Published in final edited form as:
J Infect Dis. 2010 March 15; 201(6): 881–888. doi:10.1086/650999.

Mutations in Extensively Drug Resistant *Mycobacterium tuberculosis* that do not Code for Known Drug-Resistance Mechanisms

Alifiya S. Motiwala[1], **Yang Dai**[1], **Edward C. Jones-López**[1], **Soo-Hee Hwang**[2], **Jong Seok Lee**[3], **Sang Nae Cho**[4], **Laura E. Via**[5], **Clifton E. Barry 3rd**[5], and **David Alland**[1]

[1]Division of Infectious Disease, Department of Medicine, and the Ruy V. Lourenço Center for the Study of Emerging and Reemerging Pathogens, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey [2]National Masan Tuberculosis Hospital, Masan, Republic of Korea [3]International Tuberculosis Research Center, Masan, Republic of Korea [4]Department of Microbiology, Yonsei University College of Medicine, Seoul, Republic of Korea [5]National Institutes of Health, Bethesda, Maryland

Abstract

Highly-lethal outbreaks of multi drug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis are increasing. Whole-genome sequencing of KwaZulu-Natal MDR and XDR outbreak strains prevalent in HIV patients by the Broad Institute identified 22 novel mutations which were unique to the XDR genome or shared only by the MDR and XDR genomes and not already known to be associated with drug-resistance. We studied the 12 novel mutations which were not located in highly-repetitive genes to identify mutations that were truly associated with drug-resistance or likely to confer a specific fitness advantage. None of these mutations could be found in a phylogenetically and geographically diverse set of drug–resistant and susceptible *M. tuberculosis* isolates, suggesting that these mutations are unique to the KZN clone. Examination of the 600 bp region flanking each mutation revealed 26 new mutations. We searched for a convergent evolutionary signal in the new mutations for evidence that they emerged under selective pressure, consistent with increased fitness. However, all but one rare mutation were monophyletic, indicating that the mutations were markers of strain-phylogeny rather than fitness or drug-resistance. Our results suggest that virulent XDR tuberculosis in immunocompromised HIV patients can evolve without generalizable fitness changes or other XDR-specific mutations.

Keywords

XDR tuberculosis evolution

Introduction

Multi drug-resistant (MDR) and extensively drug resistant (XDR) *Mycobacterium tuberculosis* is an expanding problem in many countries [1]. The high mortality rates associated

Corresponding Author: David Alland, MD, Professor of Medicine and Chief, Division of Infectious Disease, Assistant Dean for Clinical Research, New Jersey Medical School – UMDNJ, 185 South Orange Avenue, MSB A920C, Newark, NJ 07103, Tel: (973) 972-2179, Fax: (973) 972-0713, allandda@umdnj.edu.

All authors declare that they do not have a commercial or other association that might pose a conflict of interest.

with some drug-resistant strains are particularly worrisome. The recently described XDR tuberculosis outbreak in Tugela Ferry KwaZulu-Natal, South Africa, had a mortality rate of 98% among patients infected with the human immunodeficiency virus (HIV) [2]. It is not known whether epidemic drug-resistant strains have evolved special mechanisms that facilitate drug-resistance acquisition, maintain fitness or promote person-to-person transmission. Conventional wisdom has held that *M. tuberculosis* acquires MDR and XDR through a stepwise accumulation of chromosomal mutations that each confers resistance to individual drugs [3]. Single-step MDR mutations have not been conclusively identified. Resistance mutations have been shown to be associated with a fitness "cost" in many bacteria including *M. tuberculosis*, where the cost appears to be dependent on specific mutation and strain type [4]. Several in vitro studies have suggested that clinical *M. tuberculosis* has found ways to compensate for the fitness cost of antibiotic resistance [5]. However, except for the relatively rare occurrence of *ahpC* promoter up mutations, no molecular mechanisms that maintain fitness in drug-resistant clinical *M. tuberculosis* have been identified.

Comparative whole genome sequencing studies have the potential to identify biologically significant mutations in drug-resistant *M. tuberculosis* strains by providing an unbiased scan of the total genomic changes that accompany resistance acquisition. The Broad Institute has recently released the complete genomic sequences of a Tugela Ferry XDR outbreak isolate and two related MDR and drug susceptible (DS) isolates [6,7]. Comparing the three Tugela Ferry genomes to a representative DS isolate (strain F11) from the same geographic region revealed 15 mutations that were unique to the Tugela Ferry XDR isolate (XDR-specific mutations), and 18 mutations that were shared by both the Tugela Ferry XDR and MDR isolates but were not present in the DS isolate (XDR/MDR-specific mutations). As expected, many of these mutations mapped to known drug-resistance targets and/or had previously known associations with drug resistance. However, 22 mutations were completely novel and did not occur in genes that had been described previously in association with drug resistance. It is tempting to assume that these novel mutations represent unidentified drug resistance mutations, new mutations relevant to XDR evolution or fitness adaptations.

Mutations that are highly selected during MDR and XDR evolution should not be restricted to the Tugela Ferry XDR strain or its related MDR isolate. Rather, these biologically important mutations should occur with detectible frequencies in unrelated MDR and XDR *M. tuberculosis* strains from around the world. This hypothesis is supported by the observation that the common mutations that are known to cause resistance to the first line anti-tuberculosis drugs isoniazid, ethambutol [8], and rifampin (data not shown) can be found in different and unrelated populations, reflecting convergent evolution.

Methods

Identification of mutations specific to the extensively drug resistant (XDR) or both the XDR and multi drug resistant (MDR) isolates sequenced from Tugela Ferry KwaZulu-Natal, South Africa

Polymorphisms unique to the XDR strain or shared by the XDR and MDR strains were obtained from the publicly available comparative analysis of the XDR (KZN 605), MDR (KZN 1435), and drug-sensitive (DS) (KZN 4207) strains published by the Microbial Sequencing Center at the Broad Institute [7].

Selection of clinical *M. tuberculosis* isolates for the study

A total of 35 drug-resistant and 34 pan-susceptible isolates were selected from a collection of clinical *M. tuberculosis* isolates obtained from reference laboratories or major medical centers in Afghanistan (*n*=1), Australia (*n*=1), China (*n*=1), Colombia (*n*=14), India (*n*=6), Korea

(*n*=1), Mexico (*n*=10), New York City (*n*=6), Philippines (*n*=1), Somalia (*n*=1), Spain (*n*=3), Sudan (*n*=1), Texas (*n*=22), and Vietnam (*n*=1) [9,10]. Effort was made to include multi-drug resistant isolates where possible. Each of the isolates was assigned to one of 10 SNP cluster group/sub-group using nine SNP markers developed for this purpose [11] and then mapped onto the previously described phylogenetic tree [12] (Fig. 2). Additional six extensively drug-resistant isolates were obtained Mulago Hospital, Uganda (*n*=1), and National Masan Tuberculosis Hospital, Korea (*n*=5). All isolates were subjected to susceptibility testing as described previously [10].

Detection of mutations in *M. tuberculosis* study isolates

Primers were designed to amplify an approximately 600bp region flanking the mutations of interest per the *Mycobacterium tuberculosis* H37Rv genome sequence (GenBank Accession NC_000962). Additionally, the 1357bp Rv3616c-Rv3617 intergenic region was sequenced in all isolates. The entire length of each gene identified by the Tugela Ferry MDR-XDR mutations was sequenced in the six XDR isolates. PCR amplification was performed using Hotstar *Taq* polymerase (Qiagen Inc.). The PCR products were sequenced using standard dye terminator chemistry and analyzed on an automated DNA sequencer (3700 DNA Analyzer, Applied Biosystems). All mutations were confirmed by sequencing the reverse strand, except when they were abundant, in which case a subset of isolates were retested for each mutation.

Results

We tested for mutations that were shared by the Tugela Ferry strain and unrelated *M. tuberculosis* strains by looking for the Tugela Ferry XDR-specific and XDR/MDR-specific mutations in a diverse set of *M. tuberculosis* MDR and XDR isolates as well as DS controls. Tugela Ferry mutations that were found to be present in other sequenced DS *M. tuberculosis* genomes and mutations that were already known to confer resistance to individual antibiotics were excluded, as our interest was to discover new resistance-associated mutations. We also excluded six mutations in the PPE/PE-PGRS gene family and the other repetitive genes, because these genes are known to be hyper-variable even in DS *M. tuberculosis* and they would be unlikely to have a relationship to drug resistance [13]. Thus, our final study focused on five synonymous, five non-synonymous and two non-coding mutations in seven genes/regions with known functions and five hypothetical genes/regions (Fig. 1).

To ensure that mutations were studied in a highly diverse set of *M. tuberculosis* strains, we selected five or six drug-resistant and five DS *M. tuberculosis* isolates from each of the four major *M. tuberculosis* phylogenetic groups, and two or three drug-resistant and two or three DS isolates from each of the five phylogenetic sub-groups as described previously [12] (Fig. 2). Thirty-one of the 35 drug-resistant isolates were MDR, three were resistant to at least two drugs and one was mono-resistant (Table S1). Six XDR isolates from phylogenetic SNP cluster group (SCG) 2 and SCG 3a isolated from two different geographic locations (Korea and Uganda) were also analyzed giving a total of 41 drug-resistant and 34 drug susceptible strains.

We did not find any of the 12 Tugela Ferry XDR-specific or XDR/MDR-specific mutations in any of the 41 drug-resistant isolates in our study (Table 1). The complete absence of any of these mutations in our widely representative sample of drug-resistant isolates strongly suggests that these mutations do not have broad biological relevance in the evolution of XDR tuberculosis. We also considered the possibility that the mutations observed in the Tugela Ferry XDR strain might identify new gene regions rather than specific new mutations that play important roles in drug-resistance. To this end, we sequenced approximately 600 bp of DNA flanking each of the 12 mutations in the 41 drug-resistant isolates. We also sequenced the entire 1357bp Rv3616c-Rv3617 intergenic region. The DNA sequencing revealed 26 new mutations in five genes or intergenic regions (Rv0103c, Rv2000, intergenic-Rv3616c-Rv3617, Rv3806c

and Rv3921c) (Table 2). Nine of these mutations were present in more than one drug-resistant isolate, while 13 mutations were identified in only one drug-resistant isolate. The same regions were then sequenced in the 34 phylogenetically-matched drug-susceptible *M. tuberculosis* isolates (Table 2). We found that eight of the nine mutations previously identified in more than one drug-resistant isolate were also present in at least one drug-susceptible isolate, strongly suggesting that these mutations were not associated with drug resistance. Thus, only the −992 G to T mutation in the Rv3616c-Rv3617 intergenic region was present in more than one drug-resistant isolate but not present in any drug-susceptible isolate; however this mutation was present at a 2/40 (5%) frequency and occurred in isolates from the same SCG as described below. Three new mutations which were only present in the drug susceptible isolates were also identified. Of these, one occurred in more than one drug-susceptible isolate.

Commonly occurring drug-resistance associated mutations have a distinct phylogenetic distribution, suggestive of convergent evolution, compared to neutral mutations in *M. tuberculosis*. Common resistance-associated mutations can be shown to arise independently multiple times in broadly representative tuberculosis populations and cannot be traced back to a single common ancestor in a phylogenetic analysis. Mutations that are not strongly selected will be monophyletic and appear to have arisen only once in this population [8]. We performed a phylogenetic analysis of each of the 11 mutations which were found in more than one *M. tuberculosis* isolate, examining their distribution within the context of the entire 75 isolate study set (Fig. 3a to 3d). Each mutation either mapped to a single phylogenetic branch or mapped to adjoining branches. This included the −992 G to T mutation in the Rv3616c-Rv3617 intergenic region which was confined to SCG 1 (Fig. 3b). With one exception, when a mutation was present on adjoining branches, at least one of the branches was 100% mutant suggesting a single mutational event in a common ancestor. These results provide strong evidence that the new mutations discovered in the drug resistant isolates are associated with strain phylogeny rather than drug resistance. The lone exception occurred with the −706 T to C mutation in the Rv3616c - Rv3617 intergenic region. This mutation, which was present in both MDR and DS isolates was found in 50% of SCG 1 isolates as well as one isolate on the contiguous SCG 3a branch. The absence of a complete monophyletic distribution leaves open the possibility that this mutation has multiple ancestries and developed under selective pressure. However, the lack of any association to drug resistance, its relative paucity in the study set and its complete absence on most of the phylogeny suggest that this is not an important fitness mutation.

Eleven of the 33 Tugela Ferry MDR-XDR and XDR mutations had been previously associated with drug resistance. Two of these mutations at *katG*315 and *inhA* −15 which are known to cause resistance to the antituberculosis drug isoniazid were examined in our 75 drug-resistant and DS strain set. These known resistance-associated mutations were well represented among the drug-resistant isolates, but were not present in any of the DS isolates. The phylogenetic distribution of the *katG*315 and *inhA* −15 mutations also contrasted markedly with the distribution of the mutations that were newly identified in this study (Fig. 3e and 3f). Both the *katG* and the *inhA* mutations were distributed on multiple phylogenetic branches, consistent with previous studies [8]. The marked contrast in the distribution of established drug resistance mutations compared to the mutations newly discovered here provides further evidence that the new mutations are not associated with drug resistance or a significant fitness advantage.

We also sequenced the entire length of each gene identified by the Tugela Ferry MDR-XDR mutations in our six XDR isolates. Five additional mutations in Rv0020c, Rv0663, Rv1145 and Rv2000 were identified. Four mutations were present in multiple isolates and one mutation was only found in a single isolate. We examined our DS isolates matched by phylogenetic group for each of these mutations. All four of the mutations present in more than one XDR isolate were also found in one or more DS isolates belonging to the same SCGs as the XDR isolates (Table 3).

One of the mutations which we observed most frequently was located at position −41 (G to T) of the Rv3616c - Rv3617 intergenic region. This mutation was of interest even though it was present in both susceptible and resistant isolates because the Rv3612c-Rv3616c operon appears to be required for the secretion of ESAT-6 and CFP-10 which are vital to *M. tuberculosis* virulence [14], because the mutation was present in all of the highly virulent SCG2-Beijing clade isolates, and because the −41 mutation was in close proximity to the −55 mutation described in the Tulega Ferrry XDR isolate. These observations prompted us to investigate if this mutation had any effect on ESAT-6 secretion. However, we did not find any difference in the transcription of Rv3616c or the extra-cellular secretion of ESAT-6 using RT-PCR and western blot analysis, respectively in either −41 G to T mutant or wild type isolates (data not shown).

Discussion

This study represents the first comprehensive analysis, to our knowledge, of XDR and MDR-XDR mutations discovered by the Broad Institute KZN XDR sequencing project. It suggests that XDR tuberculosis can evolve in a virulent form without generalizable fitness changes or other XDR-specific mutations. The Broad Institute sequencing effort provided a unique opportunity to determine whether secondary mutations that predispose to MDR and/or compensate for the fitness costs of resistance-conferring mutations develop in tandem with XDR evolution. We were unable to identify any mutations that the Tugela Ferry XDR strain shared with unrelated drug-resistant *M. tuberculosis* isolates other than loci already known to be associated with drug-resistance. Thus, the Tugela Ferry genome sequence does not appear to provide new generalizable insights into the evolution of XDR tuberculosis. Furthermore, our examination of the gene and gene region surrounding the MDR-XDR and XDR mutations did not reveal any additional candidates for secondary resistance or fitness mutations. However, it was not possible to completely rule out either of these functions for mutations which were only detected one time in the study set.

It remains possible that the Tugela Ferry strain developed an unusual set of adaptations to XDR that were not present in any of the other drug-resistant strains tested. However, both heightened transmission and high mortality have been repeatedly described when immunocompromised patients are exposed to drug-resistant *M. tuberculosis*. Therefore, it is more likely that the Tugela Ferry strain does not contain unique genetic adaptations to XDR. Rather, the widespread distribution and high mortality of this strain can probably be attributed to the health status of the host and possibly, the quality and availability of medical treatment. Our results do not completely rule out the possibility that drug-resistant *M. tuberculosis* develops mutations that support MDR/XDR acquisition and maintain fitness. Selection for mutations of this type may be dictated by treatment and/or host factors that were not widespread in the Tugela Ferry XDR outbreak. For example, fitness mutations may not be required to maintain full virulence in immunocompromised hosts. Furthermore, it is possible that certain mutations in known resistance-associated genes can provide drug-resistance without attenuation (such as *katG*315 mutations [15]) or can predispose to resistance developing across drug classes (such as mutations in *embB*306 [9,16]). Both of these mutations were found in the Tugela Ferry genome, and these already established mutations rather than the new mutations of unknown function may fully explain the ability of this strain to be transmitted and cause disease. It will require further genome comparisons of additional XDR isolates, particularly from HIV negative patients to resolve these questions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was partially supported by NIH grant AI065663. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. LoBue P. Extensively drug-resistant tuberculosis. Curr Opin Infect Dis 2009;22:167–173. [PubMed: 19283912]
2. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 2006;368:1575–1580. [PubMed: 17084757]
3. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber Lung Dis 1998;79:3–29. [PubMed: 10645439]
4. Gagneux S. Fitness cost of drug resistance in Mycobacterium tuberculosis. Clin Microbiol Infect 2009;5:66–68. [PubMed: 19220360]
5. Gagneux S, Long CD, Small PM, Van T, Schoolnik GK, Bohannan BJ. The competitive cost of antibiotic resistance in Mycobacterium tuberculosis. Science 2006;312:1944–1946. [PubMed: 16809538]
6. Koenig R. Tuberculosis. Few mutations divide some drug-resistant TB strains. Science 2007;318:901–902. [PubMed: 17991835]
7. Broad Institute TB Website. www.broad.mit.edu/annotation/genome/mycobacterium_tuberculosis_spp/ToolsIndex.html
8. Hazbon MH, Motiwala AS, Cavatore M, Brimacombe M, Whittam TS, Alland D. Convergent evolutionary analysis identifies significant mutations in drug resistance targets of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2008;52:3369–3376. [PubMed: 18591265]
9. Hazbon MH, Bobadilla del Valle M, Guerrero MI, et al. Role of embB codon 306 mutations in *Mycobacterium tuberculosis* revisited: a novel association with broad drug resistance and IS6110 clustering rather than ethambutol resistance. Antimicrob Agents Chemother 2005;49:3794–3802. [PubMed: 16127055]
10. Hazbon MH, Brimacombe M, Bobadilla del Valle M, et al. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother 2006;50:2640–2649. [PubMed: 16870753]
11. Alland D, Lacher DW, Hazbon MH, et al. 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 2007;45:39–46. [PubMed: 17079498]
12. Filliol I, Motiwala AS, Cavatore M, et al. 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 2006;188:759–772. [PubMed: 16385065]
13. Cole ST. Comparative and functional genomics of the *Mycobacterium tuberculosis* complex. Microbiology 2002;148:2919–2928. [PubMed: 12368425]
14. Raghavan S, Manzanillo P, Chan K, Dovey C, Cox JS. Secreted transcription factor controls *Mycobacterium tuberculosis* virulence. Nature 2008;454:717–721. [PubMed: 18685700]
15. Pym AS, Saint-Joanis B, Cole ST. Effect of katG mutations on the virulence of *Mycobacterium tuberculosis* and the implication for transmission in humans. Infect Immun 2002;70:4955–4960. [PubMed: 12183541]
16. Safi H, Sayers B, Hazbon MH, Alland D. Transfer of embB codon 306 mutations into clinical *Mycobacterium tuberculosis* strains alters susceptibility to ethambutol, isoniazid, and rifampin. Antimicrob Agents Chemother 2008;52:2027–2034. [PubMed: 18378710]

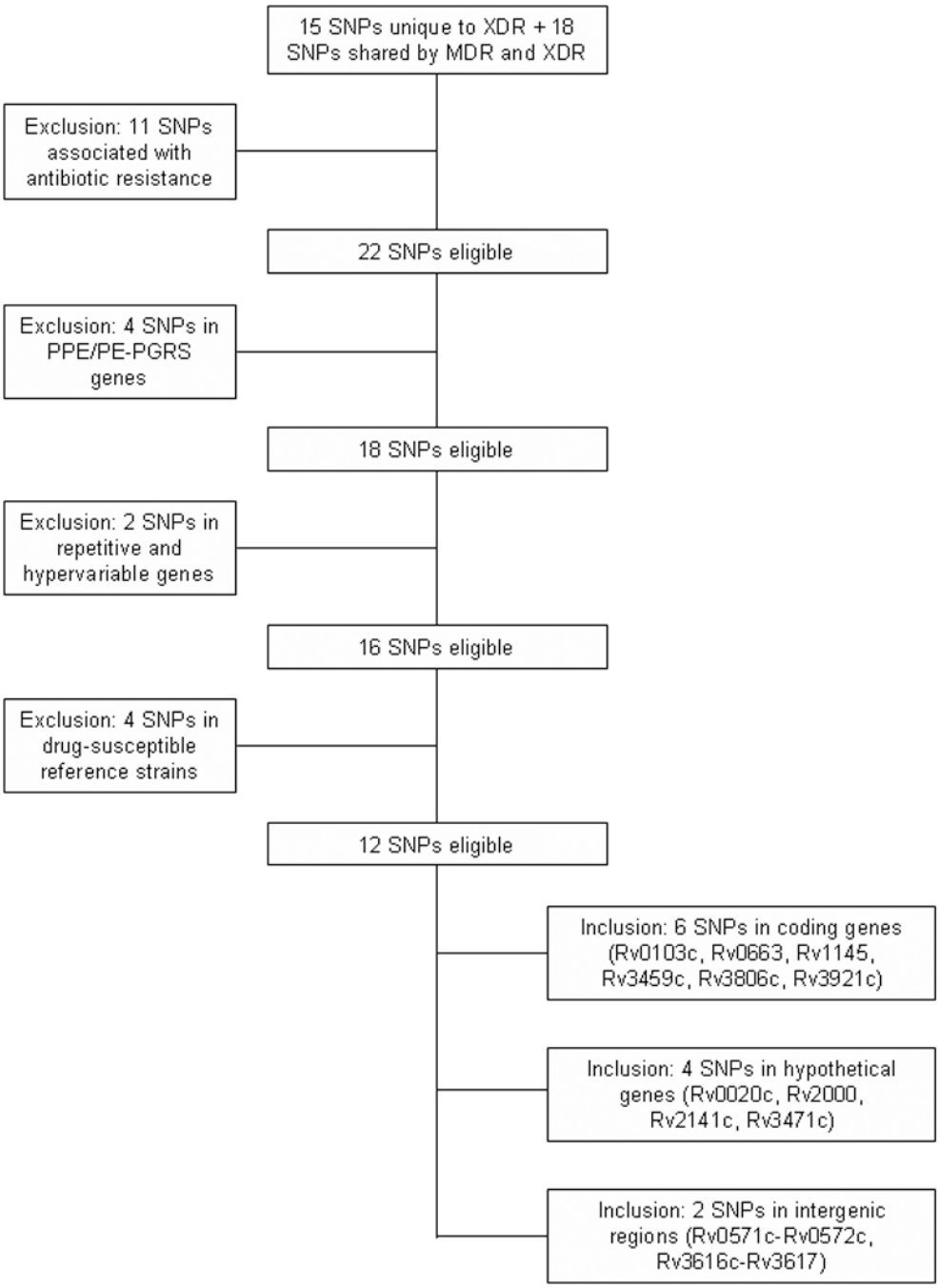

Figure 1. Flow chart for selection of genes included in the study

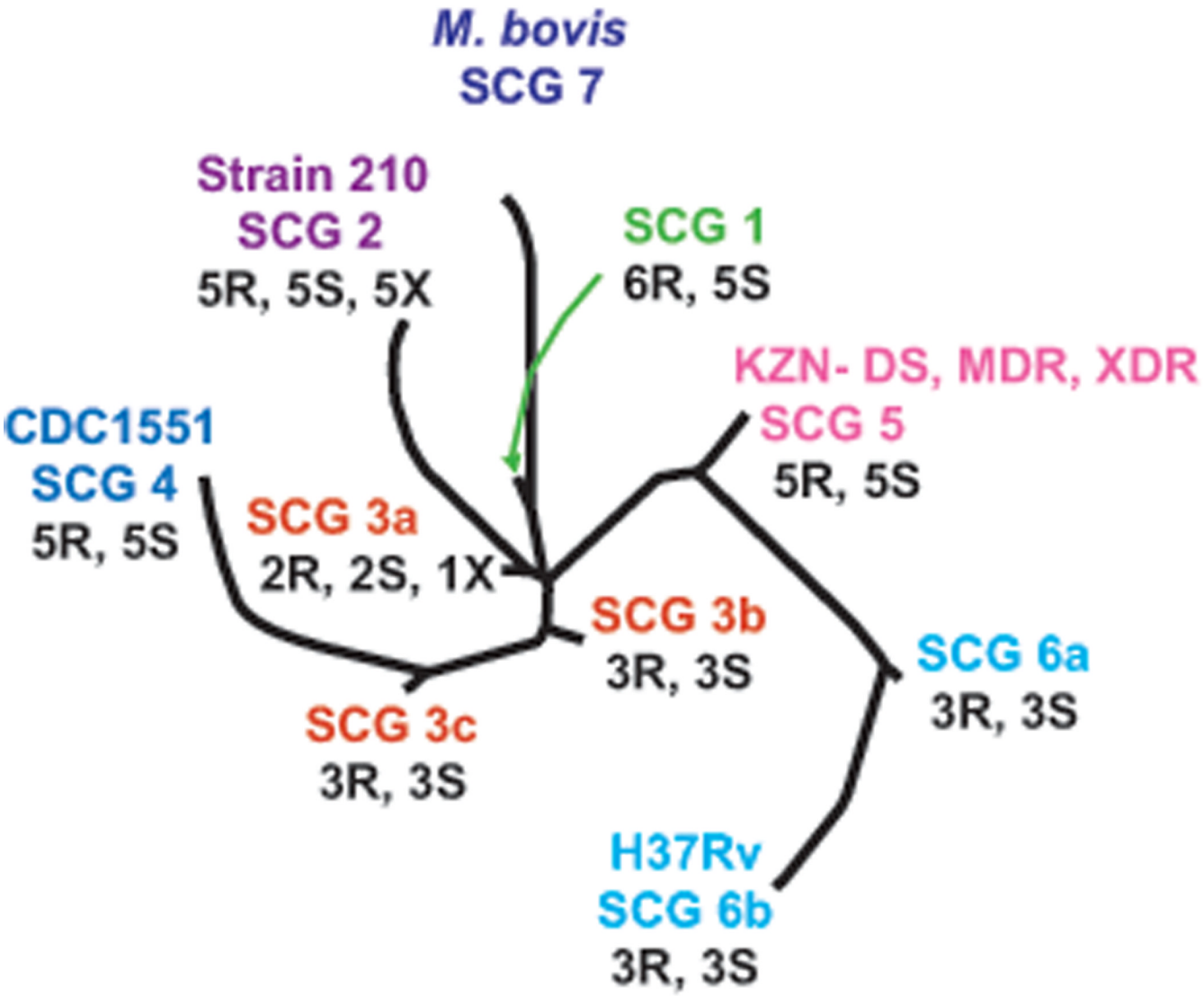

Figure 2. Phylogenetic locations of the study isolates

Each branch shows the location of SNP cluster groups (SCGs), including subgroups as defined by an analysis of 212 SNP markers on 324 *M. tuberculosis* isolates [12]. Study isolates were placed on the phylogenetic tree using 9 SNP markers identified for this purpose [11]. The positions of the *M. tuberculosis* reference strains 210, CDC1551, and H37Rv and of *M. bovis* strain AF2122/97 are indicated. Positions of the drug-susceptible (DS), multi-drug resistant (MDR) and extensively-drug resistant (XDR) are also indicated. The number of drug-resistant including MDR (R), pan-susceptible (S) and extensively drug-resistant (X) isolates from each SCG are indicated.

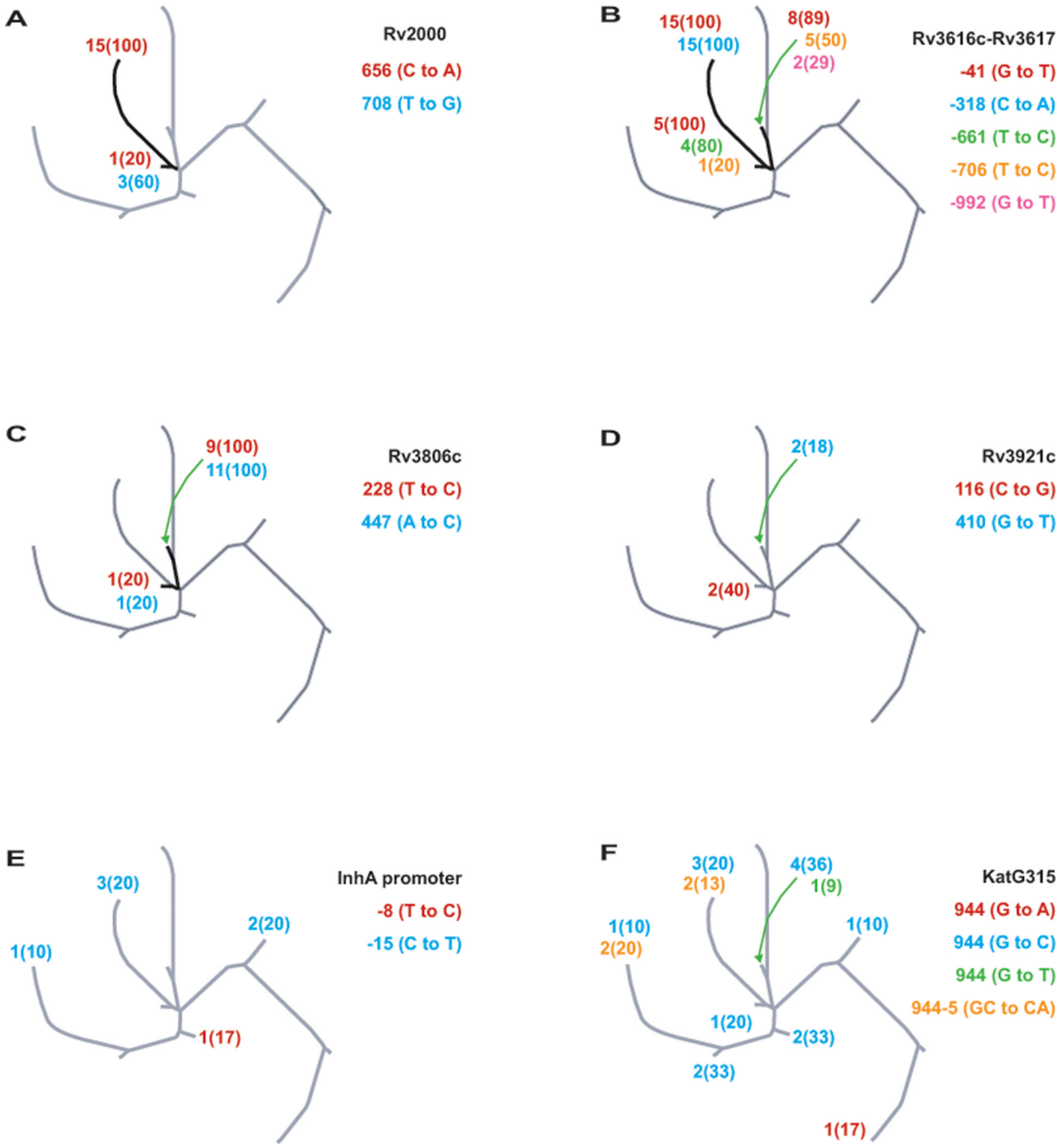

Figure 3.

Panels A–D Phylogenetic distribution of the new mutations identified in the drug-resistant and susceptible isolates. The number of isolates located on each branch of the phylogenetic tree shown in Fig. 2 (and the % of isolates on that branch which contain that mutation) is shown according to the indicated color code. Relevant linked SNP cluster groups are highlighted in dark color. Nucleotide positions indicated are relative to the start site of respective genes. **Panels E and F: Phylogenetic distribution of the inhA promoter and katG315 mutations.** The locations of each of the 75 study isolates on the phylogenetic tree shown in Fig. 2 that contained either the *katG*315 or the *inhA* promoter region SNPs are shown

according to the indicated color code. Nucleotide positions indicated are relative to the start site of respective genes.

Table 1

Frequency of the Tugela Ferry-strain XDR and XDR-MDR specific mutations in a diverse sample of 41 drug-resistant *M. tuberculosis* isolates.

H37Rv gene	H37Rv location	Mutation	#	Mutation type
Rv0020c	24125	C/T	0	S (L440L)
Rv0103c	122107	G/A	0	NS (G23S)
Rv0663	756757	C/T	0	S (Y207Y)
Rv1145	1272321	C/A	0	NS (L25I)
Rv2000	2246032	T/C	0	NS (L275P)
Rv2141c	2401402	C/A	0	S (G109G)
Rv3459c	3879441	C/G	0	S (A84A)
Rv3471c	3889150	C/A	0	NS (D64E)
Rv3806c	4269271	T/C	0	NS (V188A)
Rv3921c	4409995	C/T	0	S (Y25Y)
Rv0571c and Rv0572c^{Int}	664929	C/A	0	Intergenic
Rv3616c and Rv3617^{Int}	4056430	T/C	0	Intergenic

#: Number of mutants

Int: Intergenic

NS: Non-synonymous

S: Synonymous

Table 2

Mutations identified in study isolates by sequencing approximately 600 bp flanking the Tugela Ferry-strain XDR and XDR-MDR mutation loci.

H37Rv gene[a]	Mutation	Resistant*			Susceptible			Mutation type
		n	#	%	*n*	#	%	
Rv0103c	−4, G to T	41	1	2	33	0	-	Intergenic
	−42, T to C	41	1	2	33	0	-	Intergenic
	−119, G to A	41	1	2	33	0	-	Intergenic
Rv2000	656, C to A	41	10	24	34	6	18	NS (P219Q)
	708, T to G	41	2	5	34	1	3	S (A236A)
Rv3616c-Rv3617[Int]	−41, G to T[b]	40	18	45	33	10	30	Intergenic
	−106, del A[b]	40	1	3	33	0	-	Intergenic
	−260, A to G[b]	40	1	3	33	0	-	Intergenic
	−318, C to A[b]	40	10	25	33	5	15	Intergenic
	−661, T to C[b]	41	2	5	32	2	6	Intergenic
	−706, T to C[b]	41	4	10	32	2	6	Intergenic
	−836, G to A[b]	41	1	2	32	0	-	Intergenic
	−847, C to T[b]	41	0	0	32	1	3	Intergenic
	−992, G to T[b]	40	2	5	30	0	-	Intergenic
Rv3806c	228, T to C	41	7	17	32	3	9	S (R76R)
	447, A to C	41	7	17	34	5	15	NS (E149D)
	517, T to G	41	1	2	34	0	-	NS (S173P)
	521, A to C	41	1	2	34	0	-	NS (K174T)
	536, T to G	41	1	2	34	0	-	NS (I179S)
	713, T to C	41	1	2	34	0	-	NS (F238S)
	864, G to A	41	1	2	34	0	-	S (A288A)

H37Rv gene[a]	Mutation	Resistant*			Susceptible			Mutation type
		n	#	%	*n*	#	%	
Rv3921c	116, C to G	41	0	0	33	2	6	NS (A39G)
	289, C to T	41	1	2	33	0	-	S (L97L)
	336, G to C	41	1	2	33	0	-	NS (M112I)
	410, G to T	41	1	2	33	1	3	NS (G137V)
	468, G to A	41	0	0	33	1	3	S (P156P)
Total no. of isolates		**41**			**34**			

*Includes 6 XDR isolates

n: Number of isolates sequenced

#: Number of mutants

Int: Intergenic region

NS: Non−synonymous

S: Synonymous

[a]No mutations found in the 600bp flanking region of Rv0020c, Rv0571c–Rv0572c[Int], Rv0663, Rv1145, Rv2141c, Rv3459c, Rv3471c

[b]Position is given in reference to the translational start site of Rv3616c

Table 3

Additional mutations identified in XDR study isolates and their presence in matched DS controls.

H37Rv gene	Mutation	XDR			Susceptible			Mutation type
		n	#	%	*n*	#	%	
Rv0020c	707-24, 18bp del	6	6	100	6	6	100	6 amino acid deletion
Rv0663	1003, C to A	6	5	83	6	3	50	NS (R335S)
	1046, A to G	6	6	100	6	5	83	NS (D349G)
Rv1145	711, G to A	6	1	17	7	0	-	S (L237L)
	829, Ins A	6	6	100	7	6	86	Frame shift
Total no. of isolates		**6**			**8**			

XDR: Extensively drug resistant

n: Number of isolates sequenced

#: Number of mutants

NS: Non-synonymous

S: Synonymous