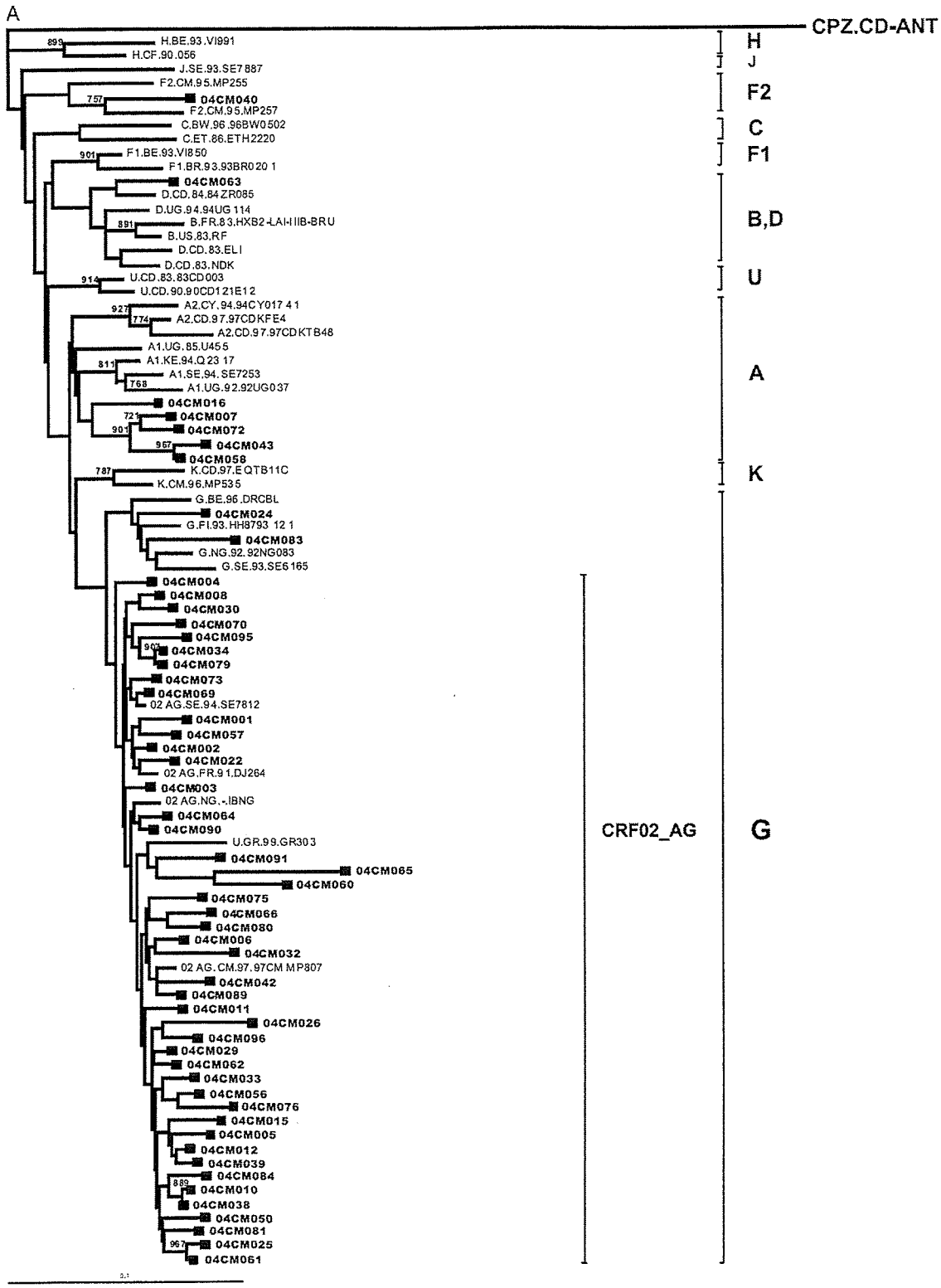

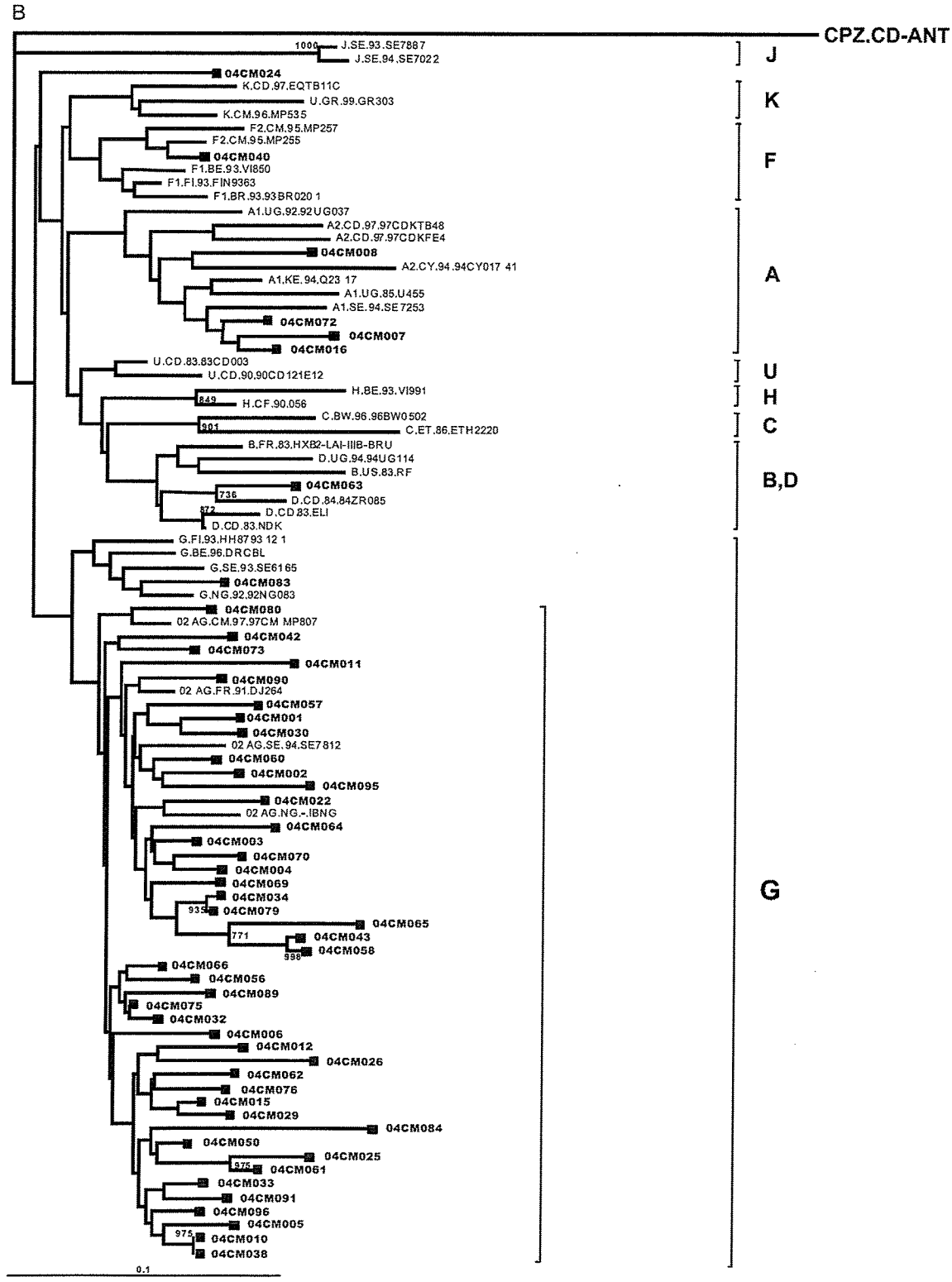

FIGURE 1. *(continued)*

As for secondary mutations, *E44D*, which is associated with multi-NRTI resistance,[29] was detected in 1 case with full clones; yet there were no coexistent primary mutations.

Secondary mutations as minor populations were omitted from the figure because they were numerous, and their importance is likely much less than that of the other mutations.

DISCUSSION

In the current study, we found PI and RTI resistance–associated primary mutations in 7.4% and 9.8%, respectively, of drug-naive HIV-1–infected individuals in Cameroon when drug-resistant minor populations were included. This is one of the first reports of the emergence of primary ART resistance–associated mutations among drug-naive, non-B subtype HIV-1–infected individuals in Cameroon.

The current study was designed to evaluate the prevalence of potential resistant strains as minor populations, so we used proviral DNA extracted from PBMCs and analyzed several clones per sample. There is no doubt that direct sequencing of plasma RNA is the gold standard for drug resistance surveillance.[31] Recently, however, it has been reported that conventional genotype testing may overlook minor virus populations if their frequencies are less than 25%.[19,20] Minor resistant strains that eventually overgrow and affect the clinical course of disease can emerge from levels of virus that are undetectable by conventional assays.[32,33] Therefore, we focused on the baseline frequency of the dormant hazard in a newly ART-promoting country.

Unlike developed countries where PI-including regimens are frequently administered, the current first line of ART in Cameroon is the combination of 2 NRTIs and 1 NNRTI, which means that PI use is limited compared with that of industrialized countries. Konings et al[23] reported that only secondary mutations associated with PI resistance were detected among drug-naive HIV-1 patients in Cameroon during 2000 to 2002. Nonetheless, in our study, primary mutations of PI resistance were detected in drug-naive patients at the rate of 7.4% as of February 2004. This is consistent with findings by Ndembi et al suggesting the emergence of the drug resistance primary mutations during the last few years (personal communication). The HIV-1 strains with primary mutations were found to be CRF02_AG, subtypes A1 and F2, which circulate in west central Africa. It would, therefore, be natural to explain this phenomenon by simple transmission of the resistant strains from patients treated with PIs in Cameroon, although PI use has been limited in this country. However, the possibility that genetic diversity gave birth to resistant strains in the drug-naive individuals cannot be excluded. In addition, the possibility of laboratory artifact that was induced by PCR error cannot be fully excluded either.

As for RTI resistance, previous reports have not detected primary mutations in sub-Saharan Africa during 1999 to 2003,[34,35] including the report by Konings et al[36] that was based on samples collected in Cameroon between 2000 and 2002. In our study, samples were collected in western Cameroon in February 2004, and 5 cases (9.8%) yielded HIV-1 strains with primary mutations, although all were found as minor populations. Recently, there have been several reports noting the importance of drug-resistant strains detected as minor populations. Minor drug-resistant HIV-1 populations have been detected both in the early phase of treatment failure[32,33] and during successful structured treatment interruption.[21] Minor drug-resistant populations undetectable by conventional assays can eventually overgrow and affect the clinical course. They also have been found to persist longer than previously expected in untreated patients, a favorable condition for wild-type virus to overgrow,[37–39] which also indicates the risk of resistance transmission even from minor strains. Thus, careful follow-up studies should be conducted to assess whether the drug-resistant mutants found in our study as minor populations might impact future ART.

Most of the published data focusing on minor populations of resistant strains are based on the analysis of subtype B strains using quantitative real-time PCR with specific primers for *V82A*, *L90M*, or *M184V* of RT.[21,40] In our study, the entire regions of PR and RT genes were amplified, cloned, and genetically analyzed to identify mutation sites other than these 3. This is particularly important in the context of analyzing non-B subtype HIV-1 strains, which often contain polymorphisms. As a result, we detected some other important mutations as minor populations, such as *V82A* in the PR gene and *V75I*/*Y188C* in the RT gene.

In our study, several non-B subtype-specific polymorphisms were confirmed in the protease gene. First, we detected *K20I/N* and *M36I/N* at relatively higher frequencies than previous reports. Because Holguin et al reported that *K20I* and *M36I* were detected in all cases of subtype G strains,[30] the higher rate can be explained by the fact that most of our samples were CRF02_AG, whose *Pol-PR* is subtype G. Other secondary mutation sites that we detected, such as L10, L63, and V77, are consistent with previous reports that consider these mutations non-B subtype natural polymorphisms. Secondly, *V82I* was detected in 2 cases, one of which belonged to subtype G; and the amino acid change could be regarded as a subtype-specific natural polymorphism.[28] In this study, there were no other subtype-specific polymorphisms that could cause considerable natural drug resistance.

The subtype distribution of HIV-1 circulating in western Cameroon was found to be slightly different from those of other districts.[16–18,23,36] The predominance of CRF02_AG was much more conspicuous in the western district (85%) than in other districts (about 60%), although the prevalences of other constituents such as A1, G, F2, and D were almost identical to those in other reports. As expected, independent recombinants were detected between CRF02_AG and subtype A1, D, or G, although the rate of recombination in this area was slightly lower (16.7%) than in other areas of Cameroon (about 21%).[18] Still, the independent recombinants carry the potential hazard of developing natural resistance.

In conclusion, as of February 2004, primary mutations of the HIV-1 protease exist in drug-naive patients in rural western Cameroon, despite little use of protease inhibitors. As the scheduled rapid provision of ART can give rise to resistant strains in developing countries, we should be careful not to overlook the emergence of resistant strains. More importantly, we also observed the emergence of minor populations of both RTI- and PI-resistant strains, which could have been

underestimated by conventional surveillance. Thus, careful drug regimen design is needed to prevent the rise of overt resistant strains from minor populations.

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