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NOTES

Antibiotic Resistance and Single-Nucleotide Polymorphism Cluster Grouping Type in a Multinational Sample of Resistant *Mycobacterium tuberculosis* Isolates[▽]

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A single-nucleotide polymorphism-based cluster grouping (SCG) classification system for *Mycobacterium tuberculosis* was used to examine antibiotic resistance type and resistance mutations in relationship to specific evolutionary lineages. Drug resistance and resistance mutations were seen across all SCGs. SCG-2 had higher proportions of *katG* codon 315 mutations and resistance to four drugs.

Isoniazid (INH) is an effective agent for treatment of infections with *Mycobacterium tuberculosis*. Increases in INH-resistant and multidrug-resistant tuberculosis jeopardize drug effectiveness (7, 24), and development of INH resistance is often a first step in multidrug resistance (2, 8). Mutations in specific genes have been linked to INH-related resistance (10, 11), including *katG* (26), *inhA* (14), codon 315 of *katG* ($katG_{315}$) (13, 16, 19, 23), *ahpC* (20), the *inhA* open reading frame and promoter (14, 17, 25), and *ndh* (22).

Recent studies of drug-resistant *M. tuberculosis* have found associations among *M. tuberculosis* strains, drug resistance, and specific gene mutations. *M. tuberculosis* strains belonging to the Beijing family were associated with drug resistance in Iran, Afghanistan, and Russia (6, 12, 15, 18), although not in Venezuela (5). These associations have also been supported through genetic laboratory studies (1, 21). Thus, it is possible that specific types of drug resistance or drug resistance mutations might occur more commonly in certain evolutionary lineages of *M. tuberculosis*.

The single-nucleotide polymorphism (SNP) cluster group (SCG) classification system defined in reference 8 gives rise to seven phylogenetically distinct groups and three subgroups that can be used to infer an evolutionary pattern in *M. tuberculosis*. Here, we analyze 428 *M. tuberculosis* isolates resistant to at least INH collected across 10 countries and report the prevalence of various INH resistance-associated mutations and prevalences of resistance to two, three, and four drugs according to the major SCG-defined phylogenetic lineages of *M. tuberculosis*.

M. tuberculosis isolates resistant to at least INH were obtained from laboratories in major medical centers in Australia, Colombia, India, Mexico, New York City, Spain, and Texas (Table 1). The study population and sample selection have been described previously (10, 11), with each collection site

TABLE 1. *M. bovis* and *M. tuberculosis* SCG types overall and by location

Location	No. (%) of isolates										
	M. bovis	*M. tuberculosis*									Total
		SCG-1	SCG-2	SCG-3a	SCG-3b	SCG-3c	SCG-4	SCG-5	SCG-6a	SCG-6b	
Australia	0	9 (30.0)	11 (36.7)	3 (10.0)	3 (10.0)	0	0	3 (10.0)	0	1 (3.3)	30 (100)
Colombia	0	0	0	1 (0.7)	33 (23.1)	1 (0.7)	6 (4.2)	84 (58.7)	17 (11.9)	1 (0.7)	143 (100)
India	0	2 (6.7)	1 (3.3)	23 (76.7)	1 (3.3)	0	0	2 (6.7)	1 (3.3)	0	30 (100)
Mexico	2 (1.6)	0	1 (0.8)	1 (0.8)	44 (35.8)	11 (8.9)	14 (11.4)	31 (25.2)	15 (12.2)	4 (3.3)	123 (100)
New York City	0	1 (3.9)	17 (65.3)	0	2 (7.7)	1 (3.9)	0	2 (7.7)	3 (11.5)	0	26 (100)
Spain	0	0	0	0	1 (9.1)	0	0	8 (72.7)	2 (18.2)	0	11 (100)
Texas	0	5 (7.7)	8 (12.3)	0	8 (12.3)	5 (7.7)	6 (9.2)	16 (24.6)	15 (23.1)	2 (3.1)	65 (100)
Total	2 (0.5)	17 (4.0)	38 (8.9)	28 (6.5)	92 (21.5)	18 (4.2)	26 (6.1)	146 (34.1)	53 (12.4)	8 (1.8)	428 (100)

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TABLE 2. Prevalence of selected genes within SCG type

Gene	No. (%) of isolates with gene:									
	M. bovis	*M. tuberculosis*								Total
		SCG-1	SCG-2	SCG-3a	SCG-3b	SCG-3c	SCG-4	SCG-5	SCG-6a	SCG-6b	
KatG	0	12 (5.0)	29 (12.1)	12 (5.0)	51 (21.3)	12 (5.0)	16 (6.7)	81 (33.9)	22 (9.2)	4 (1.8)	239 (100)
$KatG_{315}$	0	12 (5.4)	25 (11.5)	11 (5.1)	45 (20.1)	12 (5.4)	14 (6.4)	78 (35.9)	17 (7.8)	3 (1.4)	217 (100)
KasA	0	8 (16.7)	1 (2.1)	0	0	0	0	39 (81.2)	0	0	48 (100)
InhA	0	3 (7.1)	4 (9.5)	2 (4.8)	10 (23.8)	1 (2.4)	2 (4.8)	12 (28.6)	8 (19.0)	0	42 (100)
Inh promoter	0	3 (7.9)	4 (10.5)	2 (5.3)	9 (23.7)	1 (2.6)	2 (5.3)	10 (26.3)	7 (18.4)	0	38 (100)
Inh open reading frame	0	1 (11.1)	0	1 (11.1)	2 (22.2)	0	1 (11.1)	3 (33.3)	1 (11.1)	0	9 (100)
AhpC	0	1 (1.7)	1 (1.7)	17 (28.3)	10 (16.7)	0	11 (18.2)	13 (21.7)	6 (10.0)	1 (1.7)	60 (100)
Ndh	0	1 (7.1)	0	0	0	1 (7.1)	0	12 (85.7)	0	0	14 (100)

performing susceptibility testing to INH, rifampin, streptomycin, and ethambutol.

Isolates were tested for virtually all SNP mutations in the *M. tuberculosis katG*, *kasA*, *mabA*, *inhA*, *oxyR*, *ahpC*, and *ndh* genes found to be associated with INH resistance in published studies (11). A total of 204 INH resistance-associated alleles were detected (9), with confirmatory testing carried out on alleles, identified mutations, and drug resistance.

Strain types are reported in terms of SCG grouping, based on observed genomic level clustering among identified SNPs (8). SCG assignment was performed by testing each isolate for nine SNPs previously determined to replicate larger SNP-based phylogeny, as described in references 3 and 4.

The genetic, resistance, and SCG data from each set of country-specific isolates were entered into a common database. Prevalence was reported by SCG type, country, selected genes, and resistance type. Chi-square tests of differences in proportions were employed as appropriate. The STATA statistical package was used for all calculations.

The complete sample was tested for 240 alleles previously reported to be associated with INH resistance. Country-specific breakdowns by various types of resistance can be found in references 10 and 11. The overall prevalence of SCG groups is given in Table 1. The Beijing family (strongly associated with SCG-2) is present in 8.9% of the collected isolates. The most prevalent SCG types were SCG-5, SCG-3b, and SCG-6a, and the rarest (excluding *Mycobacterium bovis*) were SCG-6b, SCG-1, SCG-3c, and SCG-4. SCG prevalence by country shows various distributions. SCG-5 is among the three most prevalent SCG types in Spain, Columbia, Mexico, and Texas. SCG-3b was prevalent in Mexico, Columbia, and Texas; SCG-6a was prevalent in Texas, Spain, and Mexico; SCG-3a was prevalent in India; and SCG-2 was prevalent in Australia and New York City, perhaps reflecting Asian immigrant populations. SCG-3c, SCG-4, and SCG-6b had low prevalence in all countries.

Table 2 reports the prevalence of mutations in a selected set of genes. Only KatG and $KatG_{315}$ mutations occur across all SCG types (excluding *M. bovis*). The SCG types with the highest numbers of mutations were SCG-5, SCG-3b, and SCG-1. SCG-1 and SCG-5 had all mutations of interest. *kasA* and *ndh* mutations were found in the smallest number of SCG types (three).

As these measures could be influenced by the proportional representation of each SCG in the sample, we examined the relative proportion of each mutation within each SCG (Fig. 1). The highest proportion of $KatG_{315}$ mutations occurred in SCG-3c and SCG-2. SCG-6b, SCG-2, and SCG-3c had the highest proportion of mutations in *katG*. Mutations in *inhA* and *ahpC* promoters were found in all SCGs with $n > 17$.

To examine antibiotic resistance on a cumulative scale, we examined resistance within each SCG classification type in relation to resistance to one, two, or more antibiotics. All SCG types (excepting *M. bovis*) had at least 46% of isolates with resistance to one or two of these antibiotics. The range across all SCG types was 46% to 82%. SCG-2 had the highest prevalence (29%) of resistance to all four antibiotics.

This study provides insights into the associations among drug resistance in *M. tuberculosis*, gene mutation, and genomic SCG-based phylogenetic lineages, utilizing a large number of resistant isolates and examining patterns of relationships across SCG classification type, resistance, gene mutation, and country.

The sample contained 8.9% SCG-2 (Beijing) isolates, similar to Asian samples (18) and comparable to other SCG types, except SCG-3b, SCG-5, and SCG-6a. SCG-2 isolates accounted for more than 10% of the KatG and $KatG_{315}$ mutations in this study. Most mutations were prevalent in all SCGs.

The prevalence of resistance was high across all SCG types. Table 3 shows that SCG-2 had the highest prevalence of resistance to all antibiotics, but all SCG types display high levels of resistance to one and two antibiotics. SCG-6a displayed a high prevalence of isolates resistant to all four antibiotics. As the SCG classification reflects regions, the presence of antibiotic resistance in so many SCG classifications may reflect several ongoing evolutionary processes and implies a need to maintain a broad perspective on *M. tuberculosis* antibiotic resistance.

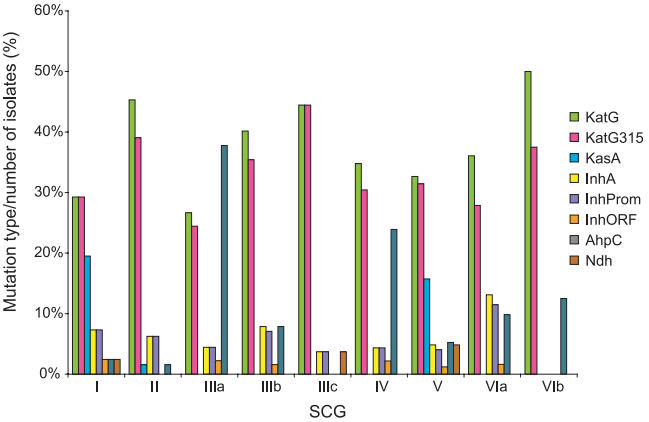

FIG. 1. Distribution of mutations by SCG.

TABLE 3. Number of isolates within each SCG type with resistance to individual antibiotics

No. of antibiotics to which isolates show resistance	No. (%) of resistant isolates									
	M. bovis	*M. tuberculosis*								Total
		SCG-1	SCG-2	SCG-3a	SCG-3b	SCG-3c	SCG-4	SCG-5	SCG-6a	SCG-6b	
1	2	12 (70.59)	12 (31.58)	12 (42.86)	26 (28.26)	8 (44.44)	8 (30.77)	28 (19.18)	14 (26.42)	3 (37.5)	125 (29.21)
2	0	2 (11.76)	10 (26.32)	9 (32.14)	30 (32.61)	4 (22.22)	12 (46.15)	39 (26.71)	16 (30.19)	2 (25.0)	124 (28.97)
3	0	2 (11.76)	5 (13.16)	6 (21.43)	21 (22.83)	5 (27.78)	4 (15.38)	53 (36.30)	10 (18.87)	3 (37.50)	109 (25.47)
4	0	1 (5.88)	11 (28.95)	1 (3.57)	15 (16.3)	1 (5.56)	2 (7.69)	26 (17.81)	13 (24.53)	0	70 (16.36)
Total	2	17 (100)	38 (100)	28 (100)	92 (100)	18 (100)	26 (100)	146 (100)	53 (100)	8 (100)	428 (100)

Limitations. This study analyzed *M. tuberculosis* isolates for mutations associated with INH resistance in previous studies. The results therefore may not be completely representative of mutations relevant to resistance. The overall patterns observed in the SCG classification may have been restricted by the method of sample selection, but the size and diversity of the sample make this unlikely.

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REFERENCES

1. **Abebe, F., and G. Bjune.** 2006. The emergence of Beijing family genotypes of Mycobacterium tuberculosis and low-level protection by bacille Calmette-Guerin (BCG) vaccines: is there a link? Clin. Exp. Immunol. **145:**389–397.
2. **Alland, D., G. E. Kalkut, A. R. Moss, R. A. McAdam, J. A. Hahn, W. Bosworth, E. Drucker, and B. R. Bloom.** 1994. Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiologic methods. N. Engl. J. Med. **330:**1710–1716.
3. **Alland, D., D. W. Lacher, M. H. Hazbón, A. S. Motiwala, W. Qi, R. D. Fleischmann, and T. S. Whittam.** 2007. Role of large sequence polymorphisms (LSPs) in generating genomic diversity among clinical isolates of *Mycobacterium tuberculosis* and the utility of LSPs in phylogenetic analysis. J. Clin. Microbiol. **45:**39–46.
4. **Alland, D., T. S. Whittam, M. B. Murray, M. D. Cave, M. H. Hazbón, K. Dix, M. Kokoris, A. Duesterhoeft, J. A. Eisen, C. M. Fraser, and R. D. Fleischmann.** 2003. Modeling bacterial evolution with comparative-genome-based marker systems: application to *Mycobacterium tuberculosis* evolution and pathogenesis. J. Bacteriol. **185:**3392–3399.
5. **Aristimuno L., R. Armengol, A. Cebollada, M. Espana, A. Guilarte, C. Lafoz, M. A. Lezcano, M. J. Revillo, C. Martin, C. Ramirez, N. Rastogi, J. Rojas, A. V. de Salas, C. Sola, and S. Samper.** 2006. Molecular characterisation of Mycobacterium tuberculosis isolates in the First National Survey of Anti-tuberculosis Drug Resistance from Venezuela. BMC Microbiol. **6:**90.
6. **Cox, H. S., T. Kubica, D. Doshetov, Y. Kebede, S. Rusch-Gerdess, and S. Niemann.** 2005. The Beijing genotype and drug resistant tuberculosis in the Aral Sea region of Central Asia. Respir. Res. **6:**134.
7. **Fang, Z., C. Doig, A. Rayner, D. T. Kenna, B. Watt, and K. J. Forbes.** 1999. Molecular evidence for heterogeneity of the multiple-drug-resistant *Mycobacterium tuberculosis* population in Scotland (1990 to 1997). J. Clin. Microbiol. **37:**998–1003.
8. **Filliol, I., A. S. Motiwala, M. Cavatore, W. Qi, M. H. Hazbón, M. Bobadilla del Valle, J. Fyfe, L. Garcia-Garcia, N. Rastogi, C. Sola, T. Zozio, M. I. Guerrero, C. I. León, J. Crabtree, S. Angiuoli, K. D. Eisenach, R. Durmaz, M. L. Joloba, A. Rendón, J. Sifuentes-Osornio, A. Ponce de Leon, M. D. Cave, R. Fleischmann, T. S. Whittam, and D. Alland.** 2006. Global phylogeny of *Mycobacterium tuberculosis* based on single nucleotide polymorphism (SNP) analysis: insights into tuberculosis evolution, phylogenetic accuracy of other DNA fingerprinting systems, and recommendations for a minimal standard SNP set. J. Bacteriol. **188:**759–772.
9. **Hazbón, M. H., and D. Alland.** 2004. Hairpin primers for simplified single-nucleotide polymorphism analysis of *Mycobacterium tuberculosis* and other organisms. J. Clin. Microbiol. **42:**1236–1242.
10. **Hazbón, M. H., M. Bobadilla del Valle, M. I. Guerrero, M. Varma-Basil, I. Filliol, M. Cavatore, R. Colangeli, H. Safi, H. Billman-Jacobe, C. Lavender, J. Fyfe, L. Garcia-Garcia, A. Davidow, M. Brimacombe, C. I. Leon, T. Porras, M. Bose, F. Chaves, K. D. Eisenach, J. Sifuentes-Osornio, A. Ponce de Leon, M. D. Cave, and D. Alland.** 2005. Role of *embB* codon 306 mutations in *Mycobacterium tuberculosis* revisited: a novel association with broad drug resistance and IS*6110* clustering rather than ethambutol resistance. Antimicrob. Agents Chemother. **49:**3794–3802.
11. **Hazbón, M. H., M. Brimacombe, M. Bobadilla del Valle, M. Cavatore, M. I. Guerrero, M. Varma-Basil, H. Billman-Jacobe, C. Lavender, J. Fyfe, L. García-García, C. Inés León, M. Bose, F. Chaves, M. Murray, K. D. Eisenach, J. Sifuentes-Osornio, M. D. Cave, A. Ponce de León, and D. Alland.** 2006. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **50:**2640–2649.
12. **Ignatova, A., S. Dubiley, V. Stepanshina, and I. Shemyakin.** 2006. Predominance of multi-drug-resistant LAM and Beijing family strains among Mycobacterium tuberculosis isolates recovered from prison inmates in Tula Region, Russia. J. Med. Microbiol. **55:**1413–1418.
13. **Kapetanaki, S. M., S. Chouchane, S. Yu, X. Zhao, R. S. Magliozzo, and J. P. Schelvis.** 2005. Mycobacterium tuberculosis KatG(S315T) catalase-peroxidase retains all active site properties for proper catalytic function. Biochemistry **44:**243–252.
14. **Musser, J. M., V. Kapur, D. L. Williams, B. N. Kreiswirth, D. van Soolingen, and J. D. van Embden.** 1996. Characterization of the catalase-peroxidase gene (katG) and inhA locus in isoniazid-resistant and -susceptible strains of Mycobacterium tuberculosis by automated DNA sequencing: restricted array of mutations associated with drug resistance. J. Infect. Dis. **173:**196–202.
15. **Pheiffer, C., J. C. Betts, H. R. Flynn, P. T. Lukey, and P. van Helden.** 2005. Protein expression by a Beijing strain differs from that of another clinical isolate and Mycobacterium tuberculosis H37Rv. Microbiology **151:**1139–1150.
16. **Pym, A. S., B. Saint-Joanis, and S. T. Cole.** 2002. Effect of *katG* mutations on the virulence of *Mycobacterium tuberculosis* and the implication for transmission in humans. Infect. Immun. **70:**4955–4960.
17. **Ramaswamy, S. V., S. J. Dou, A. Rendon, Z. Yang, M. D. Cave, and E. A. Graviss.** 2004. Genotypic analysis of multidrug-resistant Mycobacterium tuberculosis isolates from Monterrey, Mexico. J. Med. Microbiol. **53:**107–113.
18. **Ramazanzadeh, R., P. Farnia, N. N. Amirmozafari, F. Ghazi, Z. Ghadertotonchi, J. Kamran, F. Mohammadi, M. Mirsaedi, and M. Masjedi.** 2006. Comparison between molecular epidemiology, geographical regions and drug resistance in Mycobacterium tuberculosis strains isolated from Iranian and Afghan patients. Chemotherapy **52:**316–320.
19. **Saint-Joanis, B., H. Souchon, M. Wilming, K. Johnsson, P. M. Alzari, and S. T. Cole.** 1999. Use of site-directed mutagenesis to probe the structure, function and isoniazid activation of the catalase/peroxidase, KatG, from Mycobacterium tuberculosis. Biochem. J. **338:**753–760.
20. **Sherman, D. R., K. Mdluli, M. J. Hickey, T. M. Arain, S. L. Morris, C. E. Barry III, and C. K. Stover.** 1996. Compensatory ahpC gene expression in isoniazid-resistant Mycobacterium tuberculosis. Science **272:**1641–1643.
21. **Sivkov, A. I., A. N. Boldyrev, M. S. Azaev, S. A. Bodnev, E. V. Medvedeva, O. I. Baranova, A. P. Ivlev-Dantau, L. N. Blinova, A. D. Pasechnikov, and S. I. Tat'kov.** 2006. Evaluation of reasons of the MDR M. tuberculosis strains dissemination by analysis of the rifampicin and/or isoniazid resistant isolates. Mol. Gen. Mikrobiol. Virusol. **2006:**20–25.
22. **van Soolingen, D., P. E. de Haas, H. R. van Doorn, E. Kuijper, H. Rinder, and M. W. Borgdorff.** 2000. Mutations at amino acid position 315 of the katG gene are associated with high-level resistance to isoniazid, other drug resistance, and successful transmission of Mycobacterium tuberculosis in The Netherlands. J. Infect. Dis. **182:**1788–1790.
23. **Vilchèze, C., T. R. Weisbrod, B. Chen, L. Kremer, M. H. Hazbón, F. Wang, D. Alland, J. C. Sacchettini, and W. R. Jacobs, Jr.** 2005. Altered NADH/NAD^+ ratio mediates coresistance to isoniazid and ethionamide in mycobacteria. Antimicrob. Agents Chemother. **49:**708–720.
24. **Weis, S. E., J. M. Pogoda, Z. Yang, M. D. Cave, C. Wallace, M. Kelley, and P. F. Barnes.** 2002. Transmission dynamics of tuberculosis in Tarrant County, Texas. Am. J. Respir. Crit. Care Med. **166:**36–42.
25. **Wilson, R. W., Z. Yang, M. Kelley, M. D. Cave, J. M. Pogoda, R. J. Wallace, Jr., J. P. Cegielski, D. F. Dunbar, D. Bergmire-Sweat, L. B. Elliott, and P. F. Barnes.** 1999. Evidence from molecular fingerprinting of limited spread of drug-resistant tuberculosis in Texas. J. Clin. Microbiol. **37:**3255–3259.
26. **Wilson, T. M., and D. M. Collins.** 1996. ahpC, a gene involved in isoniazid resistance of the Mycobacterium tuberculosis complex. Mol. Microbiol. **19:** 1025–1034.