UTR PCR in 91 (15.7%) of 581 donors with normal ALT levels. The prevalence of TTV DNA increased with age in blood donors either with or without elevated ALT levels and aged 50–64 years without elevated ALT levels. By contrast, TTV DNA would be associated with liver damage among blood donors.

Table 1. TTV virus DNA in blood donors detected by 2 distinct polymerase chain reaction (PCR) methods.

<table>
<thead>
<tr>
<th>PCR methods and age groups</th>
<th>Alanine aminotransferase levels</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥61 U/L</td>
<td>≤45 U/L</td>
</tr>
<tr>
<td>N22 PCR</td>
<td>16–29 38/192 (19.2)</td>
<td>16/190 (8.4)</td>
</tr>
<tr>
<td></td>
<td>30–49 74/274 (27.0)</td>
<td>23/193 (11.9)</td>
</tr>
<tr>
<td></td>
<td>50–64 227/75 (36.0)</td>
<td>52/198 (26.3)</td>
</tr>
<tr>
<td>Total</td>
<td>139/547 (25.4)</td>
<td>91/581 (15.7)</td>
</tr>
<tr>
<td>UTR PCR</td>
<td>16–29 94/100 (94.0)</td>
<td>93/100 (93.0)</td>
</tr>
<tr>
<td></td>
<td>30–49 93/100 (93.0)</td>
<td>95/100 (95.0)</td>
</tr>
<tr>
<td></td>
<td>50–64 71/175 (41.7)</td>
<td>98/100 (98.0)</td>
</tr>
<tr>
<td>Total</td>
<td>258/274 (93.8)</td>
<td>286/300 (95.3)</td>
</tr>
</tbody>
</table>

NOTE. Untranslated region (UTR) PCR was performed on randomly selected 100 blood donors who were aged 16–49 years with or without elevated ALT levels and aged 50–64 years without elevated ALT levels. Table values are numbers with characteristic/total tested (%). NS, not significant.

notransferase (ALT) levels. PCR with heminested primers (NG059/NG063 and NG061/NG063), deduced from the N22 region (N22 PCR), is specific for the detection of TTV DNA of genotypes from 1 to 6 [2, 10]. The other PCR with nested primers (NG133/NG147 and NG134/NG132), deduced from well-conserved areas in the UTR, detected TTV DNA of all known genotypes from 1 to 16 [10]. Blood donors were selected who had not received transfusion and who were negative for serum markers of hepatitis B virus, hepatitis C virus, or human immunodeficiency virus type 1. Of the 1128 blood donors, 547 had elevated ALT levels (61–301 U/L; mean ± SD, 87.4 ± 33.6 U/L) and the remaining 581 had normal ALT levels (6–45 U/L; mean ± SD, 19.9 ± 7.7 U/L).

Table 1 compares the detection of TTV DNA by N22 PCR and UTR PCR in blood donors with and without elevated ALT levels, stratified by age. TTV DNA was detected by N22 PCR in 139 (25.4%) of 547 blood donors with elevated ALT levels at a frequency significantly higher (P < .0001; χ² test) than that in 91 (15.7%) of 581 donors with normal ALT levels. The prevalence of TTV DNA increased with age in blood donors either with or without elevated ALT levels. By contrast, TTV DNA was detected by UTR PCR at a high frequency (≥93%) in all age groups with no difference in prevalence between the blood donors with and without elevated ALT levels.

These results, taken along with those of Irving et al. [1], indicate that the selection of PCR primers crucially influences the detection of TTV DNA. Furthermore, these results suggest that TTV of restricted genotypes, detected by N22 PCR [2, 5], would be associated with liver damage among blood donors.

Keiichi Itoh,‡ Masaharu Takahashi,† Masato Ukita,† Tsutomu Nishizawa,† and Hiroaki Okamoto†

†Japanese Red Cross Yamaguchi Blood Center, Yamaguchi-Ken; ‡Institute of Immunology, Tokyo; †Immunology Division and Division of Molecular Virology, Jichi Medical School, Tochigi-Ken, Japan

References


Reprints or correspondence: Dr. Hiroaki Okamoto, Immunology Division and Division of Molecular Virology, Jichi Medical School, Minamikawachi-Machi, Tochigi-Ken 329-0498, Japan (immundiv@jichi.ac.jp).

The Journal of Infectious Diseases 1999;180:1750–1
© 1999 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/1999/18005-0054$02.00

Mutations in Genes Associated with Drug Resistance in Mycobacterium tuberculosis Isolates from Italy

To the Editor—Cingolani et al. [1] recently described the results of an analysis of gene mutations associated with drug resistance in isolates of Mycobacterium tuberculosis recovered from patients in a reference hospital in Italy. The authors used nucleotide sequence analysis and other molecular methods to identify mutations in 7 genes previously reported to contain mutations associated with resistance to rifampin (rpoB), isoniazid (katG, inhA, and ahpC), streptomycin (rpsL and rrs), and ethambutol (embB; reviewed in [2]). Although the authors extend understanding of the molecular genetics of drug resistance to isolates from Italy, some of the data warrant additional explanation because they differ substantially from extensive analyses conducted in many areas. With regard to katG polymorphisms associated with isoniazid resistance, all published results, in-
cluding our study of several thousand *M. tuberculosis* complex isolates from worldwide sources [2, 3], as well as our unpublished data, have shown that amino acid 463 in susceptible organisms is either an arginine or a leucine residue. In contrast, Cingolani et al. report in table 2 of their article that one isolate (T60) had an alanine residue at residue 463 in the wild-type condition that was replaced with a histidine in the isoniazid-resistant mutant organism. The authors’ basis for reporting that an isoniazid-susceptible isolate of *M. tuberculosis* had alanine at position 463 is unclear. They also report that two resistant organisms (T4 and T50) had an Arg463His amino acid replacement and one organism (T10) had an Arg463→stop mutation. (Note that two isolates are designated “T50” in their published table 2; we refer to the second T50 isolate.) Inasmuch as these changes have not been previously identified [2], it is important to report the actual nucleotide changes found. The authors report a Gln294His amino acid replacement in a resistant isolate (isolate T30; table 2 of their publication), but residue 294 in *M. tuberculosis* complex organisms is glutamic acid, not glutamine. We also presume that their suggestion in the Discussion to perform mutational analysis of region 135 of *katG* should actually refer to the codon 315 region.

The authors identified four isolates with mutations in the *ahpC* gene encoding alkylhydroperoxide reductase [4, 5]. Two isolates were reported to have Trp690Ser amino acid replacements, and one organism each had an Ala684→stop and Arg687→stop change (table 2 of their paper). The wild-type *ahpC* gene is 585 bp in length and encodes a protein with 195 amino acid residues. The polymerase chain reaction primers they used would amplify only the oxyR-*ahpC* intergenic region and 78 codons of *ahpC*. Hence, the basis for the authors’ observation that mutations exist in codons 684, 687, and 690 is not apparent.

Amino acid residue 43 in ribosomal protein S12 in wild-type *M. tuberculosis* complex isolates is lysine, not leucine as designated in their article.

The *embB* gene encodes an arabinosyl transferase that is believed to participate in the biosynthesis of arabinogalactan, a component of the *M. tuberculosis* cell wall [6, 7]. Sreevatsan et al. [8] investigated the association of ethambutol resistance, minimal inhibitory concentrations, and sequence variation in the 10-kb *embCAB* operon of *M. tuberculosis*. The analysis showed that amino acid residue 306 in EmbB was the most commonly altered position among ethambutol-resistant organisms. Cingolani et al. [1] reported that four ethambutol-resistant isolates had mutations in the 399 bp of *embB* that they characterized. However, the nucleotide substitutions and the amino acid replacements were not defined. The region they studied included codon 306 of the *embB* gene. Because relatively little information is available regarding mutations in genes associated with ethambutol resistance, it is important to report the exact nucleotide changes and amino acid replacements found.

In summary, Cingolani et al. [1] present important new data about mutations associated with *M. tuberculosis* drug resistance. Clarification of several issues will permit their data to be more fully integrated into the emerging understanding of the molecular genetic basis of drug resistance in this important human pathogen. We encourage all authors to report both the nucleotide and amino acid changes identified in analyses of this type.

James M. Musser, Amol Amin, and Srinivas Ramaswamy

Institute for the Study of Human Bacterial Pathogenesis, Department of Pathology, Baylor College of Medicine, Houston, Texas

References


Reprints or correspondence: Dr. James M. Musser, Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 S. 4th St., Hamilton, MT 59840 (jmusser@bcm.tmc.edu).

The Journal of Infectious Diseases 1999;180:1751-2
© 1999 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/1999/18005-0055$02.00

Reply

To the Editor—As reported by Musser et al. [1], we think that it is relevant and important to assess the difference in the prevalence and pattern of resistance-related mutations in *Mycobacterium tuberculosis* isolates all over the world. To answer the first objection of Musser et al., we point out that our molecular