Emergence of the H208Y mutation in the reverse transcriptase (RT) of HIV-1 in association with nucleoside RT inhibitor therapy

G. Nebbia1, Caroline A. Sabin2, D. T. Dunn3 and Anna Maria Geretti1* on behalf of the UK Collaborative Group on HIV Drug Resistance and the UK Collaborative HIV Cohort (CHIC) Study Group

1Department of Virology, Royal Free and University College Medical School, Rowland Hill Street, NW3 2PF London, UK; 2Department of Primary Care and Population Sciences, Royal Free and University College Medical School, Rowland Hill Street, NW3 2PF London, UK; 3Medical Research Council, Clinical Trial Unit, London, UK

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Objectives: The aim of the study was to determine whether mutations at RT codon 208 are associated with nucleoside RT inhibitor (NRTI) exposure, NRTI resistance patterns and HIV-1 subtype.

Methods: Six thousand three hundred and fifty two genotypic resistance tests linked to a clinical database were analysed.

Results: The prevalence of mutations at codon 208 was 6/2347 (0.3%) in treatment-naive and 165/4005 (4.1%) in treatment-experienced persons. H208Y was the most common mutation in both groups (0.2% and 3.8%, respectively) and occurred in 4.5% of treatment-experienced persons with Subtype B, 1.7% of those with Subtype C and 0.7% of those with other non-B subtypes (P < 0.001). The association with subtypes was independent of treatment experience. H208Y showed a strong association with NRTI experience, which persisted after adjusting for subtype [odds ratio (OR) 19.34; 95% confidence interval (CI) 7.87–47.54; P < 0.0001]. The prevalence of H208Y was highest in genotypes harbouring M184V and the thymidine analogue mutations (TAMs) M41L, D67N, L210W and T215Y. The median number of TAMs was 4 and 0 in genotypes with and without H208Y, respectively (P < 0.0001). The prevalence of H208Y declined over time, being highest in 1998 (9.9%) and lowest in 2003 (0.9%) (P = 0.0001).

Conclusions: There is a strong association between H208Y and NRTI experience, particularly in persons with Subtype B harbouring multiple NRTI resistance mutations. These findings indicate an accessory role for H208Y in NRTI resistance.

Keywords: resistance, mutations, lamivudine

Introduction

A number of substitutions in reverse transcriptase (RT), including those at codon 208, have been associated with nucleoside RT inhibitor (NRTI) exposure, although their role in conferring drug resistance in the absence of major NRTI resistance mutations has not been established. The H208Y mutation was first identified in HIV-1 variants resistant to foscarnet and associated with hypersusceptibility to zidovudine when present with G161L.1 The mutation was later detected in patients receiving dual therapy with zidovudine/lamivudine and shown to augment zidovudine resistance in mutants with M184V and thymidine analogue mutations (TAMs).2–3 An association between H208Y and major NRTI resistance mutations has been observed in clinical databases, and clustering with specific TAMs has been proposed.2–7 These observations suggest that H208Y has an accessory role in NRTI resistance.

Although an association between H208Y and treatment experience has been reported, data on specific NRTIs are conflicting. A study of 226 treatment-experienced persons suggested an association with the use of zidovudine, stavudine and possibly abacavir,4 whereas an analysis of 1355 persons proposed an association with stavudine, didanosine and abacavir.6 The aim of this study was to interrogate a database comprising 6352 persons...
to determine whether mutations at codon 208 increase in prevalence in NRTI-experienced persons, are associated with specific NRTIs and NRTI resistance patterns and cluster with the HIV-1 subtype. To the best of our knowledge, this is the largest data set analysed to date and the only analysis that has addressed non-B subtypes.

Methods

The data set comprised treatment-naive and treatment-experienced persons (median age 36 years, range 11–83) who underwent genotypic resistance testing in 1997–2003. RT nucleotide sequences were obtained from the UK HIV Drug Resistance Database, which contains results from all patients undergoing resistance testing in the UK. When more than one test was available from an individual, only the first genotype was retained. Clinical data were derived through a link with the UK Collaborative HIV Cohort (CHIC) study, which collects data from six centres in London and Brighton. NRTI exposure was zidovudine 40.7%, lamivudine 40.1%, stavudine 31.5%, didanosine 25.5%, zalcitabine 11.5%, abacavir 8.5%, tenofovir 1.2% and adeefovir 0.1%. Only those NRTIs to which at least 1.5% of the study population had been exposed were included in the analysis. Univariable comparisons were performed using $\chi^2$ tests and independent associations identified using multiple logistic regression. Wilcoxon rank sum test was used to determine the relationship between H208Y and number of TAMs. The study was approved by the UK Multicentre Research Ethics Committee and relevant local research ethics committees.

Results

Prevalence of mutations at RT codon 208

There were 6352 resistance tests identified. Their distribution by year was 1997, 510 (8.0%); 1998, 689 (10.9%); 1999, 957 (15.1%); 2000, 1096 (17.3%); 2001, 1242 (19.6%); 2002, 1256 (19.8%) and 2003, 602 (9.5%). Subtypes were B (71.6%), C (15.6%) and other non-B (12.8%). Among 2347 treatment-naive persons, 6 (0.3%) harboured a mutation at position 208. Five persons (0.2%), all infected with Subtype B, showed H208Y in the absence of major NRTI mutations. One person (0.04%) harboured H208L. Among 4005 treatment-experienced persons, 165 (4.1%) harboured a mutation at position 208, most commonly H208Y (150, 3.8%) as either a single mutant ($n = 112$) or an H208H/Y mixture ($n = 38$). Other mutations comprised H208Q and H208F (0.1%) and multiple other changes (N/A/C/E/K/T) each found in 0.02% to 0.05% of genotypes. H208Y was more prevalent among treatment-experienced persons infected with Subtype B (4.5%) than in those with Subtype C (1.7%) or other non-B subtypes (0.7%) ($P = 0.001$) and the relationship remained significant after controlling for treatment exposure ($P = 0.0003$). The prevalence of H208Y by year was 1997, 4.5%; 1998, 9.9%; 1999, 5.1%; 2000, 2.9%; 2001, 3.3%; 2002, 2.4% and 2003, 0.9% ($P = 0.0001$). The trend in time was independent of subtype prevalence in the database.

As a comparator, the database was interrogated for the prevalence of mutations at RT codon 207, which have not been associated with NRTI exposure. Substitutions were observed in 921 (39.2%) treatment-naive and 1493 (37.3%) treatment-experienced persons. The most common substitutions in treatment-naive/treatment-experienced persons were Q207E (23.4%/21.6%) and Q207A (6.3%/7.7%), followed by Q207D (2.0%/2.1%), Q207G (1.8%/1.5%), Q207H (0.8%/1.1%), Q207K (3.1%/2.5%), Q207N (1.3%/1.1%) and multiple other changes (L/M/P/R/S/T/V/X/Y) each found in <1% of genotypes. Among both treatment-naive and treatment-experienced persons, the prevalence of Q207A/E was higher in Subtype C (68.0%/62.4%) and other non-B subtypes (80.4%/67.4%) than in Subtype B (17.8%/16.8%) ($P = 0.0001$ for all comparisons).

Association between H208Y and treatment exposure

Comparison of treatment-naive and treatment-experienced persons showed that H208Y, but not Q207A/E, was associated with treatment experience, with an unadjusted $P$ value of 0.0001 for H208Y and 0.85 for Q207A/E. The odds ratio (OR) associated with treatment experience, adjusted for the year of testing and for whether the test was done for research or clinical reasons, was $20.50$ [95% confidence interval (CI) 8.37–50.22; $P = 0.0001$] for H208Y and $0.97$ (95% CI 0.86–1.08; $P = 0.55$) for Q207A/E. The OR adjusted for the HIV-1 subtype was $19.34$ (95% CI 7.87–47.54; $P = 0.0001$) and 1.08 (0.96–1.21; $P = 0.22$) for Q207A/E. In univariable analyses, H208Y was significantly more prevalent in persons exposed to any of the NRTIs analysed, including zidovudine, lamivudine, stavudine, zalcitabine, didanosine and abacavir (all $P = 0.0001$). Multivariable analyses showed an independent association with lamivudine and zalcitabine (Table 1). No association was found between Q207A/E and exposure to zidovudine ($P = 0.53$), lamivudine ($P = 0.19$), stavudine ($P = 0.94$), zalcitabine ($P = 0.38$), didanosine ($P = 0.97$) or abacavir ($P = 0.93$).

Relationship between H208Y and presence of M184V and TAMs

The prevalence of H208Y was higher in genotypes with M184V and TAMs than in genotypes without these mutations (all $P = 0.0001$) (Figure 1). The median (range) number of TAMs was 4 (0–6) and 0 (0–6) in genotypes with and without H208Y, respectively ($P = 0.0001$). The prevalence of H208Y was highest (>10%) in the presence of M41L, D67N, L210W and T215Y (Figure 1); all four TAMs were independently associated with H208Y after adjustment for each other ($P < 0.001$ in each case). Although the relationship with T215F was weaker than that with T215Y, both mutations were significantly associated with H208Y. The prevalence of Q207A/E was high (>20%) in genotypes with and without major resistance mutations.

Discussion

This study showed that RT position 208 is highly conserved among treatment-naive persons, thus confirming previous findings. In contrast, there was a high degree of polymorphism at codon 207, especially among non-B subtypes. Mutations at codon 208 occurred in treatment-experienced persons, although their prevalence remained relatively low at 4.1%. The highest prevalence was seen in treatment-experienced persons with Subtype B. For both Subtypes B and C, the consensus nucleotide sequence at position 208 was CAT, whereas Y was encoded
by TAT. Thus, in both subtypes, the H→Y substitution required one base change. Mechanisms underlying preferential selection in Subtype B are being evaluated in vitro.

Independently of subtypes, there was a strong association between H208Y and NRTI exposure. Lamivudine and zalcitabine were each independently associated with the mutation and the high prevalence of H208Y in genotypes with M184V was consistent with this observation. In contrast to data derived from smaller data sets,4–6 we found no independent association with zidovudine, stavudine, didanosine or abacavir, possibly reflecting the greater power of a larger data set in identifying independent associations. When using a stepwise selection procedure to select final variables, a weak association emerged with didanosine exposure (OR 1.62; 95% CI 1.09–2.39; P = 0.02). However, given the fact that the variables may be highly correlated, it cannot be ruled out that associations exist with NRTIs other than lamivudine and zalcitabine. This may partly explain the association found with the presence of TAMs, despite the apparent lack of direct association with exposure to the thymidine analogues zidovudine and stavudine.

The prevalence of H208Y was significantly higher in genotypes containing multiple TAMs than in genotypes without these mutations, indicating emergence with prolonged NRTI-selective pressure. Consistent with these findings, preliminary data from clonal analysis indicate that H208Y is present on viral genomes containing multiple TAMs and M184V (data not shown). A preferential association with the pattern comprising M41L, L210W and T215Y (referred to as TAM1) has been proposed.2–7 In this study, the prevalence of H208Y was highest in genotypes with TAM1 mutations and D67N, but was also significantly higher in genotypes with K70R, K219Q/E and T215F than in those without, indicating a preferential but not absolute association with the TAM1 pathway. Given the association between H208Y and the presence of major NRTI resistance mutations, it is uncertain whether including the mutation in interpretative algorithms would add to the prediction of drug resistance. From a more mechanistic perspective, however, the data suggest that significant interactions occur between H208Y and key RT positions, which may explain the apparently contradictory effects on zidovudine susceptibility in combination with G161L1 or M184V and TAMs.2 The mutation is located in the palm domain of RT and is unlikely to make direct contact with the enzyme catalytic residues or the incoming dNTP.8 Its involvement in resistance to foscarnet (a pyrophosphonate) suggests a possible influence of incorporated nucleotides on pyrophosphorylation.8 Reversal of zidovudine hypersusceptibility induced by M184V can be postulated, explaining the association with both lamivudine and presence of TAMs. Given the structural proximity with L210W and T215Y/F, a possible compensatory effect can also be proposed that rescues drug-resistant virus crippled by major resistance mutations.3,6 Consistent with this hypothesis, treatment failure in the presence of H208Y has been associated with high HIV-1 plasma RNA load and low CD4 counts.6 Arguing against this hypothesis, the double mutant H208Y/T215Y shows reduced replicative capacity relative to both wild-type virus and the single mutants.9 Recently, H208Y has also been associated with non-NRTI (NNRTI) hypersusceptibility when present with T215Y, but the mechanism of the effect has not been elucidated.9,10

There was a significant decline in the prevalence of H208Y between 1997 and 2003 when analysing the first genotype from each patient. This could reflect changes in prescription patterns, especially reduced use of zalcitabine. Although the prevalence of non-B subtypes has increased in recent years in the UK, the trend remained significant after adjusting for subtype. A likely determinant is the decline in genotypes with multiple TAMs (1997, 13%; 2003, 4.2%), reflecting improved use of therapy and prompt management of treatment failure.

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UK Collaborative Group on HIV Drug Resistance Steering Committee: S. Burns, City Hospital, Edinburgh; S. Cameron, Gartnavel General Hospital, Glasgow; P. Cane, Health Protection Agency, Porton Down; I. Chrystie, St Thomas’ Hospital, London; D. Churchill, Brighton and Sussex University Hospitals; V. Delpech, D. Pillay, Health Protection Agency, London; D. Dunn, E. Fearnhill, H. Green, K. Porter, MRC Clinical Trials Unit,* London; P. Easterbrook, M. Zuckerman, King’s College Hospital, London; AM Geretti, Royal Free Hospital, London; P. Kellam, D. Pillay, A. Phillips, C. Sabin, Royal Free and University College Medical School, London; D. Goldberg, Health Protection Scotland, Glasgow; M. Gompels, Southmead Hospital, Bristol; T. Hale, Health Protection Agency, Leeds; S. Kaye, St Mary’s Hospital, London; A. Leigh-Brown, University of Edinburgh; C. Orkin, St Bartholomew’s Hospital, London; A. Pozniak, Chelsea and Westminster Hospital, London; G. Robb, Department of Health, London; E. Smit, Health Protection Agency, Birmingham Heartlands Hospital; P. Tilston, Manchester Royal Infirmary; I. Williams, Mortimer Market Centre, London. *Coordinating Centre.

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Transparency declarations

None to declare.

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