Genetic defects in the nef gene are associated with Korean Red Ginseng intake: monitoring of nef sequence polymorphisms over 20 years

Young-Keol Cho 1*, Jung-Eun Kim 1, Jun-Hee Woo 2

1 Department of Microbiology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea
2 Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

A R T I C L E   I N F O

Article history:
Received 18 December 2015
Received in Revised form 19 February 2016
Accepted 19 February 2016
Available online 3 March 2016

Keywords:
AIDS/HIV-1
Genetic defects
Ginseng
Korean Red Ginseng
nef

A B S T R A C T

Background: The presence of gross deletions in the human immunodeficiency virus nef gene (gΔnef) is associated with long-term nonprogression of infected patients. Here, we investigated how quickly genetic defects in the nef gene are associated with Korean Red Ginseng (KRG) intake in 10 long-term slow progressors. Methods: This study was divided into three phases over a 20-yr period; baseline, KRG intake alone, and KRG plus highly active antiretroviral therapy (ART). nef gene amplicons were obtained using reverse transcription polymerase chain reaction (PCR) and nested PCR from 10 long-term slow progressors (n = 1,396), and nested PCR from 36 control patients (n = 198), and 28 ART patients (n = 157), and these were then sequenced. The proportion of gΔnef, premature stop codons, and not in-frame insertion or deletion of a nucleotide was compared between three phases, control, and ART patients. Results: The proportion of defective nef genes was significantly higher in on-KRG patients (15.6%) than in baseline (5.7%), control (5.6%), on-KRG plus ART phase (7.8%), and on-ART patients (6.6%; p < 0.01). Small in-frame deletions or insertions were significantly more frequent among patients treated with KRG alone compared with controls (p < 0.01). Significantly fewer instances of genetic defects were detected in samples taken during the KRG plus ART phase (7.8%; p < 0.01). The earliest defects detected were gΔnef and small in-frame deletions after 7 mo and 67 mo of KRG intake, respectively. Conclusion: KRG treatment might induce genetic defects in the nef gene. This report provides new insight into the importance of genetic defects in the pathogenesis of AIDS.

© 2017 The Korean Society of Ginseng, Published by Elsevier Korea LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The introduction of highly active antiretroviral therapy (ART) from 1996 has dramatically reduced human immunodeficiency virus (HIV)-associated morbidity and mortality and transformed HIV infection into a chronic, manageable condition [1]. Even during sustained ART-mediated viral suppression, low-level viremia persistently occurs [2]. As a result of viremia, chronic immune activation and inflammation are accompanied by microbial translocation [3]. By contrast, there is no microbial translocation or immune activation in the well-adapted natural simian immune deficiency virus in hosts such as the sooty mangabeys (Cercocebus atys) [4], which is somewhat similar to the attenuated immune activation and slow progression observed in HIV-1-infected patients who have been treated with Korean Red Ginseng (KRG) for an extended period [5,6]. Interestingly, the saponin fraction of ginseng downregulates proinflammatory mediators in lipopolysaccharide-stimulated cells and protects mice against endotoxic shock [7–9] as well as anti-inflammatory activity [10]. In general, it is known that red ginseng has significantly higher biological effects and fewer side effects compared with fresh and white ginseng [11].

Among HIV-infected patients, many reports suggest that long-term nonprogressors (LTNPs) harbor grossly mutated copies of HIV nef more frequently than progressors [5,12–16], although there is some debate about the association between gross deletions in the nef gene (gΔnef) and long-term nonprogression or slow progression [17,18]. In an Australian LTNP study group, three of 70 LTNPs were infected only with viruses containing nef-defective genomes, with no wild-type (WT) virus present [11,12]; all had viral loads <200 copies/mL [15]. Some reports indicate that defects in the nef
gene are more common during the late stages of disease [19]; however, the proportions of $g\Delta nef$ and small in-frame deletions (SDs) have never been investigated in relation to the effect of treatment in Western patient groups.

We had an opportunity to treat HIV-1-infected patients with KRG from 1991. Data analysis over a period of 60 mo revealed that KRG intake significantly delayed the decrease in CD4+ T cells compared with zidovudine monotherapy [20,21]. Using KRG, many patients maintained their CD4+ T cell counts for more than 10 yr without receiving ART. In addition, there was a significant inverse correlation found between KRG and the annual decrease of CD4+ T cells and between KRG and RNA copy number in our previous study [22]. Furthermore, we reported that, in addition to clinically beneficial effects, a high frequency of genetic defects, including $g\Delta nef$ [5,6,22–25], are associated with the long-term intake of KRG [26]. Consequently, we reported many long-term slow progressors (LTSPs) among a small Korean cohort of HIV-1 patients who have taken KRG for more than 10 yr [26]. Among the LTSPs, two patients have remained healthy for 26 yr after diagnosis in the absence of ART [26,27]. In the literature, Patient 87–05 might be the longest follow-up case in the absence of ART. Interestingly, all Korean LTSPs treated with KRG had viral loads of more than 3,000 copies/mL and revealed a high frequency of $g\Delta nef$ (18.8%) [5]. Moreover, recently we found that there is an association between taking KRG and gross deletions in the pol gene [28]. In addition, we reported for the first time that KRG intake has a synergistic antiviral effect in combination with ART in vivo, as well as acting to delete HIV-1 strains carrying resistant mutations [29].

Previously, we reported a possible association between KRG intake and $g\Delta nef$ [5,23,24] and the difference of the nature of $g\Delta nef$ in our LTSPs compared with Western patient groups [5]. Here, we investigated the time frame in which genetic defects in nef occur in response to KRG intake. We additively obtained sequence data at baseline (prior to KRG intake), after 1–2 yr of regular KRG intake and on KRG plus ART that were not included in the previous study [5]. In conclusion, these data show that genetic defects are associated with KRG intake and unlike SD, the detection of $g\Delta nef$ is decreased during ART. As the longest follow-up study of changes in the nef gene, spanning 20 yr, this report provides new insight into the importance of genetic defects and variation in the pathogenesis of AIDS.

2. Materials and methods

2.1. Patients

Ten patients included in this study who were infected with HIV subtype B were defined as LTSPs, with annual decreases in CD4+ T cells of <20/μL over 10 yr [5]. The clinical characteristics of these patients, including changes in CD4+ T cell counts, RNA copy numbers, KRG therapy, and frequent genetic defects in nef and the 5’LTR/gag, have been described elsewhere [5,6]. However, the follow-up period of this study was extended to include the KRG plus ART period (Fig. 1). Control patients (n = 36) were selected from 216 available patients [30] using the following two criteria: first, patients that had not been exposed to KRG or any ART (zidovudine) at the sampling time point; and second, peripheral blood mononuclear cells (PBMCs) were used for HIV gene amplification. Twenty-eight ART only patients were also included for comparison with KRG plus ART group. This study was approved by the Institutional Review Board of the Asan Medical Center.

2.2. KRG intake

KRG capsules were manufactured from the roots of 6-yr-old fresh ginseng plants, Panax ginseng Meyer, harvested in the Republic of Korea by the Korea Ginseng Corporation, Seoul, Korea. KRG was made by steaming fresh ginseng at 90–100°C for 3 h and then drying at 50–80°C. One capsule contained 300-mg powder without any additives. There was an interruption to KRG intake of 4–5 mo after the first 6-mo pilot study.

2.3. Amplification of the nef gene

We used nested polymerase chain reaction (PCR) to amplify the proviral nef gene from PBMCs, as previously described [5,22,23]. Total RNA was extracted from 300 μL of serum samples using a QiAamp Ultra sense Viral RNA kit (Qiagen, Hilden, Germany), as previously described [30,31]. We compared sequence data at baseline (prior to KRG intake) and after 1–2 yr of regular KRG intake using reverse transcription-PCR analysis of data generated from serum, because the proportion of $g\Delta nef$ detectable in serum does not differ to that in PBMCs [32].

2.4. Definition of defective nef genes

A defective nef gene was defined as one with a premature stop codon or one lacking an initiation codon, and a $g\Delta nef$ was defined as a deletion of more than 15 nucleotides outside the variable region [5] as well as not in-frame insertion or deletion of a nucleotide. In this study, the amplicons revealing SDs, including that of the last cysteine, were considered intact.

2.5. Statistical analysis

Data are expressed as the mean ± two standard deviations (for continuous variables) or as counts and percentages (categorical variables). Proportions were compared between groups using the Chi-square or Fisher exact tests and logistic regression controlling for the subject effect (SPSS package version 12.0; SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

2.6. Sequences

Genbank accession numbers for nef sequences are KM871217–KM871789, KM884898–940, and KU588425–KU588857.

3. Results

3.1. Effects of KRG during the follow-up period prior to ART administration

Ten LTSP patients received follow up for a mean of 199 ± 45 mo (16.6 yr; range: 164–314 mo) following HIV-1 diagnosis prior to ART (Table 1). During this period, KRG therapy was administered for over a mean 176 ± 42 mo and, thereafter, KRG plus ART was administered for 76 ± 44 mo (Fig. 1). The average total amounts of KRG administered per patient during these periods were 14,680 ± 5,940 g and 7,981 ± 6,050 g, respectively (Table 1). CD4+ T cells decreased from 453/μL to 169/μL for 173 ± 34 mo, corresponding to an annual decrease of 20.8 ± 14.8/μL.

3.2. Effects of KRG on the nef gene

Using samples from 10 LTSPs, we obtained 88 amplicons from sera at baseline, 962 amplicons (254 and 708 from sera and PBMCs, respectively) during KRG therapy only, and 346 amplicons from PBMCs during KRG plus ART. At baseline, five amplicons revealed defective nef genes (5.7%). For one patient (Patient 93–60), however, although a 7-bp deletion was detected at baseline, the virus had been previously exposed to KRG, because she was infected with
Fig. 1. Changes in the CD4+ T cell count, plasma viral load, and genetic defects according to Korean Red Ginseng (KRG) intake and highly active antiretroviral therapy (ART). The periods of KRG intake and ART, and duration of survival from diagnosis to initiation of ART are shown using a bar at the upper and middle parts, respectively. To our knowledge,
obtained 67 amplicons from the samples which were obtained respectively (5.6%). In the KRG-treated group, all 10 patients defective, which was signiﬁcantly higher compared with baseline.

First two digits before the hyphen in a patient code denotes the year of HIV-1 diagnosis.

Patient 87-05 took wild ginseng twice in 1983–1984.

Patients 89-17 and 93-04 revealed a single resistance mutation to lamivudine and zidovudine, respectively. From the untreated control group (n = 36), we obtained 198 amplicons from PBMC samples, eight (4.0%) and three (1.5%) of which were grossly deleted and 150 (15.6%) of 962 amplicons were grossly deleted for ART Highest viral load (copy/mL) prior to ART Amount of KRG supplied prior to & on ART(g) Initiation of ART Duration of ART (mo)

Table 1
Characteristics of 10 long-term slow progressors treated with Korean Red Ginseng (KRG)

| Patient code | HIV-1 subtype | Genotyping for ART | Highest viral load (copy/mL) prior to ART | Amount of KRG supplied prior to & on ART(g) | Initiation of ART | Duration of ART (mo) |
|--------------|---------------|-------------------|------------------------------------------|---------------------------------------------|------------------|---------------------|
| 87-05        | B             | WT                | 69,600                                   | 21,258                                      | Aug 2015         | 5                   |
| 89-17        | KSB           | M184V(A)          | 162,000                                  | 5,076                                        | Mar 2005         | 123                 |
| 90-05        | KSB           | WT                | 94,376                                   | 25,602                                       | Jul 2008         | 87                  |
| 90-18        | KSB           | WT                | 319,000                                  | 13,182                                       | Apr 2004         | 135                 |
| 90-50        | KSB           | WT                | 244,000                                  | 18,916                                       | May 2007         | 101                 |
| 91-20        | KSB           | WT                | 17,800                                   | 14,336                                       | Aug 2007         | 98                  |
| 92-13        | KSB           | WT                | 14,600                                   | 13,470                                       | Jun 2007         | 45                  |
| 93-04        | KSB           | K70R(Δ)WT         | 656,000                                  | 9,660                                        | Nov 2006         | 9                   |
| 93-60        | KSB           | WT                | 121,000                                  | 10,710                                       | May 2008         | 90                  |
| 96-51        | KSB           | WT                | 386,543                                  | 14,587                                       | Dec 2009         | 66                  |

Note. From “High frequency of grossly deleted nef genes in HIV-1 infected long-term slow progressors treated with Korean Red Ginseng,” by Cho et al., 2006, Current HIV Research, 4, pp. 447–57. Copyright 2006, Bentham Science Publishers. Adapted with permission.

ART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; KSB, Korean subclade of subtype B; WT, wild type.

1) Number in parenthesis were obtained from sera by reverse transcription PCR.

HIV-1 in 1993 via her husband (Patient 89-17) who had taken KRG since 1991 (Table 2). From the untreated control group (n = 36), we obtained 198 amplicons from PBMC samples, eight (4.0%) and three (1.5%) of which were ganef and premature stop codons (SC), respectively (5.6%). In the KRG-treated group, all 10 patients revealed ganef and 150 (15.6%) of 962 amplicons were grossly defective, which was signiﬁcantly higher compared with baseline (p < 0.05; Table 2) and control patients (p < 0.001; Fig. 2).

Focusing on how quickly ganef and other defects occur, we obtained 67 amplicons from the samples which were obtained 3–6 mo after treatment, none of which was a defective gene (0%). However, alterations gradually and signiﬁcantly increased to 10.2% (6/59), 15.3% (13/85), and 17.4% (131/751) during 7–12 mo, 13–24 mo, and 24 mo after KRG intake, respectively (p < 0.001; Fig. 3). Even when the only ganef was analyzed, there was a statistically signiﬁcant diﬀerence to the baseline results.

The time for initial detection of all nef gene defects, including SC was 7 mo. However, the actual median time is likely to be even earlier. The proportion of ganef in this study was slightly lower than that in another study population treated with KRG for 3 yr without interruption [5,23]. Patient 90-50, who took a higher dose of KRG (9 g/d) since February 1993, was the ﬁrst in which a ganef was detected in September 1993. Thus, the ﬁrst occurrence of ganef was after 7 mo of KRG intake, which is the earliest detection of ganef to date. ganef was not detected within the 1st 6 mo of KRG intake even when other patients were included. However, there were no diﬀerences in SCs and not in-frame insertions between the three phases.

3.3. Effect of ART on nef

Detection of genetic defects including ganef was signiﬁcantly decreased during the time when patients received KRG plus ART (7.8%) compared with the KRG-only period (15.6%, p < 0.001; Table 2), although the average amount of KRG/mo was higher on KRG plus ART (7,981 g/76 mo; 105 g) than in KRG only (14,680 g/176 mo; 84 g). Even excluding amplicons containing SCs and not in-frame insertions, the results remain statistically signiﬁcant (14.8% vs. 7.4%, p < 0.001). However, four patients (89–17, 90–50, 92–13, and 93–60) with poor compliance or several interruptions for ART

Table 2
Distribution of defective nef genes among the 10 long-term slow progressors

| Patient code | Baseline | KRG only | KRG plus ART | Interruptions for ART |
|--------------|----------|----------|--------------|-----------------------|
|              | No. of PCR | No. of defective | No. of PCR (from serum) | No. of defective | No. of defective |
| 87-05        | 18       | 1ins     | 157 (18)     | 19 + 1ins            | 18          | 1Presence         |
| 89-17        | 16       |           | 63 (11)      | 11                   | 29          | 1 + 1ins          |
| 90-05        | 2        |           | 165 (40)     | 26 + 2ins + 2ins     | 28          | 1 + 1ins          |
| 90-50        | 11       |           | 77 (29)      | 12                   | 65          | 2                 |
| 91-20        | 8        | 1 + 1ins  | 71 (31)      | 11                   | 59          | 3                 |
| 92-13        | 16       |           | 96 (44)      | 13 + 2ins + 1ins     | 18          | 2Prese             |
| 93-04        | 5        | 1        | 96 (16)      | 4                    | 14          | 1                 |
| 93-60        | 7        | 1        | 54 (17)      | 18 + 1ins            | 20          | 12                 |
| 96-51        | ND       | ND       | 94 (14)      | 11                   | 58          | 1                 |
| Total        | 88       | 5 (5.7%) | 962 (254)    | 150 (15.6%)          | 346         | 27(7.8%)**         |

All nef amplicons at baseline and from controls were obtained by reverse transcription PCR and nested PCR, respectively.

*p < 0.05 compared with baseline.

**p < 0.001 compared with Korean Red Ginseng only.

ART, highly active antiretroviral therapy; ins, insertion of a nucleotide; KRG, Korean Red Ginseng; ND, not determined; PCR, polymerase chain reaction; pre, premature stop codon.

1) Number in parenthesis were obtained from sera by reverse transcription PCR.
compared with baseline (0/68). Genetic defects increased significantly on KRG alone and 0.6% (2/346) on KRG plus ART (Table 3). These proportions were significantly lower than in controls or at baseline and decreased significantly during GCT [KRG plus antiretroviral therapy (ART)]. In 28 ART patients, the numbers of gross deletions in the nef gene and stop codons were six (3.6%) and 0.9% on KRG alone and 0.6% (2/346) on KRG plus ART.

The proportion of genetic defects in the nef gene, not in-frame insertion or deletion, and premature stop codon. The proportion of genetic defects was significantly higher during Korean Red Ginseng (KRG) intake than in controls or at baseline and decreased significantly during CCT [KRG plus antiretroviral therapy (ART)]. In 28 ART patients, the numbers of gross deletions in the nef gene and stop codons were six (3.6%) and five (3.0%) of 165 amplicons, respectively.

(revealing viremia < 20 copies/mL) revealed unusually high proportions of gΔnef (19/104; 18.3%) even on KRG plus ART. In contrast, the remaining six patients with good compliance (no viremia < 20 copies/mL) revealed a very low proportion of gΔnef (6/242; 2.5%, p < 0.001; Table 2). These data suggest that detection of gΔnef might be used as an indicator for poor compliance for ART on KRG plus ART.

Analysis of the nef gene revealed SCs in six of 708 amplicons (0.9%) on KRG alone and 0.6% (2/346) on KRG plus ART (Table 3). These proportions were significantly lower than 3.6% (6/168) in 28 ART only patients (p < 0.05).

3.4. Small deletions ranging from 6 bp to 18 bp and insertions of 6 bp to 9 bp

Patient 87-05 was found to carry a virus with a 6-bp deletion in nef at the baseline, although the patient reported two periods of wild ginseng intake in 1983–1984 and 2002. We obtained 10 amplicons from the nef gene from the earliest sample from this patient (April 1991 prior to KRG intake). Of these, five were WT, two contained a 6-bp deletion of nucleotides encoding amino acids (AAs) 8–9, two had a 9-bp deletion of nucleotides encoding AAs 9–11, and one had WT (Fig. S1). After about 20 yr, only a smaller deletion of 3 bp (encoding AA 10) was detected. We hypothesize that these SDs could be related to the long-term slow progression of this patient who remained healthy for >28 yr in the absence of ART. Various other gene alterations were identified in viral material from this patient, including a SC in December 1994, gΔnef in August 2007 and July 2010, and a deletion of 2 bp (nucleotides 2,977 and 2,978) in HIV-1 N4-3 in the pol gene, although we did not classify these SDs as defective genes (n = 63; Fig. S1). In addition, G-to-A hypermutations in the nef gene were observed in 22 sequences amplified from this patient after December 2009 (Fig. 1). The deletion of nef AAs 9–11 is very rare, although it has previously been reported in GenBank sequence AY265085 (Cameroon CRF02 Ag).

In addition, Patient 90-18 had 30 nef amplicons with two different 6-bp deletions; encoding AAs 50–51 67 mo after baseline and AAs 47–48 132 mo after baseline. All 19 samples obtained during KRG intake contained both WT and deleted amplicons. The most common deletion in nef is of nucleotides encoding AAs 60–61 and this was reported in two patients by Alexander et al [34]. These deletions occurred on KRG intake and were similar to the 9-bp deletion in the vif gene in samples from the same patient [33]. Samples from Patient 91-20 and Patient 92-13 demonstrated 9-bp (AAs 151–153; n = 1) and 18-bp (AAs 151–156; n = 1) deletion after 10 yr and 14 yr of KRG intake in November 2001 and April 2007, respectively. An amplicon from Patient 90-05 (January 2003) revealed a novel insertion of 3 bp between the codons for AAs 25 and 26 and a further 15 amplicons (July 2005) with 6-bp insertions between AAs 27 and 28 (Fig. S1). Amplicons with a deletion of the last cysteine in nef were identified in three patients (Patient 89-17, Patient 92-13, and Patient 93-60; Fig. S1). SDs appeared in both serum and PBMCs regardless of the type of specimen as shown for gΔnef.

In summary, except for Patient 87-05, there were no such deletions or insertions at baseline (0/70; p = 0.068) or in the control group (0/159; p < 0.01), compared with KRG alone (47/962).

4. Discussion

Our previous reports have shown that long-term intake of KRG slows depletion of CD4 T cells in HIV-1-infected patients irrespective of HLA Class I alleles [35]. In addition to clinical relevance, sequence data have indicated a strong association between KRG treatment and gΔnef in regard to dosage and duration [5,24,25]. However, previous data did not clarify when and how early the gΔnef can be induced by KRG treatment. In this study, we found that the gΔnef was first observed after 7 mo of KRG intake. gΔnef was not detected within the first 6 mo of KRG intake even when other patients were included. However, we still do not know the mechanism of gΔnef by KRG treatment. The reasons are as follows:

![Fig. 2](image-url) Comparison of the proportion of genetic defects such as gross deletion in the nef gene, not in-frame insertion or deletion, and premature stop codon. The proportion of genetic defects was significantly higher during Korean Red Ginseng (KRG) intake than in controls or at baseline and decreased significantly during Community Care Treatment (CCT) [KRG plus antiretroviral therapy (ART)]. In 28 ART patients, the numbers of gross deletions in the nef gene and stop codons were six (3.6%) and five (3.0%) of 165 amplicons, respectively.

![Fig. 3](image-url) Proportion of genetic defects (%) in the nef gene during different periods with KRG. The proportion of genetic defects increased significantly after 12 mo compared with baseline (p < 0.05).
firstly, ginseng contains many active components and we applied whole ginseng for patients. Secondly, the occurrence of $g_{\Delta nef}$ is <30% in the level of PCR amplicons, although it was detected 100% in patient levels over the long term. Thirdly, it is well documented in literature that ginseng modulates nearly all kinds of immune cells, although the main direction is via cell mediated immunity and innate immunity [9,10] than humoral immunity. Fourthly, there is no similar report on any therapeutic agents, including medicinal food, that deletes or attenuates the “microorganisms or invader gene,” although antibiotics or antiviral drugs develop specific resistance mutations or insertions.

With respect to the possible mechanism of KRG, a few reports also support our data. For example, polyacetyleneginsenside-Ro, xylanase, and quinqueginsin of ginseng have been shown previously to have inhibitory effects on HIV-1 reverse transcriptase [36–38]. It is possible that these inhibitory effects on RT might decrease its fidelity and thereby result in a high frequency of genetic defects.

Our data showed that the proportion of detected $g_{\Delta nef}$ is significantly decreased in KRG plus ART. Effective ART reduces the virus concentration from about 10,000 copies/mL to <20 copies/mL. Deleted genes comprise a minor portion compared with intact genes in vivo. Thus, ART significantly decreases the chance of detecting deleted genes. This is therefore a kind of “marginal dilution effect” rather than actual inhibition of occurrence of $g_{\Delta nef}$ [25].

In addition to the SD mentioned above, we found similar SDs (6–15 bp) in 11 further Korean patients among the 216 analyzed [30]. In detail, nine of these were in amplicons from patients taking KRG, whereas another two were in patients not taking KRG. Among the nine patients, four patients carried viruses with SDs (two nucleotide-encoding AAs 10–11 and two of AAs 8–12) compared with baseline samples (Fig. S2). The position of the SD was very similar to that in amplicons from Patient 87-05 (AAs 9–11). The remaining five patients had a WT nef sequence at baseline with SD appearing after at least 37 mo of KRG intake; deletion of codons for AAs 47–48 in one patient after 37 mo (Patient KYa; Fig. S2). This occurrence was earlier than after 76 mo in Patient 90-18 and after 12 yr in Patient 92-13 in the vif gene [33]. This may indicate that the nef gene is more variable than vif [30,33]. Interestingly, one patient (Patient JHS) was infected with WT virus via her husband (Patient CHR). Her husband took more KRG (4,620 g for 8 yr) than her (2,820 g for 8 yr), and a SD was observed earlier in the husband, suggesting a dose–relationship between KRG intake and SD occurrence. In conclusion, four out of nine patients revealed a deletion of the cysteine prior to the stop codon (Fig. S2). By contrast, SD was detected in two patients in the absence of KRG intake. In conclusion, our data suggest the possibility of associations between KRG intake and SD, although SD occurred relatively rarely and later than $g_{\Delta nef}$.

The report by Alexander et al [34] supports the findings of the current study. Although there is no consensus on the association of SDs with prognosis, an elite suppressor (ES10-53) revealed two SDs in nef [39], and it is particularly interesting that the position of these deletions was the same or adjacent to those of five patients in this study.

Regarding the immunological mechanism underlying the occurrence of $g_{\Delta nef}$, we would like to put a high value on the potentiation of cytotoxic T lymphocyte activity, $g_{\Delta nef}$ and SD might result indirectly from immune modulation toward a Th1 cytokine profile, an anti-inflammatory response [7–10], in addition to viral suppression [29].

In addition to this immunological pressure on proviral DNA within the host chromosome, many cellular factors could be involved in provirus latency. For example, it is well known that chromatin remodeling enzymes like histone deacetylases (HDACs) recruited to the HIV promoter play an important role in HIV latency. HDAC inhibitors might lead to the activation of HIV in latently infected cells and result in the fragmentation of proviral DNA. Recently, Compound K, which is a major metabolite of ginseng saponin, has been found to act as an HDAC inhibitor [40].

In conclusion, this report provides new insights into the importance of genetic defects and variations in the nef gene in the pathogenesis of AIDS.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This work was supported by the 2014 grant from the Korean Society of Ginseng. This manuscript was presented at the 11th International Symposium on Ginseng (OP44, Konkuk University, Seoul, Korea, on October 27–30, 2014).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.gjrr.2016.02.005.

References

[1] Palereal Jr, FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. New Engl J Med 1998;338:853–60.
[2] Hatano H, Delwart EL, Norris PJ, Lee TH, Neiandls TB, Kelley CF, Hunt PW, Hoh R, Linnen JM, Martin JN, et al. Evidence of persistent low-level viremia in long-term ART-suppressed, HIV-infected individuals. AIDS 2010;24:2535–9.
[3] Hunt PW. HIV and inflammation: mechanisms and consequences. Curr HIV/ AIDS Rep 2012;9:139–47.
[4] Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Attmann D, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12:1365–71.
[5] Cho YK, Lim JY, Jung YS, Oh SK, Lee HJ, Sung H. High frequency of grossly deleted nef genes in HIV-1 infected long-term slow progressors treated with Korean Red Ginseng. Curr HIV Res 2006;4:447–57.
[6] Cho YK, Jung YS, Lee J, Kim JH, Yang WS, Kwak YS, Kim SY, Cheong ES, Rhee MH, Cho JY. Molecular mechanism of macrophage activation by red ginseng acidic polysaccharide from Korean Red Ginseng. Mediators Inflamm 2012. http://dx.doi.org/10.1155/2012/723860.
[7] Baek KS, Hong YD, Kim Y, Sung NY, Yang S, Lee KM, Park JY, Park JS, Rho HS, Shin SS, et al. Anti-inflammatory activity of AP- SF, a ginsenoside-enriched fraction, from Korean ginseng. J Ginseng Res 2015;39:155–61.
[8] Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, Kwak YS. Characterization of Korean Red Ginseng (Panax ginseng Meyer): history, preparation method, and chemical composition. J Ginseng Res 2015;39:384–91.
[9] Deacon NJ, Tyas CN, Solomon A, Smith K, Ludford-Menting M, Hooker DJ, McPhee DA, Greenway AL, Ellett A, Chatelain P, et al. Immunologic and virologic sequences in a long-term survivor with nonprogressive HIV-1 infection. Curr HIV Res 2010;4:447–57.
[10] Baek KS, Hong YD, Kim Y, Sung NY, Yang S, Lee KM, Park JY, Park JS, Rho HS, Shin SS, et al. Anti-inflammatory activity of AP-SF, a ginsenoside-enriched fraction, from Korean ginseng. J Ginseng Res 2015;39:155–61.
[11] Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, Kwak YS. Characterization of Korean Red Ginseng (Panax ginseng Meyer): history, preparation method, and chemical composition. J Ginseng Res 2015;39:384–91.
[12] Deacon NJ, Tyas CN, Solomon A, Smith K, Ludford-Menting M, Hooker DJ, McPhee DA, Greenway AL, Ellett A, Chatelain P, et al. Immunologic and virologic sequences in a long-term survivor with nonprogressive HIV-1 infection. Curr HIV Res 2010;4:447–57.
Cho YK, Jung Y, Sung H, Joo CH. Frequent genetic defects in the HIV-1 5

Cho YK, Jung YS, Sung H. Frequent gross deletion in the HIV type 1

Cho YK, Jung YS. Dosage and duration effects of Korean Red Ginseng intake on

Cho YK, Sung HS, Kim TK, Lim JY, Jung YS, Kang SM. Korean Red Ginseng

Cho YK, Sung H, Lee HJ, Joo CH, Cho GJ. Long-term intake of Korean Red

Cho YK, Lee HJ, Kim YB, Oh WI, Kim YK. Sequence analysis of C3-V3 region of

Kirchhoff F, Easterbrook PJ, Douglas N, Troop M, Greenough TC, Weber J,

Salvi R, Garbuglia AR, Caro AD, Pulciani S, Montella F, Benedetto A. Grossly

Miura T, Brockman MA, Brumme CJ, Brumme ZL, Carlson JM, Pereyra F,

Cho YK, Kim JE, Foley BT. Phylogenetic analysis of the earliest nef gene from human immunodefi cits and deletions with disease progression. J Virol 2010;84:3644–53.

Kirchhoff F, Easterbrook PJ, Douglas N, Troop M, Greenough TC, Weber J, Carl S, Sullivan JI, Daniels RS. Sequence variations in human immunodefi ciency virus type 1 Nef are associated with different stages of disease. J Virol 1999;73:5497–508.

Cho YK, Lee HJ, Kim YB, Oh WI, Kim YK. Sequence analysis of C3-V3 region of human immunodefi ciency virus type 1 gp120 and its correlation with clinical signifi cance: the effect of long-term intake of Korean Red Ginseng on env gene variation. J Korean Soc Microbiol 1997;32:611–23.

Cho YK, Sung H, Lee HJ, Joo CH, Cho GJ. Long-term intake of Korean Red Ginseng in HIV-1 infected patients: development of resistance mutation to zidovudine is delayed. Int Immunopharmacol 2001;1:1295–305.

Cho YK, Kim JE, Foley BT. Phylogenetic analysis of near full-length HIV-1 type 1 genomic sequences from 21 Korean individuals. AIDS Res Hum Retroviruses 2013;29:738–43.

Cho YK, Kim BR, Kim JE. Frequent genetic defects in long-term survivors for more than 26 years in the absence of antiretroviral therapy in Korea: its association with ginseng treatment. Retrovirology 2013;10(Suppl. 1):14.

Cho YK, Kim JE, Kim BR. Frequent gross deletions in pol gene in 10 HIV-1 infected patients treated with Korean red ginseng for 3 years: dosage dependence. Retrovirology 2013;10(Suppl. 1):15.

Cho YK, Kim BR, Kim JE, Woo JH, Foley BT. First report on a T69-ins insertion in CRF06_cpx HIV-1. AIDS Res Hum Retroviruses 2013;29:1079–84.

Cho YK, Kim JE, Foley BT. Phylogenetic analysis of the earliest nef gene from human immunodeficiency virus and local controls in Korea. Biore Med Access Open 2012;1:41–9.

Kim BR, Kim JE, Sung H, Cho YK. Long-term follow up of HIV-infected Korean hemophiliacs, after infection from a common source of virus. Haemophilia 2015;21:e1–11.

Brambilla A, Turchetto L, Gatti A, Bovolenta C, Veglia F, Santagostino E, Gringeri A, Clementi M, Poli G, Bagarelli P, et al. Defective nef alleles in a cohort of hemophiliacs with progressing and nonprogressing HIV-1 infection. Virology 1999;259:349–68.

Cho YK, Kim BR, Chang MS, Kim JE. Effects of Korean Red Ginseng and ART on vif gene in 10 long-term slow progressors over 20 years: high frequency of deletions and G-to-A hypermutation. Evid Based Complement Alternat Med 2013. http://dx.doi.org/10.1155/2013/871648.

Alexander L, Weiskopf E, Greenough TC, Gaddis NC, Auerbach MR, Malim MH, O’Brien SJ, Walker BD, Sullivan JL, Desrosiers RC. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. J Virol 2000;74:4361–76.

Sung HS, Kang SM, Lee MS, Kim TG, Cho YK. Korean Red Ginseng slows depletion of CD4 T cells in human immunodeficiency virus type 1-infected patients: association with HLA. J Ginseng Res 2004;28:173–82.

Cho YK, Jung YS. Dosage and duration effects of Korean Red Ginseng intake on frequency of gross deletions in the nef gene. J Ginseng Res 2010;34:219–25.

Cho YK, Jung YS, Sung H. Frequent gross deletion in the HIV type 1 nef gene in hemophiliacs treated with Korean Red Ginseng: inhibition of detection by highly active antiretroviral therapy. AIDS Res Hum Retroviruses 2009;25:419–24.

Cho YK, Jung Y, Sung H, Joo CH. Frequent genetic defects in the HIV-1 5’LTR/gag gene in hemophiliacs treated with Korean Red Ginseng: decreased detection of genetic defects by highly active antiretroviral therapy. J Ginseng Res 2011;35:413–20.

Cho YK, Sung H, Lee HJ, Joo CH, Cho GJ. Long-term intake of Korean Red Ginseng in HIV-1 infected patients: development of resistance mutation to zidovudine is delayed. Int Immunopharmacol 2001;1:1295–305.

Cho YK, Sung H, Kang SM, Lee MS, Kim TG, Cho YK. Korean Red Ginseng slows depletion of CD4 T cells in human immunodeficiency virus type 1-infected patients. Clin Diagn Lab Immunol 2005;12:497–501.

Zhang H, Lu Z, Tan GT, Qiu S, Farnsworth NR, Pezzuto JM, Fong HHS. Polyacetylene ginsenoside-Ro, a novel triterpene saponin from Panax ginseng, Tetradodon Lept 2002;43:973–7.

Lam SK, Ng TB. Quinqueginsin, a novel protein with anti-human immuno deficiency virus-1 reverse transcriptase. Life Sci 2002;70:3049–58.

Wang HX, Ng TB. Quinqueginsin, a novel protein with anti-human immuno deficiency virus, antifungal, ribonuclease and cell-free translation-inhibitory activities from American ginseng roots. Biochem Biophys Res Commun 2000;269:203–8.

Blankson JN, Bailey JR, Thyail S, Yang HC, Lassen K, Lai J, Gandhi SK, Brien SJ, Walker BD, Sullivan JL, Desrosiers RC. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. J Virol 2000;74:4361–76.

Sung HS, Kang SM, Lee MS, Kim TG, Cho YK. Korean Red Ginseng slows depletion of CD4 T cells in human immunodeficiency virus type 1-infected patients. Clin Diagn Lab Immunol 2005;12:497–501.

Zhang H, Lu Z, Tan GT, Qiu S, Farnsworth NR, Pezzuto JM, Fong HHS. Polyacetylene ginsenoside-Ro, a novel triterpene saponin from Panax ginseng, Tetradodon Lept 2002;43:973–7.

Lam SK, Ng TB. Sanchi ginseng (Panax notoginseng) with inhibitory effects on human immunodeficiency virus-1 reverse transcriptase. Life Sci 2002;70:3049–58.

Wang HX, Ng TB. Quinqueginsin, a novel protein with anti-human immuno deficiency virus, antifungal, ribonuclease and cell-free translation-inhibitory activities from American ginseng roots. Biochem Biophys Res Commun 2000;269:203–8.

Blankson JN, Bailey JR, Thyail S, Yang HC, Lassen K, Lai J, Gandhi SK, Brien SJ, Walker BD, Sullivan JL, Desrosiers RC. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. J Virol 2000;74:4361–76.

Cho YK, Kim BR, Kim JE. Frequent genetic defects in the HIV-1 5’LTR/gag gene in 10 long-term slow progressors over 20 years: high frequency of deletions and G-to-A hypermutation. Evid Based Complement Alternat Med 2013. http://dx.doi.org/10.1155/2013/871648.

Alexander L, Weiskopf E, Greenough TC, Gaddis NC, Auerbach MR, Malim MH, O’Brien SJ, Walker BD, Sullivan JL, Desrosiers RC. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. J Virol 2000;74:4361–76.

Sung HS, Kang SM, Lee MS, Kim TG, Cho YK. Korean Red Ginseng slows depletion of CD4 T cells in human immunodeficiency virus type 1-infected patients. Clin Diagn Lab Immunol 2005;12:497–501.

Zhang H, Lu Z, Tan GT, Qiu S, Farnsworth NR, Pezzuto JM, Fong HHS. Polyacetylene ginsenoside-Ro, a novel triterpene saponin from Panax ginseng, Tetradodon Lept 2002;43:973–7.

Lam SK, Ng TB. Sanchi ginseng (Panax notoginseng) with inhibitory effects on human immunodeficiency virus-1 reverse transcriptase. Life Sci 2002;70:3049–58.

Wang HX, Ng TB. Quinqueginsin, a novel protein with anti-human immuno deficiency virus, antifungal, ribonuclease and cell-free translation-inhibitory activities from American ginseng roots. Biochem Biophys Res Commun 2000;269:203–8.

Blankson JN, Bailey JR, Thyail S, Yang HC, Lassen K, Lai J, Gandhi SK, Brien SJ, Walker BD, Sullivan JL, Desrosiers RC. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. J Virol 2000;74:4361–76.

Cho YK, Kim BR, Kim JE. Frequent genetic defects in the HIV-1 5’LTR/gag gene in 10 long-term slow progressors over 20 years: high frequency of deletions and G-to-A hypermutation. Evid Based Complement Alternat Med 2013. http://dx.doi.org/10.1155/2013/871648.