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katG Mutations in Isoniazid-Resistant *Mycobacterium tuberculosis* Isolates Recovered from Finnish Patients

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***katG* and *inhA* genes from isoniazid-resistant *Mycobacterium tuberculosis* strains isolated in Finland were examined by PCR or sequencing. By PCR, *katG* was not detected in 3 of 54 strains. Sequencing of *katG* from 13 strains showed small point mutations or insertions; a previously described mutation causing a Ser-to-Thr change at position 315 was found in 4 strains, and there were nine new missense mutations of *katG*. A 209-bp segment of *inhA* from 17 strains was sequenced, but no mutations were observed. This result indicates that different mutations prevail in different geographical areas.**

Isoniazid (INH) has been used extensively as an essential part of antituberculous treatment and chemoprophylaxis of tuberculosis since the early 1950s. Soon after the introduction of the drug, INH-resistant *Mycobacterium tuberculosis* strains began to emerge. Middlebrook (8) noted that some of the INH-resistant strains had lost their catalase expression. It took 40 years before the molecular mechanisms of catalase negativity were unveiled (18). The finding of the *katG* gene and its deletion in certain INH-resistant strains have led to intensified investigation of the gene.

katG has a length of 2,223 bp and encodes catalase-peroxidase, which is believed to play a crucial role in mediating the antituberculous effect of INH (6). Several mutations of the gene that are seemingly highly diversified have been identified recently, and some of them seem to occur more often than others (1, 3, 5, 9, 10, 14, 15).

We examined deletions and mutations of *katG* of INH-resistant *M. tuberculosis* strains isolated from Finnish patients. In Finland, the annual incidence of tuberculosis is 10/100,000, the prevalence of INH resistance among *M. tuberculosis* strains has been about 2% in recent years, and multidrug resistance is negligible (11). Our aim was to compare gene alterations with those observed in strains originating from areas in which the prevalence of resistance was high.

A total of 54 INH-resistant *M. tuberculosis* isolates was recovered from patients by hospital laboratories all over the country from 1989 until April 1995. All isolates were shown to be either moderately (0.4 μg/ml) or highly (1.0 μg/ml) resistant to INH by the disk elution method (Sensi-Disk; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.). Two INH-susceptible isolates (one catalase positive and one negative) were chosen as positive controls. Thirteen isolates were also resistant to another antituberculous drug, and two isolates were resistant to three drugs. All isolates were tested for catalase production by the drop method, while the semiquantitative catalase test was used for isolates with major deletions in *katG* and for isolates subjected to sequence analysis (12). Table 1 shows the oligonucleotide primers used for PCR and sequencing. In addition to the listed primers, some sequencing primers (not shown) were synthesized to cover the 3′ end of *katG* reliably. The listed primers were also synthesized in biotinylated versions in which a biotin amidite group (Biodite; Pharmacia Biotech, Espoo, Finland) was added to the oligonucleotide at the 5′ end. Amplification was carried out with a DNA thermal reactor (HB-TR1; Hybaid Ltd., Middlesex, United Kingdom). A total of 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and synthesis at 72°C for 1 min was carried out. The biotinylated PCR products were purified with streptavidin-coated Dynabeads (Dynabeads M-280 streptavidin; Dynal AS, Oslo, Norway).

The DNA sequence was determined with the *Taq* DyeDeoxy terminator cycle sequencing kit and the 373-18 DNA sequencer (Applied Biosystems). The data were assembled and edited with SeqEd v. 1.0.3 software (Applied Biosystems), and the sequences were compared with the published consensus sequence of the *katG* gene (6) from GenBank. The observed mutations were confirmed by sequencing the complementary strand of *katG*.

Fifty-four INH-resistant *M. tuberculosis* isolates and two INH-susceptible ones were analyzed by PCR with five sets of primers covering the entire *katG* gene (Table 1). The sizes of the PCR products varied from 496 to 620 bp. Three strains (5.4%) failed to amplify, indicating deletion of *katG*. This result supports earlier studies (4, 15, 17) showing that the total deletion of *katG* is relatively rare in INH-resistant *M. tuberculosis*. In addition, one strain had a large, about 60-bp-long deletion in the middle of the gene. All isolates with deletions were catalase negative. Successful extraction of DNA from the bacteria was verified by amplifying a 423-bp segment of the gene encoding the 32-kDa mycobacterial protein (16).

TABLE 1. Primers used in the study of *katG*

Primer	Sequence (origin position)	Direction
P1	AACGGCTTCCTGTTGGACGAG (−127)	Forward
P2	CATGAGAGCTTCTTGCCGTAC (482)	Reverse
P3	ACCTACCGCATCCACGACGG (334)	Forward
P4	CGCCATGGGTCTTACCGAAAG (829)	Reverse
P5	GGTCGACATTCGCGAGACGTT (732)	Forward
P6	CGGTGGATCAGCTTGTACCAG (1250)	Reverse
P7	CGACGATGCTGGCCACTGAC (1124)	Forward
P8	TTGTTCCTGCGACGCATCGTG (1719)	Reverse
P9	TTCGCCGACCTCGCTGTGCT (1603)	Forward
P10	GGGTCTGACAAATCGCGCCG (2332)	Reverse

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TABLE 2. *katG* mutations found in 19 isolates of *M. tuberculosis*

Isolate	Status of isolate for INH[a]	Other drugs to which isolate is resistant[b]	Mutation(s)	Amino acid change	Ht (mm) of bubbles in catalase test	History of INH therapy[c]
1	S				10	No
2	S		C-2073→T		0	No
3	I		A-1574→C	Gln→Pro	0	Yes
4	I		T-1760→C	Leu→Pro	20	NA
5	I	PZA			27	Yes
6	I				9	No
7	R		C-195→CCCCC	Reading frame	0	Yes
8	R		C-883→T and G-1464→C	Gln→Stop Lys→Asn	0	Yes
9	R		T-898→G	Trp→Gly	0	Yes
10	R	RMP	G-944→C	Ser→Thr	0	NA
11	R	PZA and SM	G-944→C and G-1388→T	Ser→Thr Arg→Leu	23	No
12	R	SM	G-944→C C-945→A	Ser→Thr	10	No
13	R	RMP and SM	G-944→C and C-945→A G-1501→C	Ser→Thr Pro→Ala	22	Yes
14	R		C-1849→CAC	Reading frame	0	Yes
15	R		T-2098→C	Ser→Pro	0	Yes
16	R	SM	Total deletion		0	Yes
17	R		Total deletion		0	Yes
18	R		Total deletion		0	No
19	R		60-bp deletion		0	Yes

[a] S, susceptible; I, resistant to INH at 0.4 μg/ml and susceptible to INH at 1.0 μg/ml; R, resistant to INH at 1.0 μg/ml.
[b] PZA, pyrazinamide; RMP, rifampin; SM, streptomycin.
[c] NA, not available.

katG was sequenced from 13 INH-resistant strains without deletions detectable by PCR and from the INH-susceptible control strains. Eleven of the 13 isolates had *katG* mutations (Table 2). The remaining two isolates, which had no *katG* changes, were only moderately resistant to INH.

All mutations in *katG* were relatively small insertions or point mutations; the largest insertion was a CCCC insertion in one strain, and a dinucleotide mutation occurred in two isolates. In general, more mutations affected the 3′ end than the 5′ end of the gene. Although the mutations were widely scattered along the gene, mutations affecting certain amino acids prevailed. The most common deduced alteration, found in four strains, was a Ser-to-Thr change at position 315 (Ser-315→Thr), which is partially in agreement with the findings of earlier studies (3, 5, 9, 10, 14). This substitution resulted from two kinds of point mutations in bases 944 and 945. On the other hand, we observed only one Arg-463→Leu mutation. Such mutations have been rather a common finding in many studies (3, 5, 9, 10, 14). Interestingly, the isolate with the codon 463 mutation had also undergone a mutation in codon 315. A silent mutation was observed in the INH-susceptible, catalase-negative control strain.

Earlier studies have shown that *M. tuberculosis* strains can, regardless of the MICs of INH for the strains, be either catalase positive or negative, although highly resistant strains are more often catalase negative (4, 17). Our results support this concept.

Nine of the 13 patients with highly INH-resistant *M. tuberculosis* isolates harboring *katG* mutations had received INH earlier. Three previously untreated patients showed a mutated isolate, possibly as a result of primary infection by a resistant organism.

In addition to being associated with *katG* gene alterations, INH resistance has also been associated with mutations in a two-gene locus containing *mabA* and *inhA* genes (2, 5, 9, 10, 13, 14). *inhA* encodes a structure which appears to be a target site of catalase-activated INH (7). The isolates from which *katG* was sequenced and isolates with major deletions were subjected to a further study in which a 209-bp segment of *inhA* was sequenced (13). The segment contains the region encoding amino acids Ser-94 and Ile-95 that have been associated with resistance (2, 13). No perturbations were observed. Earlier studies have reported point mutations in a presumed ribosomal binding site located in the region upstream of the locus, and it has been suggested that these substitutions alter the regulation of MabA or InhA in certain resistant strains (9, 10, 14). Analysis of the region would be warranted, especially in the two INH-resistant strains with intact *katG*.

In conclusion, it seems that mutations are distributed in various parts of the *katG* gene of INH-resistant *M. tuberculosis* strains. The gene was not present in 3 of 54 resistant strains, and 1 isolate had a large deletion in the middle of the gene. All nine highly resistant isolates subjected to sequence analysis showed a *katG* mutation. Among those isolates recovered from patients living in Finland, the Ser-315→Thr alteration was seen in four strains. The Arg-463→Leu change, reportedly rather common earlier, was found in one strain only. We also observed nine new missense mutations. However, previous studies have not included samples from northern Europe (1, 3, 5, 9, 10, 14, 15). These results indicate that different mutations are prevailing in different geographical areas. A reliable clinical application of genotypic testing for INH resistance seems to be a remote goal at this stage because of the diversity of the mutations. Screening for the most common genetic alterations in the *katG* gene causing amino acid changes in codons 315 and 463 could, however, already be feasible.

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