



Single Transmitted Drug Resistance Mutations: Not Always an Indicator of Transmission of More Extensive Resistance Profiles

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Transmission of drug resistance

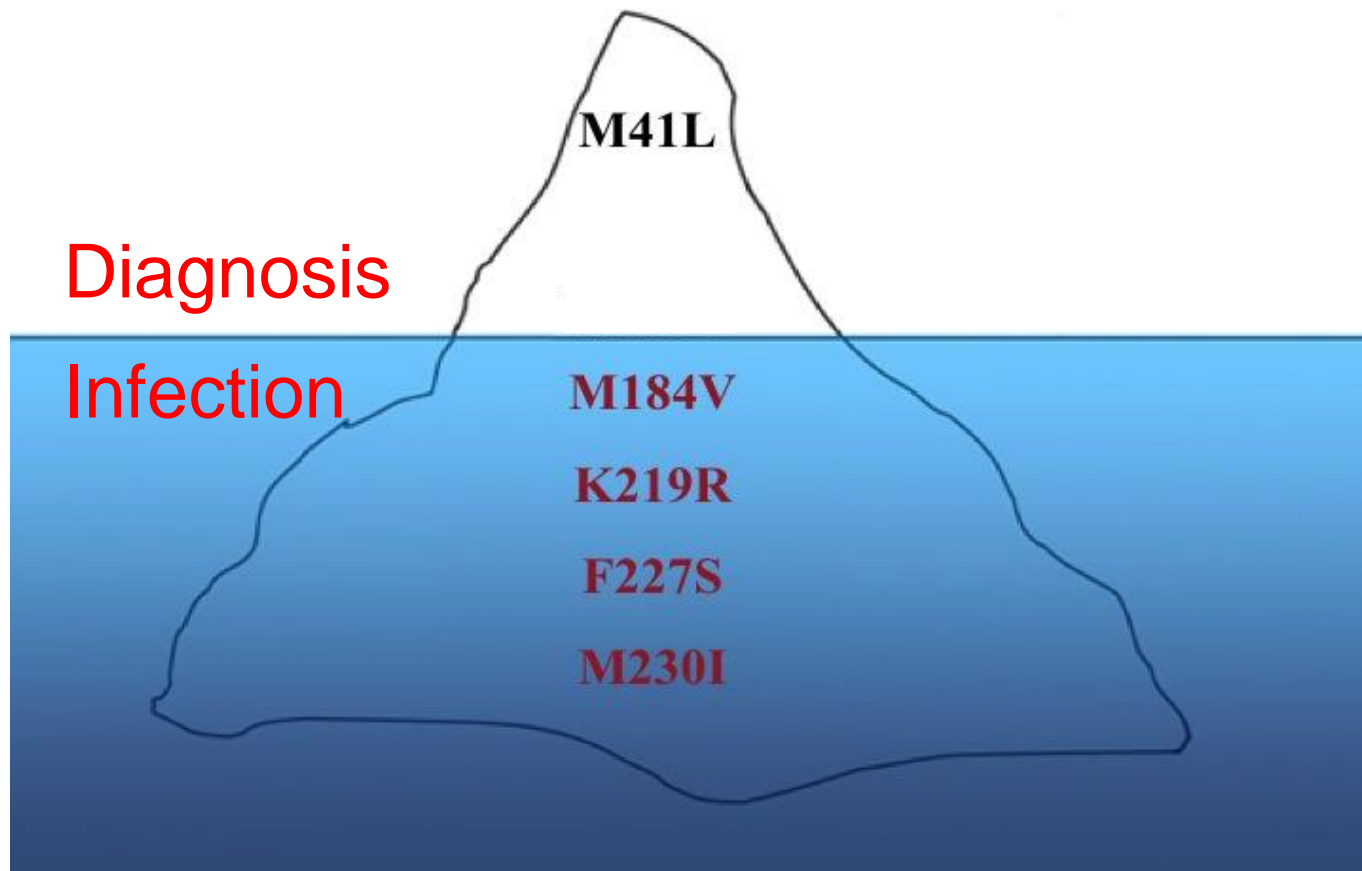
- Prevalence of transmitted drug resistance 8 -10% ¹

- Majority of transmitted drug resistance is limited to single mutations ²

1: Vercauteren et al JID 2008, Wensing et al JID 2005.

2: Wensing et al AIDS 2008.

Are single mutations still the tip of the iceberg, or do they represent true singletons?



Approach

- 10 patients identified in 2003-2008 with HIV harbouring a single mutation at a resistance-related codon detected by population sequencing
- Minority variation analysis of protease and RT was performed on plasma using 454 amplicon sequencing

Population based genotypic analysis

Patient	Resistance profile	Viral load
A	M41L	> 1,0 e5
B	T69N	> 1,0 e5
C	M184V	> 1,0 e5
D	T215E	> 1,0 e5
E	T215E	> 1,0 e5
F	T215L	> 1,0 e5
G	T215S	> 1,0 e5
H	T215S	> 1,0 e5
I	T215S	> 1,0 e5
J	K219Q	> 1,0 e5

454 amplicon sequencing results

Patient ID	Resistance mutations population sequencing	Codon	Patient ID	Resistance mutations population sequencing	Reverse Transcriptase						
					Codon	%Resistant	reads (F+R)	Codon	%Resistant	reads (F+R)	
A	M41L	41L	A	M41L	41L	81.7	2623				
B	T69N	69A	B	T69N	69A	2.0					
C	M184V	69N			69N	11.7	2516	<u>103N</u>	7.5	2967	
D	T215E	69D			69D	2.6					
E	T215E		C	M184V							
F	T215L		D	T215E	215E	95.0	2582				
G	T215S		E	T215E	215E	87.1	2115				
H	T215S		F	T215L	215L	98.2					
I	T215S		G	T215S	215S	98.5	2246				
J	K219Q		H	T215S	215S	96.7	2685				
			I	T215S	215S	98.5	2680				
			J	K219Q	219Q	96.9	2562	118I	99.7	2787	

454 pyrosequencing results

- Average number of reads (f+r): ~ 2500
- For 9/10 patients, no additional mutations could be identified using 454 amplicon sequencing
- Subsequently we looked at therapy outcome

Conclusions

- These data suggest that recently diagnosed patients infected with singletons may represent onward transmission of drug-resistant HIV variants
- Further clinical studies are warranted to determine the implication of singletons at baseline for the choice of initial therapy

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