Codon 215 Mutations in Human Immunodeficiency Virus–Infected Pregnant Women

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In 1994, the Pediatric AIDS Clinical Trials Group (PACTG) Protocol 076 demonstrated a two-thirds reduction of perinatal human immunodeficiency virus (HIV) type 1 transmission with zidovudine chemoprophylaxis. However, zidovudine alone does not fully suppress HIV replication, and chemoprophylaxis with zidovudine alone might select for zidovudine-resistant viral variants, decreasing the efficacy of zidovudine prophylaxis and affecting future responses to combined antiretroviral regimens. Sixty-two HIV-infected pregnant women consecutively enrolled in the ongoing Swiss HIV and Pregnancy Study were prospectively evaluated for the presence or development of zidovudine resistance by analysis of codon 215 of the reverse transcriptase gene. Six women (9.6%) harbored a codon T215Y/F mutation, which is associated with high-level resistance to zidovudine. Postnatal evaluation was completed in all children of mothers harboring the mutation. None was HIV-infected. The observed prevalence of codon 215 mutations of 9.6% raises important concerns regarding the future use of the PACTG 076 regimen.

Vertical transmission remains the major cause of human immunodeficiency virus (HIV) infection in children, and the reduction of transmission is a major concern worldwide. In February 1994, the results of Pediatric AIDS Clinical Trials Group (PACTG) Protocol 076 demonstrated that zidovudine chemoprophylaxis during pregnancy, delivery, and to newborns reduced perinatal HIV-1 transmission by two-thirds [1]. These dramatic results have been confirmed in numerous epidemiologic studies [2] and in the final PACTG 076 study analysis [3], and although more aggressive combination regimens are actually recommended for HIV-infected pregnant women [4], the PACTG 076 regimen as recommended by the US Public Health Service Task Force in 1994 [5] was the reference standard for general clinical practice when this study was started. As zidovudine monotherapy cannot achieve complete suppression of viral replication, there is a concern that it might select for zidovudine-resistant viral variants and thus decrease the efficacy of the prophylaxis on transmission and affect future responses to combined antiretroviral regimens.

A high rate of viral replication in conjunction with a high genetic variability due to low fidelity of viral reverse transcriptase (RT) readily leads to the emergence of viral variants resistant to antiretroviral drugs under selective pressure, especially with incompletely suppressive regimens. Several mutations of the RT gene have been identified in patients treated with zidovudine, the most common being the codon 215 mutation [6]. Vertical transmission of a zidovudine-resistant virus was first reported in 1994 [7], raising important concerns about the efficacy of prophylactic zidovudine chemotherapy.

To evaluate the prevalence and the appearance of codon 215 mutation associated with zidovudine-resistance in HIV-infected pregnant women and its consequences on vertical transmission, we prospectively analyzed the presence or emergence of mutations at codon 215 of the RT gene in 62 consecutive HIV-infected pregnant women enrolled in the Swiss HIV and Pregnancy Study.

Patient Population and Methods

The Swiss HIV and Pregnancy Study enrolls HIV-infected pregnant women at any time during pregnancy until delivery or termination. A detailed history is obtained at enrollment; physical examinations are performed and blood samples are collected for immunologic, virologic, and hematologic evaluations at each trimester, at delivery, and at 6 weeks and 6 months thereafter, according to a standard protocol [8].

The infants are followed by the Swiss Neonatal HIV Study [9], and virologic analyses are done during the first week of life and at ages 2 and 6 months. Negative virologic test results are confirmed serologically by seroreversion at age 18 months. Children are classified as infected if they have detectable HIV antibodies beyond age 24 months or if they have positive virus detection test results

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by at least 2 methods (antigen after heat-mediated disruption of immune complexes, polymerase chain reaction [PCR]) in ⩾2 different samples. For classification as uninfected, the absence of HIV-related symptoms and either no detectable HIV antibodies beyond age 15 months or negative virus detection tests in ⩾2 samples after the age of 1 month by 2 different methods are required. All other children are classified as indeterminate.

Sixty-two consecutively enrolled women were prospectively included in a subprotocol started in March 1995 to evaluate zidovudine resistance by the detection of mutations at codon 215 of the RT gene. The RT gene was sequenced in all patients with mutation at codon 215.

Analysis of RT gene codon 215. Blood was collected without anticoagulant, centrifuged twice for 5 min at 1500 *g,* aliquoted, and stored at −75°C within 2 h. Total RNA in 50 μL of serum was extracted using an automated system (model 540; Autogen Instrument, Beverly, MA). RNA purification was based on the single-step method of acid guanidinium thiocyanate–phenol–chloroform extraction [10]. Total RNA was reverse transcribed and amplified by selective PCR [11]. In brief, the first PCR step was done with 0.25 μg of primers A (5′-AATTTTCCCATTAGTCCTATT) and NE (5′-TATGTCATTGACAGTCCAGCT) with reaction mixture that contained 50 m*M* KCl, 10 m*M* Tris-HCl (pH 8.3), 3 m*M* $MgCl_2$, 10% glycerol, 200 μ*M* of each dNTP, and 2.5 U of Amplitaq polymerase (Perkin-Elmer Cetus, Norwalk, CT). One microliter of a 1:1- and 1:100-diluted PCR product was reamplified in a second selective PCR. To identify wild type mutant at codon 215, primer pair B/3W was used, and primer B was paired with 3M. The reactions were subjected to 35 cycles of 20 s at 95°C, 30 s at 50°C, and 30 s at 72°C by DNA thermal cycler (Perkin-Elmer Cetus). PCR products were separated through 2% Nu-Sieve agarose. An alternative primer pair was used in first PCR for patients with negative results (NA 5′-CCTATTGAAACTGTACCAGT and NNE 5′-ACTGTCCATTTATCAGGATG).

Direct sequencing of RT gene. Amplicons of the first PCR (5 μL) were reamplified with 0.25 μg of primers NNA 5′-AAGCCAGGAATGGATGGCCCA and E biotinylated 5′-CCATTTATCAGGATGGAGTTC in a reaction mixture containing 50 m*M* KCl, 10 m*M* Tris-HCl (pH 8.3), 3 m*M* $MgCl_2$, 250 μ*M* of each dNTP, and 2.5 U of Amplitaq polymerase. The reactions were subjected to 30 cycles of 20 s at 95°C, 30 s at 50°C, and 30 s at 72°C. For single-strand sequencing, 45 μL of nested PCR product was mixed with 250 μg of streptavidin-coated magnetic beads (Dynabeads M280; Dynal, Oslo) in 40 μL of 10 m*M* Tris-HCl (pH 7.5), 1 m*M* EDTA, and 2 *M* NaCl and kept at room temperature for 20 min, before the product was denatured with 16 μL of NaOH 0.1 *M* for 10 min and bead-bound single strand was recovered for sequencing. The complementary strand was recovered from the supernatant after purification by CentriSep column (Applied Biosystems, Foster City, CA). Primer NNA was used to analyze the 30–130 codon region and primer E for the 130–228 codon region. The nucleotide sequence was determined by terminator cycle sequencing kit (AmpliTaq FS dyedeoxy; Applied Biosystems) and automatic sequencer (model 373; Applied Biosystems). Sequence alignments were done with PCGENE software (IntelliGenetics, Mountain View, CA).

Results

Sixty-two consecutively enrolled HIV-infected women (64 newborns, 62 deliveries, 2 pairs of twins, 1 abortion, 1 woman with 2 pregnancies) were included in this study. In total, 116 blood samples (mean, 1.8/woman; range, 1–5) were collected for analysis of RT gene codon 215. Negative PCR results were obtained in 19 samples (16%), most with <1000 HIV-1 RNA copies/mL. A T215Y/F mutation associated with high-level zidovudine resistance was found in 12 (12%) of 97 samples, corresponding to 6 (9.6%) of 62 women. However, the prevalence of zidovudine-resistant variants may be underestimated, since only the presence of mutations at codon 215 was analyzed.

Fifty-eight (95%) of 62 women were naive for antiretroviral drugs at inclusion. Prophylactic zidovudine chemotherapy was given to 51 women and their 52 neonates, including 1 pair of twins. Seven women (11%) refused antiretroviral treatment. For the remaining 4 pregnancies, no information regarding zidovudine chemoprophylaxis was available.

The 6 women harboring mutation at codon 215 tended to be in a more advanced stage of HIV infection than patients with 215 wild type virus with mean CD4 cells/μL of 333 ± 218 for the former and 439 ± 264 for the latter (no statistical difference). Results of the codon 215 analysis and of antiretroviral therapy in these 6 women is shown in figure 1.

Mutation at codon 215 was detected in the first available sample from patients 1, 2, 5, and 6. Patient 1 received zidovudine for 4 years before study entry. Patient 2 began zidovudine treatment 2 weeks after her last menstrual period because of immunologic deterioration, and codon 215 mutation was detected after 24 weeks of treatment. In this patient, because of the detection of mutant virus, lamivudine was added to zidovudine 1 month before delivery. Mutation at codon 215 was detected in the first available sample from patient 5 after only 2 weeks of zidovudine treatment. She had received intravenous zidovudine during a previous delivery (11 months earlier) and had not been treated with zidovudine before or between pregnancies. The retrospective analysis of a plasma sample collected at the time of her first delivery revealed 215 wild type virus. Patient 6 was infected about the time of conception, probably with a mutant virus strain, as the 215 mutation was detected on the first available sample before initiation of zidovudine prophylaxis, within 1 year after seroconversion. Her neonate was given oral didanosine in addition to postnatal zidovudine treatment (weeks 2–6 after birth).

Development of mutation at codon 215 during zidovudine therapy was observed in at least 1, possibly 2 formerly zidovudine-naive patients: patient 3 after 17 weeks (11 weeks in combination with lamivudine) and likely in patient 4 after 22 weeks of zidovudine monotherapy. This patient showed a mixed population of wild type and mutant virus in the first available sample.

HIV infection status was determined for 46 children (72%);

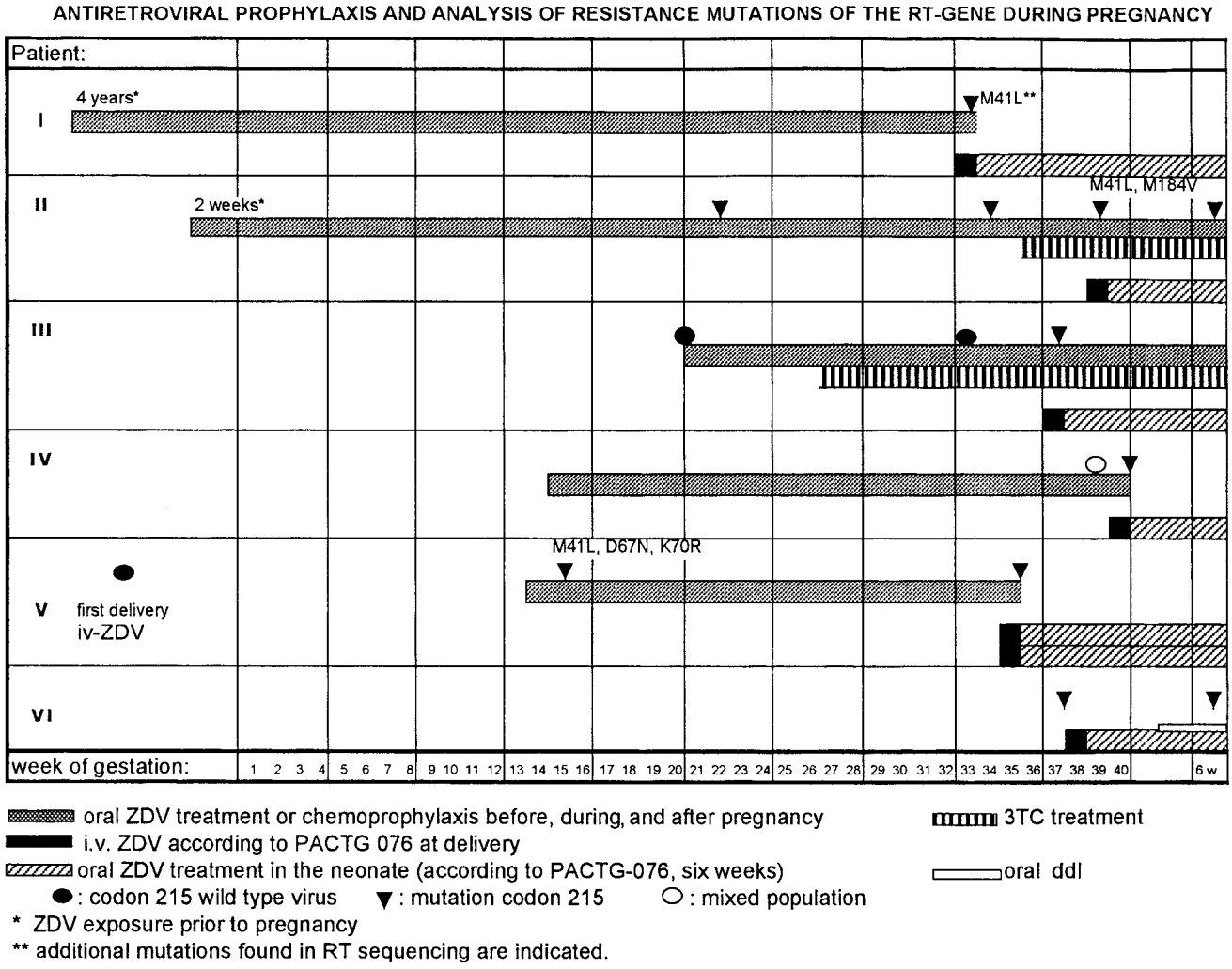

Figure 1. Antiretroviral prophylaxis and analysis of resistance mutations of reverse transcriptase (RT) gene in 6 pregnant HIV-infected women. iv, intravenous; ddI, didanosine; PACTG, Pediatric AIDS Clinical Trials Group; 3TC, lamivudine; ZDV, zidovudine.

the remaining 18 children could not be classified because of young age or because the parents refused testing. Forty-three children are known to be HIV-negative and 3 children are infected, resulting in a transmission rate of 6.5%. Characteristics of the mothers who transmitted the infection are shown in table 1. None of the 7 children born to mothers with a mutation at codon 215 was infected (95% confidence interval, 0%–41%).

Discussion

Zidovudine was the most widely used antiretroviral drug between 1987 and 1995, and HIV variants with decreased susceptibility to zidovudine were first reported in 1989 [12]. Sequencing of the HIV RT gene revealed a number of mutations that influence viral sensitivity to zidovudine and thus can be

Table 1. Characteristics of the 3 HIV-transmitting women at delivery.

Child	HIV RNA (copies/mL)	CD4 (cells/L)	CDC stage	Mode of delivery	Age (weeks)	Time from membrane rupture to birth	Zidovudine (weeks)[a]	Remarks
A	68,517	16	B3	SC	28	160 h	1 week	
B	NA	NA	A1	V	42	15 min	28 weeks	Methadone during pregnancy
C	NA	443	A2	SC	34	18 h	6 weeks	HIV-1 and -2 coinfection in mother

NOTE. NA, not available; SC, secondary cesarean; V, vaginal.
[a] Duration of zidovudine therapy before delivery.

used as genotypic markers for the presence of zidovudine resistance. Horizontal transmission of zidovudine-resistant virus was first reported in 1993 [13].

Our data demonstrate that virus bearing T215Y/F mutant codon associated with high-level zidovudine resistance is frequently detected in HIV-infected pregnant women, since it was found in 6 of 62 consecutive pregnant women (prevalence, 9.6%). Our data suggest that mutant variants may develop under the selective pressure of zidovudine prophylaxis during pregnancy.

The mechanisms for the reduction of vertical transmission with zidovudine chemoprophylaxis according to PACTG 076 are still not fully understood. Results of a large study demonstrate that the effect on maternal HIV-1 RNA level cannot fully account for the observed efficacy of zidovudine in reducing transmission and that preexposure prophylaxis of the fetus/infant could be an important protective component [3]. In a previous evaluation of genotypic resistance in women of the PACTG 076 study [14], none of the women showed high-level zidovudine resistance at study entry or delivery.

None of the 7 children born to mothers harboring mutation at codon 215 was vertically infected. However, given an actual transmission rate of <10% with chemoprophylaxis alone and even less when combined with a primary cesarean section [9], much larger numbers of mother-child pairs will be needed to estimate the effect of mutations associated with resistance on the efficacy of zidovudine prophylaxis. It also remains to be seen whether the emergence of resistance mutations due to zidovudine chemotherapy during pregnancy could adversely influence the subsequent evolution of HIV infection in women and in infected children.

In conclusion, although emergence of mutations associated with zidovudine resistance are of concern, as they are present in 9.6% of our subjects, they were not associated with infection in newborns. This might support zidovudine prophylaxis in countries where access to antiretroviral drugs is limited. Current recommendations for antiretroviral treatment in pregnant women [4] include protease inhibitor–containing regimens. Recent data indicate that these regimens may be associated with potential toxicity in the newborn and, thus, optimal drug regimens remain to be defined [15].

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