MD, Guerra-Assunção JA, Warry A, Hill-Cawthorne G, Mallard K, Nair M, Miranda A, Alves A, Perdigão J, Viveiros M, Portugal I, Hasan Z, Hasan R, Glynn JR, Martin N, Pain A, Clark TG. 2015. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. *Genome Med* 7:51. <https://doi.org/10.1186/s13073-015-0164-0>.
- Zhang Y, Yew WW, Barer MR. 2012. Targeting persisters for tuberculosis control. *Antimicrob Agents Chemother* 56:2223–2230. <https://doi.org/10.1128/AAC.06288-11>.
- Tortoli E, Fedrizzi T, Meehan CJ, Trovato A, Grottola A, Giacobazzi E, Serpini GF, Tagliazucchi S, Fabio A, Bettua C, Bertorelli R, Frascaro F, De Sanctis V, Pecorari M, Jousson O, Segata N, Cirillo DM. 2017. The new phylogeny of the genus *Mycobacterium*: the old and the news. *Infect Genet Evol* 56:19–25. <https://doi.org/10.1016/j.meegid.2017.10.013>.

10. Brown-Elliott BA, Nash KA, Wallace RJ. 2012. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. *Clin Microbiol Rev* 25:545–582. <https://doi.org/10.1128/CMR.05030-11>.
11. Griffith DE, Infectious Disease Society of America, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huit G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Winthrop K. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 175:367–416. <https://doi.org/10.1164/rccm.200604-571ST>.
12. van Ingen J, Boeree MJ, van Soolingen D, Mouton JW. 2012. Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. *Drug Resist Updat* 15:149–161. <https://doi.org/10.1016/j.drug.2012.04.001>.
13. Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF, Leitch A, Loebinger MR, Milburn HJ, Nightingale M, Ormerod P, Shingadia D, Smith D, Whitehead N, Wilson R, Floto RA. 2017. British Thoracic Society guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *BMJ Open Respir Res* 4:e000242. <https://doi.org/10.1136/bmjresp-2017-000242>.
14. Adams KN, Szumowski JD, Ramakrishnan L. 2014. Verapamil, and its metabolite norverapamil, inhibit macrophage-induced, bacterial efflux pump-mediated tolerance to multiple anti-tubercular drugs. *J Infect Dis* 210:456–466. <https://doi.org/10.1093/infdis/jiu095>.
15. Hartkoorn RC, Sala C, Neres J, Pojer F, Magnet S, Mukherjee R, Uplekar S, Boy-Röttger S, Altmann K-H, Cole ST. 2012. Towards a new tuberculosis drug: pyridomycin—nature’s isoniazid. *EMBO Mol Med* 4:1032–1042. <https://doi.org/10.1002/emmm.201201689>.
16. Nessar R, Cambau E, Reyat JM, Murray A, Gicquel B. 2012. Mycobacterium abscessus: a new antibiotic nightmare. *J Antimicrob Chemother* 67:810–818. <https://doi.org/10.1093/jac/dkr578>.
17. Luthra S, Rominski A, Sander P. 2018. The role of antibiotic-target-modifying and antibiotic-modifying enzymes in Mycobacterium abscessus drug resistance. *Front Microbiol* 9:2179. <https://doi.org/10.3389/fmicb.2018.02179>.
18. Zhao X, Hersleth H-P, Zhu J, Andersson KK, Magliozzo RS. 2013. Access channel residues Ser315 and Asp137 in Mycobacterium tuberculosis catalase-peroxidase (KatG) control peroxidatic activation of the pro-drug isoniazid. *Chem Commun (Camb)* 49:11650–11652. <https://doi.org/10.1039/c3cc47022a>.
19. Stover CK, de la Cruz VF, Fuerst TR, Burlein JE, Benson LA, Bennett LT, Bansal GP, Young JF, Lee MH, Hatfull GF, Snapper SB, Barletta RG, Jacobs WR, Bloom BR. 1991. New use of BCG for recombinant vaccines. *Nature* 351:456–460. <https://doi.org/10.1038/351456a0>.
20. Rominski A, Roditscheff A, Selchow P, Böttger EC, Sander P. 2017. Intrinsic rifamycin resistance of Mycobacterium abscessus is mediated by ADP-ribosyltransferase MAB_0591. *J Antimicrob Chemother* 72:376–384. <https://doi.org/10.1093/jac/dkw466>.
21. Becker K, Haldimann K, Selchow P, Reinau LM, Dal Molin M, Sander P. 2017. Lipoprotein glycosylation by protein-O-mannosyltransferase (MAB_1122c) contributes to low cell envelope permeability and antibiotic resistance of Mycobacterium abscessus. *Front Microbiol* 8:2123. <https://doi.org/10.3389/fmicb.2017.02123>.
22. Rominski A, Schulthess B, Muller DM, Keller PM, Sander P. 2017. Effect of beta-lactamase production and beta-lactam instability on MIC testing results for Mycobacterium abscessus. *J Antimicrob Chemother* 72:3070–3078. <https://doi.org/10.1093/jac/dkx284>.
23. Rominski A, Selchow P, Becker K, Brulle JK, Dal Molin M, Sander P. 2017. Elucidation of Mycobacterium abscessus aminoglycoside and capreomycin resistance by targeted deletion of three putative resistance genes. *J Antimicrob Chemother* 72:2191–2200. <https://doi.org/10.1093/jac/dkx125>.
24. Dal Molin M, Gut M, Rominski A, Haldimann K, Becker K, Sander P. 2018. Molecular mechanisms of intrinsic streptomycin resistance in Mycobacterium abscessus. *Antimicrob Agents Chemother* 62:e01427-17. <https://doi.org/10.1128/AAC.01427-17>.
25. Reller LB, Weinstein MP, Woods GL. 2000. Susceptibility testing for mycobacteria. *Clin Infect Dis* 31:1209–1215. <https://doi.org/10.1086/317441>.