JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009, p. 223–226 Vol. 47, No. 1
0095-1137/09/$08.00+0 doi:10.1128/JCM.01237-08

Single-Nucleotide Polymorphisms in Rv2629 Are Specific for *Mycobacterium tuberculosis* Genotypes Beijing and Ghana but Not Associated with Rifampin Resistance▽†

Susanne Homolka,[1] Claudio Köser,[2] John Archer,[2] Sabine Rüsch-Gerdes,[1] and Stefan Niemann[1]*

Forschungszentrum Borstel, National Reference Center for Mycobacteria, Borstel, Germany,[1] and Department of Genetics, University of Cambridge, Cambridge CB2 3EH, United Kingdom[2]

Received 1 July 2008/Returned for modification 27 October 2008/Accepted 11 November 2008

Sequence analysis of 58 multidrug-resistant *Mycobacterium tuberculosis* complex strains from Germany and 55 susceptible strains from a reference collection comprising major phylogenetic lineages confirmed that variations in Rv2629, 191A/C and 965C/T, are specific for genotypes Beijing and Ghana, respectively, but not involved in the development of rifampin (rifampicin) resistance.

Pathogens of the *Mycobacterium tuberculosis* complex (MTBC) are still one of the leading causes of death from infectious diseases worldwide. One-third of the global population is infected with these pathogens, and there are an estimated 2 million deaths caused by tuberculosis (TB) annually (13). Additionally, the situation is aggravated by the emergence of drug-resistant MTBC strains. According to a recent report of the World Health Organization, it is estimated that there are 490,000 multidrug-resistant (MDR) TB cases (resistant against isoniazid and rifampin [rifampicin; RMP]) every year, causing more than 110,000 deaths (14).

These statistics clearly demonstrate the need to detect resistance of clinical isolates rapidly in order to ensure effective treatment and avoid further spread of resistant strains (8). The most advanced technologies rely on the detection of chromosomal mutations conferring the resistance phenotype by nucleic acid amplification methods based on PCR and reverse hybridization (6, 8). However, mechanisms of resistance to several drugs are not fully understood, and further investigations are urgent and indispensable.

The mode of action of the key first-line drug RMP and the molecular mechanism of resistance have been explored in detail in previous studies (4, 8, 15). Several investigations showed that mutations in the 81-bp hot spot region (codon 426 to 452) of the *rpoB* gene are solely responsible for resistance in at least 90% of all RMP-resistant strains (4, 8, 15).

Wang et al. recently described a potential new RMP resistance mechanism that is based on the 191A/C (D64A) mutation in the Rv2629 gene (12). Initially identifying this mutation by using comparative proteomics/genomics, the authors detected this mutation specifically in RMP-resistant strains (111 out of 112) but not the 30 RMP-susceptible strains investigated (12). Accordingly, overexpression of the variant protein in *M. smegmatis* led to a significant increase in the MIC of RMP. Thus, the Rv2629 191A/C mutation appears to be involved in the development of RMP resistance and could serve as an excellent marker for RMP resistance in clinical isolates.

However, a very recent study by Chakravorty and coworkers questioned the association of the Rv2629 191C allele with RMP resistance (2). In their study, the 191C allele was not correlated with RMP resistance but appeared to be a phylogenetic marker for Beijing genotype strains. The reasons for these contradicting findings are not fully appreciated, and further studies are urgently needed to investigate the role of genomic variations of Rv2629 in the development of RMP-resistant clinical MTBC isolates.

To address this question, we determined Rv2629 sequence variations in 58 MDR strains isolated in Germany in 2006 and in 55 strains from a reference collection representing the major MTBC species and phylogenetic lineages, such as *M. tuberculosis* Beijing or *M. africanum* West African 1 (1), as well as the type strains of *M. tuberculosis* (H37Rv; ATCC 27294), *M. bovis* (ATCC 19210), and *M. africanum* (ATCC 25420). In contrast to Chakravorty et al., who analyzed only position 191 by targeted real-time PCR, we extended our analyses to the entire 1,125-bp open reading frame of Rv2629 to identify additional polymorphisms in this potentially important gene. Also, all strains were analyzed for mutations in the 81-bp hot spot region of the *rpoB* gene. Strains were genotyped with IS*6110* DNA fingerprinting, spoligotyping, and 24-locus mycobacterial interspersed repetitive-unit–variable-number tandem-repeat typing as described in the supplemental material. All genetic data were correlated with the presence of phenotypic RMP resistance as determined by conventional susceptibility testing.

Among the 58 MDR strains investigated, 36 (62%) had the 191A/C mutation (codon 64, Asp to Ala) in Rv2629 (Fig. 1). At a glance, these data seem to support an association with RMP resistance. However, when the MDR strains were stratified in different phylogenetic lineages, it became evident that the 191C allele was exclusively present in Beijing genotype strains. This applies to Beijing genotype strains showing a classical Beijing IS*6110* restriction fragment length polymorphism pattern (see the upper part of Fig. 1) as well as for two strains with

* Corresponding author. Mailing address: Molecular Mycobacteriology Group, National Reference Center for Mycobacteria, Research Center Borstel, Parkallee 1, Borstel, Germany. Phone: 49-4537188762. Fax: 49-4537188311. E-mail: sniemann@fz-borstel.de.

† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 19 November 2008.

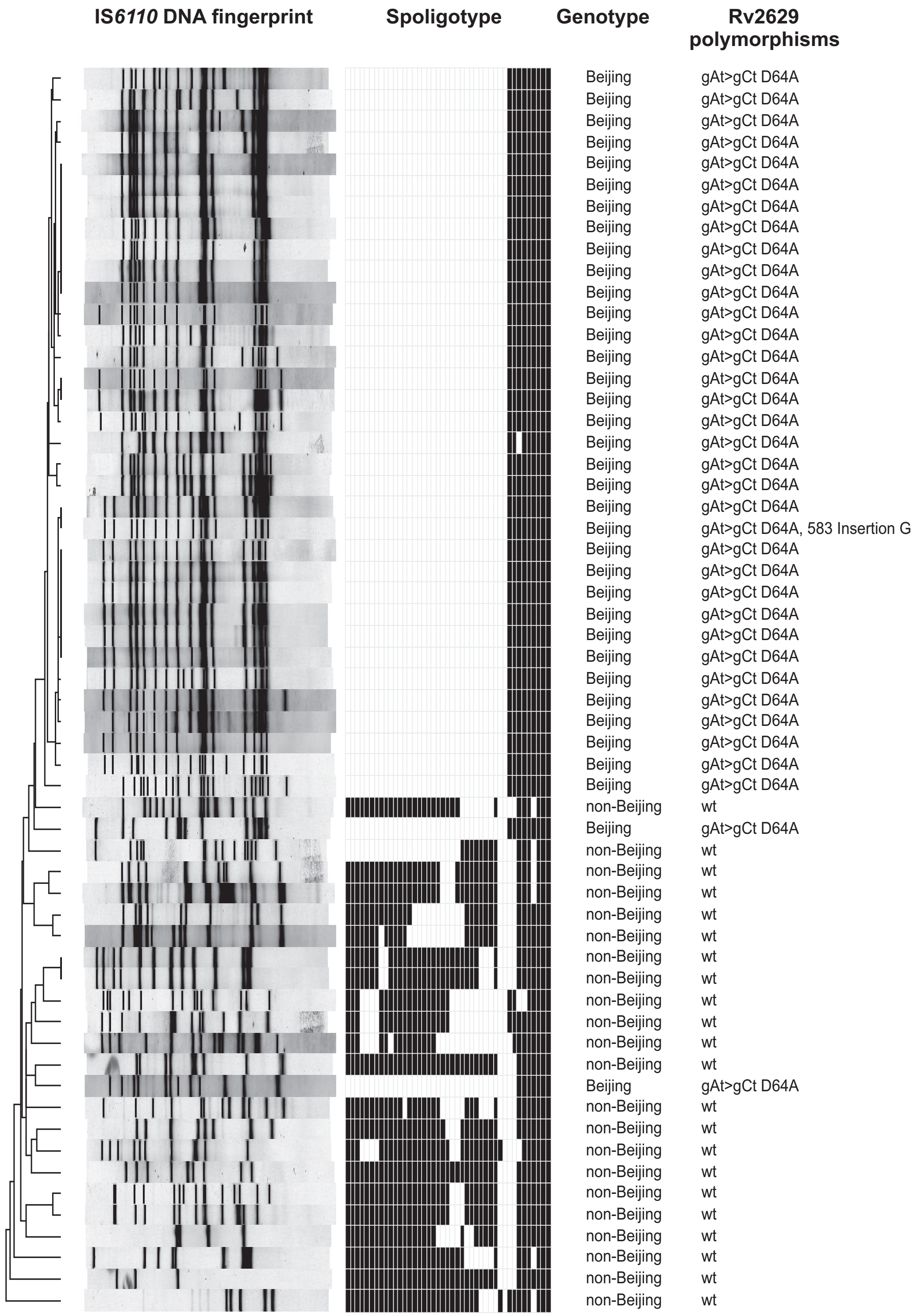
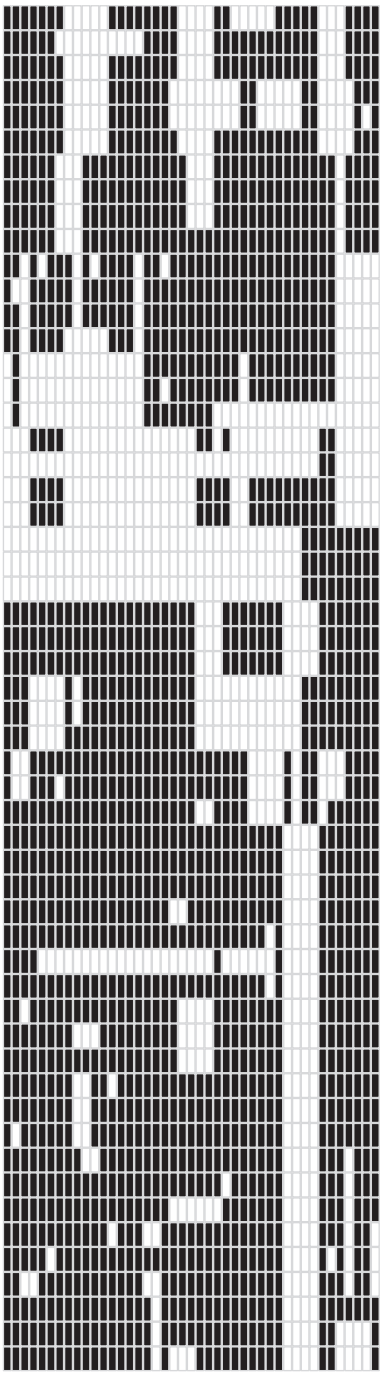


FIG. 1. IS*6110* DNA fingerprint and spoligotype patterns as well as Rv2629 polymorphisms of the 58 MDR strains investigated. The position of each IS*6110* band is normalized so that banding patterns of all strains are mutually comparable. The strains/genotypes are ordered in a dendrogram based on the similarity of their IS*6110* DNA fingerprint patterns. wt, wild-type sequence of full-length gene.

distinct IS*6110* restriction fragment length polymorphism profiles, however, being confirmed as the Beijing genotype by a highly specific Beijing/non-Beijing genotype real-time PCR (5). Thus, our data further support the hypothesis that this single-nucleotide polymorphism is specific for Beijing strains and not involved in RMP resistance (2). This notion is additionally confirmed by the fact that the RMP MIC was not enhanced for three fully susceptible Beijing genotype strains



Spoligotype	Species	Genotype	Rv2629 polymorphisms
	M. africanum	West African 1	wt
	M. africanum	West African 1	wt
	M. africanum	West African 1	wt
	M. africanum	West African 1	wt
	M. africanum	West African 1	wt
	M. africanum	West African 1	wt
	M. africanum	West African 2	wt
	M. africanum	West African 2	wt
	M. africanum	West African 2	wt
	M. africanum	West African 2 (ATCC)	wt
	M. bovis	Bovis (ATCC)	wt
	M. bovis	Bovis	wt
	M. bovis	Bovis	wt
	M. bovis	Bovis	wt
	M. caprae	Caprae	wt
	M. caprae	Caprae	wt
	M. caprae	Caprae	wt
	M. microti	llama	wt
	M. microti	vole	wt
	M. pinnepedii	Seal	wt
	M. pinnepedii	Seal	wt
	M. tuberculosis	Beijing	gAt>gCt D64A
	M. tuberculosis	Beijing	gAt>gCt D64A
	M. tuberculosis	Beijing	gAt>gCt D64A
	M. tuberculosis	Cameroon	wt
	M. tuberculosis	Cameroon	wt
	M. tuberculosis	Cameroon	wt
	M. tuberculosis	Dehli/CAS	wt
	M. tuberculosis	Dehli/CAS	wt
	M. tuberculosis	Dehli/CAS	wt
	M. tuberculosis	EAI	wt
	M. tuberculosis	EAI	wt
	M. tuberculosis	EAI	wt
	M. tuberculosis	Ghana	cCg>cTg P322L
	M. tuberculosis	Ghana	cCg>cTg P322L
	M. tuberculosis	Ghana	cCg>cTg P322L
	M. tuberculosis	H37Rv (ATCC)	wt
	M. tuberculosis	Haarlem	wt
	M. tuberculosis	Haarlem	wt
	M. tuberculosis	Haarlem	wt
	M. tuberculosis	LAM	wt
	M. tuberculosis	LAM	wt
	M. tuberculosis	LAM	wt
	M. tuberculosis	S-type	wt
	M. tuberculosis	S-type	wt
	M. tuberculosis	S-type	wt
	M. tuberculosis	Uganda	wt
	M. tuberculosis	Uganda	wt
	M. tuberculosis	Uganda	wt
	M. tuberculosis	Uganda	caC>caT Codon 126 silent
	M. tuberculosis	Uganda	wt
	M. tuberculosis	Uganda	wt
	M. tuberculosis	X-type	wt
	M. tuberculosis	X-type	wt
	M. tuberculosis	X-type	wt

FIG. 2. Spoligotype patterns and Rv2629 polymorphisms of 55 strains of an MTBC reference collection. The strains are ordered according to species and genotypes according to the MIRU-VNTR*plus* database (www.miru-vntrplus.org). wt, wild type; EAI, *M. tuberculosis* East African Indian; LAM, *M. tuberculosis* Latin American Mediterranean.

carrying the mutation compared with reference strain H37Rv (data not shown).

Likewise, 53 of the 58 MDR strains (91%) showed a polymorphism in the 81-bp hot spot region of *rpoB*, most frequently in codon 450 (43 of all MDR strains; 74%). Further mutations were detected in codon 445 ($n = 5$; 9%) and in codon 435 ($n = 3$; 5%). One strain showed a variation in codon 432, and another isolate showed variations in codons 429 and 445. These data indicate that, at least in our study collection of MDR strains from Germany, RMP resistance is most likely

based on mutations in the 81-bp hot spot region of the *rpoB* gene and not influenced by mutations in Rv2629.

To investigate whether mutations in Rv2629 are specific for particular phylogenetic lineages, we compared the full-length Rv2629 sequences of a reference collection of 55 RMP-susceptible strains comprising the major phylogenetic lineages of the MTBC (Fig. 2). Again, the 191A/C substitution was found exclusively in Beijing genotype strains (Fig. 2). Interestingly, our sequence analyses revealed a further nonsynonymous substitution (CCG to CTG in codon 322) that was specific for strains of the Ghana genotype (1). None of the strains showed a variation in the 81-bp *rpoB* hot spot region, consistent with susceptibility to RMP (data not shown).

In conclusion, our data confirm that the 191A/C substitution in the Rv2629 gene is unlikely to be directly associated with the development of RMP resistance in clinical isolates. It proves to be a valid phylogenetic marker that can be used to distinguish between Beijing and non-Beijing genotype strains. Furthermore, our data revealed the presence of a second single-nucleotide polymorphism at codon 322 that appears to be specific for strains of the Ghana genotype (1). Although not directly involved in RMP resistance, the functional consequence of nonsynonymous Rv2629 sequence polymorphism in Beijing genotype strains that were reported to be hypervirulent (3, 9) and are frequently found to be associated with high rates of drug resistance (3) warrants further investigations. It is interesting to note that Rv2629 is a member of the *dosR* dormancy regulon (7) and was found to be upregulated under dormancy conditions (11). Recently, the *dosR* regulon was described to be constantly upregulated in Beijing genotype strains (10), a phenotype that might confer a selective advantage under microaerophilic and anaerobic conditions occurring during the infection process. The function of Rv2629 within this framework, however, is not clear and needs further investigations that are actually ongoing in our laboratory.

We thank I. Radzio, T. Ubben and P. Vock for excellent technical assistance. We are grateful to Kyle Rhode for critical review of the manuscript. This work was supported by the Germany Ministry of Health and the German Federal Ministry for Education and Research (BMBF) within the PathoGenomikPlus Network. C.K. is a recipient of a Gates Cambridge Scholarship and received additional funding from the Cambridge European Trust and Clare Hall, Cambridge, United Kingdom. J.A. gratefully acknowledges the support of the Cambridge-MIT Institute.

REFERENCES

1. **Allix-Béguec, C., D. Harmsen, T. Weniger, P. Supply, and S. Niemann.** 2008. Evaluation and strategy for use of MIRU-VNTR*plus*, a multifunctional database for online analysis of genotyping data and phylogenetic identification of *Mycobacterium tuberculosis* complex isolates. J. Clin. Microbiol. **46:** 2692–2699.
2. **Chakravorty, S., B. Aladegbami, A. S. Motiwala, Y. Dai, H. Safi, M. Brimacombe, D. Helb, and D. Alland.** 2008. Rifampin resistance, Beijing-W clade-single nucleotide polymorphism cluster group 2 phylogeny, and the Rv2629 191-C allele in *Mycobacterium tuberculosis* strains. J. Clin. Microbiol. **46:** 2555–2560.
3. **Glynn, J. R., J. Whiteley, P. J. Bifani, K. Kremer, and D. van Soolingen.** 2002. Worldwide occurrence of Beijing/W strains of Mycobacterium tuberculosis: a systematic review. Emerg. Infect. Dis. **8:**843–849.
4. **Heep, M., B. Brandstatter, U. Rieger, N. Lehn, E. Richter, S. Rusch-Gerdes, and S. Niemann.** 2001. Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. J. Clin. Microbiol. **39:**107–110.
5. **Hillemann, D., R. Warren, T. Kubica, S. Rusch-Gerdes, and S. Niemann.** 2006. Rapid detection of *Mycobacterium tuberculosis* Beijing genotype strains by real-time PCR. J. Clin. Microbiol. **44:**302–306.
6. **Hillemann, D., M. Weizenegger, T. Kubica, E. Richter, and S. Niemann.** 2005. Use of the genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* complex isolates. J. Clin. Microbiol. **43:**3699–3703.
7. **Park, H. D., K. M. Guinn, M. I. Harrell, R. Liao, M. I. Voskuil, M. Tompa, G. K. Schoolnik, and D. R. Sherman.** 2003. Rv3133c/dosR is a transcription factor that mediates the hypoxic response of Mycobacterium tuberculosis. Mol. Microbiol. **48:**833–843.
8. **Parsons, L. M., A. Somoskovi, R. Urbanczik, and M. Salfinger.** 2004. Laboratory diagnostic aspects of drug resistant tuberculosis. Front. Biosci. **9:**2086–2105.
9. **Reed, M. B., P. Domenech, C. Manca, H. Su, A. K. Barczak, B. N. Kreiswirth, G. Kaplan, and C. E. Barry III.** 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature **431:**84–87.
10. **Reed, M. B., S. Gagneux, K. Deriemer, P. M. Small, and C. E. Barry III.** 2007. The W-Beijing lineage of *Mycobacterium tuberculosis* overproduces triglycerides and has the DosR dormancy regulon constitutively upregulated. J. Bacteriol. **189:**2583–2589.
11. **Starck, J., G. Kallenius, B. I. Marklund, D. I. Andersson, and T. Akerlund.** 2004. Comparative proteome analysis of Mycobacterium tuberculosis grown under aerobic and anaerobic conditions. Microbiology **150:**3821–3829.
12. **Wang, Q., J. Yue, L. Zhang, Y. Xu, J. Chen, M. Zhang, B. Zhu, H. Wang, and H. Wang.** 2007. A newly identified 191A/C mutation in the Rv2629 gene that was significantly associated with rifampin resistance in Mycobacterium tuberculosis. J. Proteome Res. **6:**4564–4571.
13. **World Health Organization.** 2007. W.H.O. report 2007: global tuberculosis control—surveillance, planning, financing. World Health Organization, Geneva, Switzerland.
14. **World Health Organization.** 2008. Anti-tuberculosis drug resistance in the world. Report number 4. World Health Organization, Geneva, Switzerland.
15. **Zhang, Y., and A. Telenti.** 2000. Genetics of drug resistance in *Mycobacterium tuberculosis*, p. 235–251. *In* G. F. Harfull and W. R. Jacobs, Jr. (ed.), Molecular genetics of mycobacteria. ASM Press, Washington, DC.