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# Molecular Analysis of *katG* Gene Mutations in Strains of *Mycobacterium tuberculosis* Complex from Africa

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**A sample of 124 isoniazid (INH)-resistant and 88 susceptible strains of *Mycobacterium tuberculosis* complex from south, central, and west Africa was analyzed by direct sequence analysis and PCR-restriction fragment length polymorphism analysis of their catalase-peroxidase (*katG*) genes. Point mutations at codon 315 were found in the genomes of 64% of INH-resistant strains, but no complete deletions were identified. Mutations at codon 463 were independent of INH resistance and were linked to the geographic origins of the strains.**

The emergence of drug-resistant strains of *Mycobacterium tuberculosis* complex is an increasing problem for populations and tuberculosis control programs in developed and developing countries alike. Today, throughout the world isoniazid (INH) and rifampin together form the backbone of short-course chemotherapy for *M. tuberculosis* infection. However, strains resistant to INH are seen with increasing frequency. In South Africa the rate of INH resistance among 16,430 *M. tuberculosis* isolates collected between 1980 and 1988 was 14.2% (15).

The *katG* gene is involved in detoxification of endogenously generated or exogenously supplied hydrogen peroxide, and recently, the enzyme that it encodes has been shown to be the only enzyme of *M. tuberculosis* capable of activating the prodrug INH (11). In high-level (MIC, >50 μg/ml) INH-resistant isolates of *M. tuberculosis*, the *katG* gene was found to be deleted from the chromosome (16). However, in most INH-resistant strains either simple base pair changes resulting in missense mutations or small deletions in the *katG* gene are associated with resistance (1, 2, 6, 10).

Aiming to learn more about the nature of *katG* gene mutations in INH-resistant mycobacterial isolates from different geographical regions and focusing on gene mapping of mutations for development of rapid molecular diagnostics, we chose for analysis an 894-bp central fragment of the *katG* gene in 212 African strains of the *M. tuberculosis* complex.

Heat-killed culture samples of 87 multidrug-resistant (MDR) strains of *M. tuberculosis* were received from the National Tuberculosis Research Programme, Medical Research Council, Pretoria, South Africa. This set originated from six southern African regions: Free State ($n$ = 36; F), Gauteng ($n$ = 28; G), Swaziland ($n$ = 8; S), Natal ($n$ = 9; N), Lesotho ($n$ = 4; L), and Eastern Cape ($n$ = 2; C). Samples of 37 INH-resistant strains of *M. africanum* originating from Sierra Leone ($n$ = 28) and Uganda ($n$ = 9) and 88 INH-susceptible strains of *M. africanum* from Sierra Leone ($n$ = 53) and Uganda ($n$ = 35) were provided by the Armauer-Hansen-Institute, Würzburg, Germany.

Amplification of the *katG* gene and direct nonradioactive sequence determination were performed by a modification of the technique described by Telenti et al. (13). The oligonucleotides (Applied Biosystems GmbH, Weiterstadt, Germany) used for amplification and sequencing were Gfor3 (5′-TTTC GGCGCATGGCCATGA-3′) and Grev2 (5′-ACAGCCACC GAGCACGAC-3′). Amplification was carried out by using standard cycling conditions and resulted in an 894-bp fragment spanning codons 275, 315, 328, and 463.

For restriction fragment length polymorphism (RFLP) analysis, the 894-bp amplicon was digested with *Nci*I (Boehringer, Mannheim, Germany), and the resulting fragments were analyzed by electrophoresis on a 2% agarose gel (FMC BioProducts, Rock, Germany land, Maine).

Molecular subtyping was done for all strains by the mixed-linker PCR method, described in detail by Haas et al. (4, 5).

No complete deletions of the *katG* gene were found among the 124 INH-resistant strains analyzed. Direct sequencing showed 100% sequence identity among the different isolates and to the published sequence for *M. tuberculosis* (GenBank accession no. x68081), apart from codons 315 and 328.

At codon 315 the missense mutation AGC→ACC (Ser.Gen BankThr) was found in 52 of 87 (60%) MDR strains of *M. tuberculosis* from South Africa. Five strains showed an AGC→AAC (Ser→Asn) mutation at this position. Two strains had, to our knowledge, previously undescribed changes at codon 315: AGC→ATC (Ser→Ile) and AGC→CGC (Ser→Arg). Thus, for 68% of INH-resistant *M. tuberculosis* strains from South Africa, mutations at *katG* gene position 315 could be found (Table 1). For *M. africanum* strains from Sierra Leone or Uganda, we found that 20 of 37 isolates (54.1%) exhibited mutations at codon 315 (Table 1). Previously undescribed mutations at codon 328 were found in two strains of *M. tuberculosis* from South Africa: TGG→TTG (Trp→Leu) and TGG→TGC (Trp→Cys).

PCR-RFLP analysis of the sample of 124 INH-resistant *M. tuberculosis* complex strains revealed a remarkable degree of polymorphism in *katG* gene codon 463 (Table 1). Of 87 MDR strains from South Africa, 81 had the Arg-463 variant; the other 6 strains had a nucleotide exchange in codon 463, resulting in the loss of the *Nci*I restriction site. Two of these strains were harboring an additional mutation at codon 315 (ACC).

Restriction analysis of 37 INH-resistant and 88 INH-susceptible *M. africanum* isolates revealed an Arg at position 463 for all 44 strains originating from Uganda, while 77 of 81 strains

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TABLE 1. Frequency of mutations in *katG* gene codons 315 and 463 in 124 INH-resistant and 88 INH-susceptible strains of *M. tuberculosis* complex

| INH susceptibility | Species | Origin | No. of isolates | No. of strains | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *katG* gene position 315 | | | | | Position 328[a] | | | Position 463 | |
| | | | | AGC | ACC | AAC | ATC | CGC | TGG | TTG | TGC | CGG | Mutation[b] |
| Resistant | *M. tuberculosis* | South Africa | 87 | 28 | 52 | 5 | 1 | 1 | 68 | 1 | 1 | 81 | 6 |
| | *M. africanum* | Sierra Leone | 28 | 10 | 11 | 4 | 3 | | 21 | | | 1 | 27 |
| | *M. africanum* | Uganda | 9 | 7 | 2 | 0 | 0 | | 8 | | | 9 | 0 |
| Susceptible | *M. africanum* | Sierra Leone | 53 | NA[c] | NA | NA | NA | NA | NA | NA | NA | 3 | 50 |
| | *M. africanum* | Uganda | 35 | NA | NA | NA | NA | NA | NA | NA | NA | 35 | 0 |

[a] Of 124 INH-resistant isolates, 99 were analyzed for codon 328. All remaining 25 isolates contained a mutation at codon 315.
[b] PCR-RFLP analysis, with loss of the *Nci*I restriction site.
[c] NA, not analyzed.

(95.1%) originating from Sierra Leone had a mutation at position 463, with loss of the restriction site. Sequence analysis of three randomly selected strains showed the CGG→CTG mutation, which has been described previously (8).

DNA fingerprint analysis of the 87 *M. tuberculosis* strains from South Africa by mixed-linker PCR showed 69 individual fingerprints (79.3%), and 23 isolates belonged to 9 clusters (16.1%) with identical IS*6110* patterns. The *katG* mutations of clustered strains are listed in Table 2. Four of six MDR *M. tuberculosis* strains from South Africa with a CTG mutation at position 463 had unrelated IS*6110* DNA fingerprints with multiple bands (between 8 and 14 copies of IS*6110*). The DNA fingerprint analysis of our sample of *M. africanum* strains has been described in detail before (4).

Understanding of the genetic basis of INH resistance is far from complete. Changes in the *katG* gene, the *inhA* gene, and the *ahpC* gene have been described in INH-resistant mycobacterial isolates (7, 8, 11). Focusing on the *katG* gene, we did not find complete deletions of the *katG* gene among 124 INH-resistant African strains of the *M. tuberculosis* complex. In addition, our results showed a highly conserved *katG* gene fragment, with restriction of allelic variation to codons 315 and 328.

Missense mutations at codon 315 were found in almost two-thirds of the isolates analyzed. The AGC→ACC mutation (Ser→Thr), a nucleotide exchange already described by other investigators (8), was encountered most frequently in our sample, with 65 of 79 (82%) strains having the mutation. Five strains carried, to our knowledge, previously undescribed mutations at codon 315. The high percentage of mutations at position 315 and the occurrence of different nonsynonymous substitutions demonstrate the importance of this codon for the development of INH resistance among strains from Africa. Mutations at this position have also been reported for single isolates from other geographical regions (7–9, 14). The number of mutations at codon 315 among resistant strains might be even higher, considering that culture isolation was not performed on INH-containing media. Thus, the number of resistant cells carrying the mutation might, in some cases, have been below our limit of detection.

Mutation at codon 328 seems to be a rare event and has only been reported once (9). In our sample two mutations were encountered at this position. Because no other *katG* gene mutations were observed, we suggest that codon 328 is involved in the resistance mechanism in these isolates.

The analysis of position 463 for all 212 African strains of *M. tuberculosis* complex suggested geographical differences between strains of *M. africanum* from Uganda and Sierra Leone, and INH resistance was not associated with substitution of Arg-463. In our sample the geographical origins of strains could serve as a predictor of the conformation of codon 463. Analysis of the DNA fingerprint patterns and the biochemical properties of these strains of *M. africanum* provide additional support for this observation and have been described in detail

TABLE 2. Epidemiological data and mutations found among clustered isolates

| Cluster no. | No. of bands | Codon 315 | Codon 463 | Resistance profile[a] | Patient data | | |
|---|---|---|---|---|---|---|---|
| | | | | | Age (yr) | Gender | Region/ hospital[b] |
| 1 | 5 | ATC | CGG | RHSEt | 32 | Male | L/7 |
| | | ACC | CGG | RHSEEt | 38 | Male | S/2 |
| | | ACC | CGG | RHS | 24 | Male | S/6 |
| | | ACC | CGG | RHSEt | 23 | Female | F/1 |
| 2 | 3 | AAC | CGG | RHE | 28 | Female | F/1 |
| | | wt[c] | CGG | HSE | 54 | Male | G/8 |
| | | ACC | CGG | RH | 22 | Male | G/4 |
| | | ACC | CGG | RH | 44 | Male | G/3 |
| | | ACC | CGG | RHS | 29 | Male | S/5 |
| 3 | 11 | wt | CGG | RH | 45 | Male | N/9 |
| | | wt | CGG | RH | 34 | Male | F/1 |
| 4 | 11 | wt | CGG | RHS | 54 | Male | G/4 |
| | | ACC | CGG | RH | 32 | Male | F/1 |
| 5 | 7 | ACC | CGG | RHSE | 39 | Male | F/1 |
| | | ACC | CGG | RHE | 31 | Female | F/1 |
| 6 | 13 | AAC | CGG | RH | 55 | Male | G/8 |
| | | AAC | CGG | RH | 55 | Male | G/8 |
| 7 | 15 | ACC | CGG | RHS | NA[d] | Male | G/3 |
| | | ACC | CGG | RHS | NA | Male | G/3 |
| 8 | 8 | wt | CGG | RH | 36 | Male | G/8 |
| | | ACC | CGG | RHSE | 44 | Male | G/3 |
| 9 | 6 | wt | CTG | RH | 31 | Female | F/1 |
| | | wt | CTG | RHSEEt | 40 | Male | F/1 |

[a] R, rifampin; H, isoniazid; S, streptomycin; E, ethambutol; Et, ethionamide.
[b] Regions are indicated by a one-letter code as described in the text, and hospitals are assigned individual numbers; i.e., identical letters/numbers are equivalent to the same region/hospital.
[c] wt, wild type.
[d] NA, not available.

elsewhere (4). These results add further evidence to reports by Musser et al. (9) and Sreevatsan et al. (12), who suggest an ancestral *katG* CTG genotype for 127 isolates of *M. microti*, *M. bovis*, and *M. africanum*. In addition, this is the first description of a geographical subtype of *M. africanum* that exhibits the CGG sequence at position 463, which has so far been described only for *M. tuberculosis*. Numerical taxonomy by using biochemical characteristics has been found to result in two separate groups of *M. africanum* which are related to either *M. tuberculosis* or *M. bovis* (3). On the basis of our results, we suggest that strains of *M. africanum* derived from different geographical origins are polyphylogenetic.

Determination of the frequency of mutations underlying drug resistance in isolates from different geographical areas is the basis for developing rapid and specific techniques for the detection of drug-resistant strains by molecular genetic techniques. In addition, the analysis of phylogenetically preserved sequence motifs among members of the *M. tuberculosis* complex in combination with geographical and epidemiological data will contribute important information for tracing the phylogenetic spread of these pathogens.

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