Mutations At RT Codon 103: Phenotypic Resistance To NNRTIs And Clinical Correlates

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Background

- A K->N substitution conferring decreased NNRTI susceptibility is the most commonly occurring substitution at codon 103 of the HIV RT.
- Occasionally, however, other mutations with unknown significance are observed.
- We determined the prevalence and phenotypic effect on NNRTIs of these unusual mutations at RT codon 103.

Methods

- The occurrence of K103 substitutions among >60,000 clinical samples submitted to Virco for routine resistance testing between 1998-2003 was assessed.
- Phenotypic resistance profiles for clinical isolates carrying rare K103 variants in the absence of other known NNRTI resistance associated mutations were retrieved from Virco's correlative genotype/phenotype database.
- An additional 15,000 clinically derived samples from a nearly independent dataset where clinical histories were generally known from British Columbia, Canada were also examined.
- PCR products containing the more commonly observed amino acid changes at codon 103 were constructed in an HIV HXB2 amino acid background by the overlap-extension polymerase chain reaction method [Ho et al., Gene 77 (1989) 51-57].
  - Complementary forward- and reverse-primer containing nucleotide changes were designed to generate two PCR fragments having overlapping ends.
  - PCR products were mixed, denatured, re-annealed and extended. The resulting amplicons were utilized to construct recombinant viruses, and assess phenotypic susceptibility via the Virco Antivirogram®.

Results

Part 1: Prevalence of Mutations and Polymorphisms at RT Codon 103

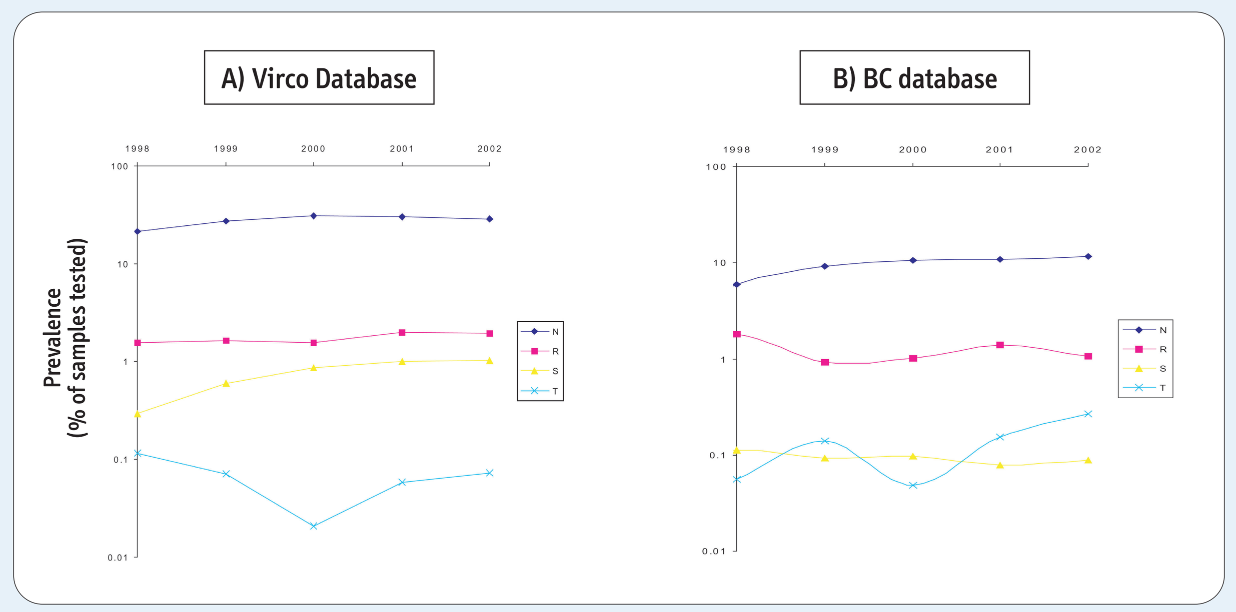
- Prevalence of all polymorphisms at codon 103 in the BC Centre for Excellence database.
  - The vast majority of samples (~90%) consisted of the wildtype AAA or mutant AAC (K103N).
  - The most common amino acid substitution was K103N, with two relatively common variants (AAT and AAC).
  - Other rare variants were also observed (see Table 1).
- Not likely to be due to differences in Clade, since only 1 amino acid substitution at codon 103 other than N was observed in a non-B subtype, out of over 300 non-B samples tested.

Table 1. Prevalence of Mutations and Substitutions at Codon 103 in BC Centre for Excellence Database (excluding isolates encoding amino acid mixtures). N>15,000.

Amino Acid	Sequence	NucleotidePrevalence (BC Data)
K103K	AAA	73.2%
K103N	AAC	12.9%
K103K	AAG	5.2%
K103N	AAT	2.1%
K103K	AAR	1.4%
K103R	AGA	1.0%
K103N	AAY	0.99%
K103S	AGC	0.16%
K103T	ACA	0.04%
K103R	AGG	0.03%
K103S	AGT	0.03%
K103S	AGY	0.02%
K103Q	CAA	0.01%
K103E	GAA	0.01%
K103T	ACM	0.01%
K103H	CAC	0.01%

- Prevalence Of All Polymorphisms At Codon 103 In Virco Database.
  - K103N occurred at a consistent prevalence of approximately 25% in isolates collected over the period 1998-2003 in the Virco Database.
  - Other mutations observed at codon 103 included K103R (6% of K103N prevalence); K103S (3%); and, more rarely, K103T; K103Q and K103H (<1% each).
  - Only the K103S mutation appears to be increasing in prevalence over time (0.3% to 1.2% from 1998 to 2003).
  - Similar patterns were observed over the same period in the BC database, though with a lower prevalence of K103N and K103S, which may be related to the rare use of efavirenz in British Columbia.

Figure 1. Changes In Prevalence Of Mutations At Codon 103 Over Time In Virco And British Columbia Databases



Part 2: Phenotypic Effect of Unusual 103 Mutations and Polymorphisms in Clinical Isolates and Site Directed Mutants

Clinical Isolates

- Median phenotypic NNRTI susceptibility was assessed in samples without other mutations likely to confer NNRTI resistance
  - NNRTI resistance mutations defined as any mutation at codon 98, 100, 101, 106, 108, 179, 181, 188, 190, 225, 227, 230, 234, 236, or 318.
  - Susceptibility reduced for K103S, K103T, and K103H, but not for K103R. (Table 2).

Table 2. Median Fold Decrease in NNRTI Phenotypic Susceptibility in clinical isolates without other key NNRTI resistance mutation in the Virco database.

	EFV	Del	NVP	N
K103R	<2	<2	<2	115
K103Q	<2	<2	<2	5
K103S	10	19	>50	16
K103T	15	>50	>50	2
K103H	>50	>50	>50	4

Site directed Mutants

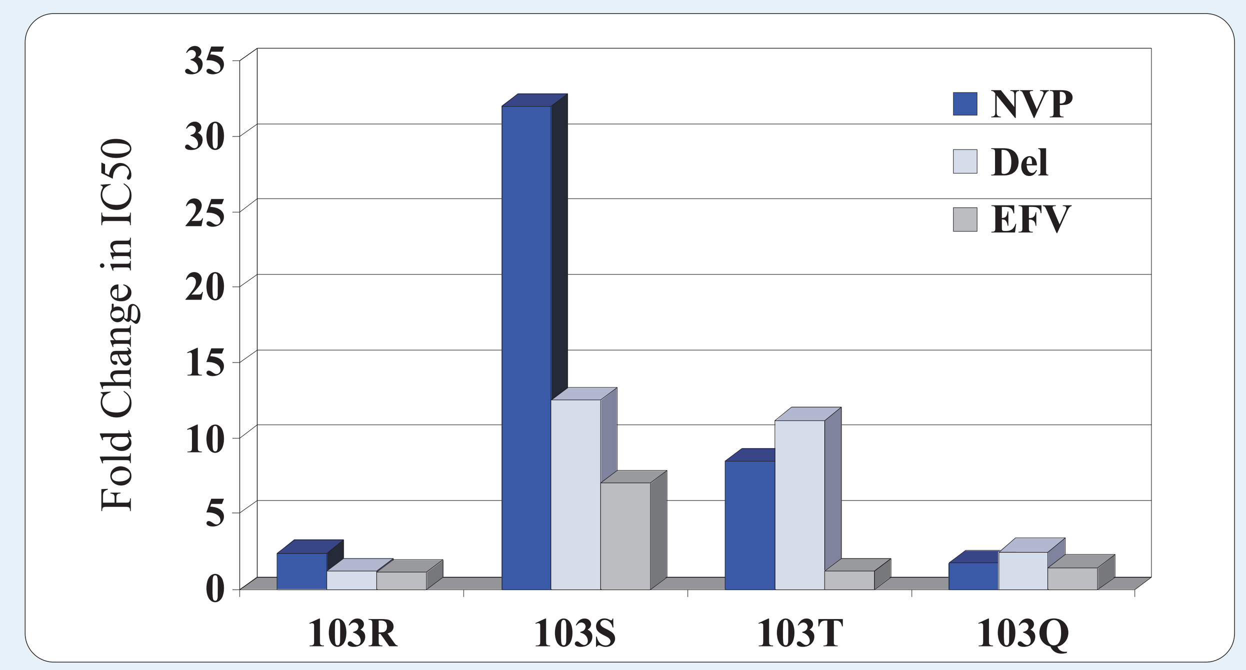
- The phenotypic effect of the rare mutations K103S and K103T was considerably reduced when present in a HXB2 amino acid background compared to clinical isolates (compare Table 2 with Figure 2, Table 3).
- Site directed mutagenesis confirmed that K103R conferred < 3-fold changes in IC50 for all three NNRTIs, consistent with other data suggesting this mutation has little or no effect on NNRTI resistance (data not shown).

Table 3. Fold change in NNRTI susceptibility of different variants at codon 103 in an HIV HXB2 genetic background

Mutation	Sequence	NVP	Del	EFV
K103R	AGA	2.4	1.2	1.1
K103R	AGG	n/a	n/a	n/a
K103S	AGT	29.1	14	7.5
K103S	AGC	35	10.8	6.5
K103T	ACA	8.5	12.9	1.3
K103T	ACG	n/a	9.5	1
K103Q	CAA	1.9	2.2	1.3
K103Q	CAG	1.6	2.9	1.5
K103H	CAC	n/a	n/a	n/a

Each amino acid substitution (except K103H) was encoded by two different nucleotides. As expected, the specific sequence was not related to the degree of phenotypic NNRTI susceptibility. The phenotypic effect of some substitutions could not be assessed due to poor virus growth in this genetic background (eg. K103H).

Figure 2. Average Phenotypic Effect Of Mutations K103R, S, T, And Q In An HIV HXB2 Background



These substitutions at RT codon 103 had little phenotypic effect on the other antiretrovirals tested, including the nucleos(t)ides (Table 4) and the PIs (data not shown)

Table 4. Little influence of rare 103 variants on other RT inhibitors.

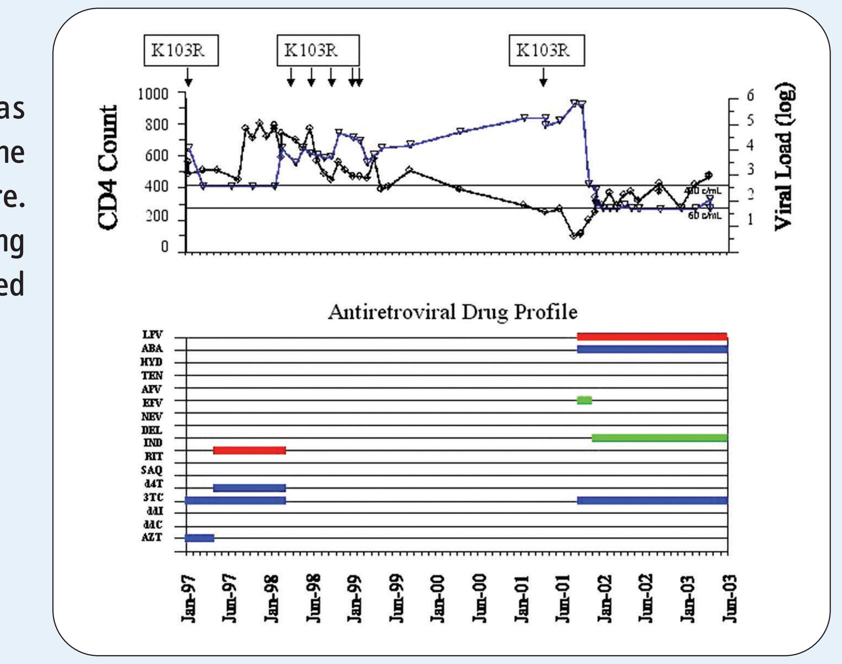
Drug	Mean Fold Change in IC50	Range (N=7)
AZT	0.67	(0.6-0.8)
3TC	1.0	(0.4-1.6)
ddI	0.39	(0.2-0.6)
ddC	1.0	(0.7-1.3)
D4T	0.6	(0.5-0.9)
ABC	0.54	(0.4-0.8)
TDF	0.91	(0.2-1.4)

Part 3: Clinical Correlates of 103 Variants

- In a clinical cohort of patients with known treatment history, K103 S, H and T variants were observed predominantly in NNRTI experienced subjects.
- The detection of K103R did not appear to be related to NNRTI therapy, as it was detected in a significant minority of individuals who had never been exposed to NNRTIs (data not shown).
- K103S, Q and H were seen mainly in patients failing EFV-containing therapy while K103T was more commonly seen in subjects failing NVP-containing therapy.
- Examination of the time course of emergence of these rare 103 variants during NNRTI treatment revealed that these could occur before and/or after selection of K103N. Three illustrative case studies are shown as examples:

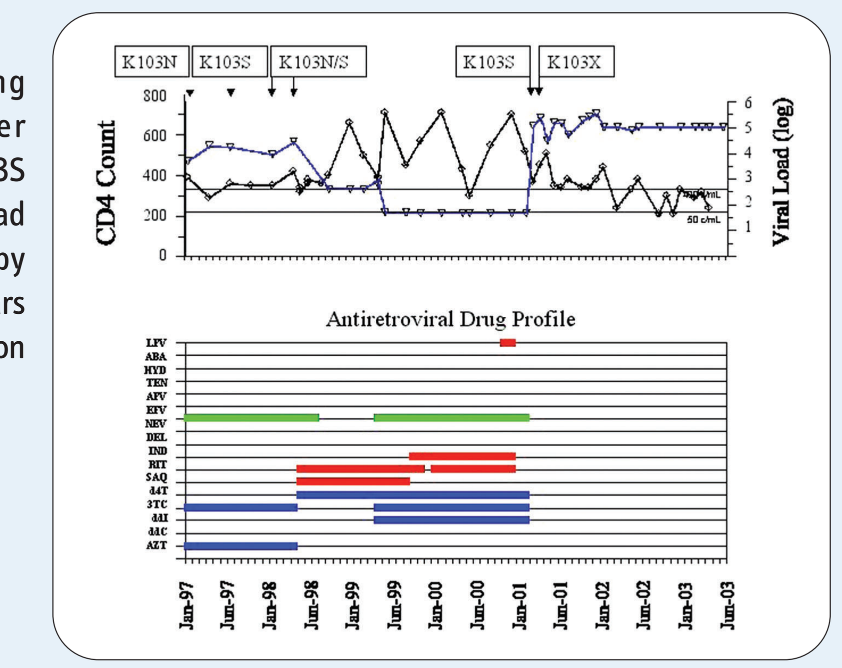
Case A)

a K103R polymorphism was consistently detected despite the lack of any NNRTI exposure. This is consistent with this being a rare polymorphism unrelated to NNRTI usage.



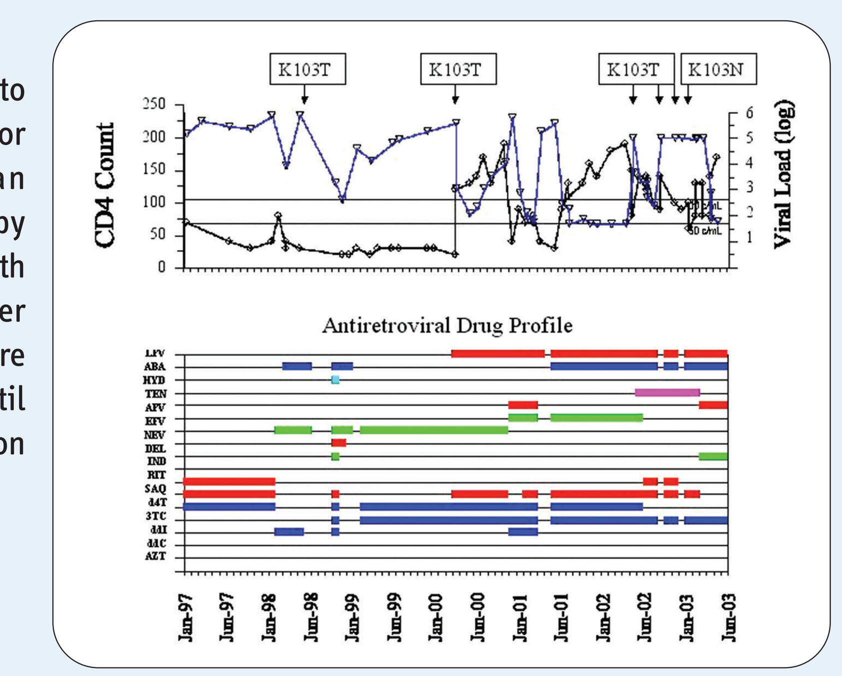
Case B)

a K103S arises following selection of K103N after Nevirapine exposure. The K103S is also detected after viral load rebound after suppression by multiple drug therapy. It appears to revert upon the total cessation of therapy.



Case C)

K103T arises after exposure to Nevirapine and is retained for several years despite an exceedingly complex therapy history, before replacement with K103N. Importantly, no other NNRTI specific mutations were detected at other codons until three years after the detection of the K103T.



Conclusions

- Variants at position 103 other than K103N are relatively rare in clinical isolates
- Some (including K103 S, H, and T) may confer dramatically decreased susceptibility to individual NNRTIs
- In some cases, these rare mutations may arise before the classical K103N
- This data may be informative for interpretive algorithms and the design of assays specific to RT codon 103

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