医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
			2007年2月13日	該当なし	

一般的名称	①乾燥抗 HBs 人免疫グロブリン ②ポリエチレングリコール処理抗 HBs 人免疫グロブリン	研究報告の公表状況	Vox Sanguinis 2007; 92: 113-120	公表国 韓国
販売名（企業名）	①ヘブスブリン（ベネシス） ②静注用ヘブスブリンーIH（ベネシス）			

研究報告の概要

20 例の血友病患者が、1990 年初頭以降、韓国で製造された血液凝固第 IX 因子の投与を受けてから 1～2 年後にヒト免疫不全ウイルス-1(HIV-1)に感染していると診断された。本研究は血漿ドナーで見出されたウイルスと血友病患者で見出されたウイルスとの間の遺伝子の関連性を調べたものである。感染した血友病患者、韓国製の凝固因子製剤製造に用いられた血漿のドナー、韓国外で感染した血友病患者、および韓国ローカルの対照から得られたウイルスの nef 遺伝子と pol 遺伝子の配列を nestedPCR および直接的な DNA 配列分析で調べた。それらの配列の間の関連性を調べるために系統学的分析を用いた。その結果、血漿ドナーと血友病患者の双方とも、HIV-1 サブタイプ B の韓国 subclade での感染であることが判明した。即ち、これらのデータは、20 例の血友病患者への HIV-1 の伝播が韓国製の凝固因子製剤の静脈内注射を介して起こったことを示している。
更に筆者らは、この報告の中で、当該ロットの血液凝固第 IX 因子製剤を製造した韓国 X 社の製造工程について、以下の問題点があったことを指摘している。
1) 製造法に、New York Blood Center から導入した SD 処理技術を採用していたが、当時レトロウイルスを不活化することが明らかにされていた加熱処理は行なっていなかった。
2) 1991 年半ばに、韓国 X 社の製造施設及び製造工程の査察が行われ、New York Blood Center の専門家の指摘により、以下の工程改善が行われたが、これらの改善は韓国製血液凝固第 IX 因子製剤による HIV 集団感染が起こった後に行われたものであった。
・SD 試薬添加時に二人のサインを求める。
・SD 試薬添加の直前にろ過する。
・SD 試薬添加前にあらかじめ定めたたん白濃度まで希釈する。

報告企業の意見	今後の対応
工程管理の不備により、HIV-1 が SD 処理血液凝固第 IX 因子から伝播したとの報告である。 弊社で製造工程に SD 処理を導入している製剤には、血液凝固第 IX 因子製剤であるクリスマシン-M のほか血液凝固第 IX 因子製剤であるコンコエイト HT-Wf、トロンビン-ヨシトミ及びフィブリノゲン-HT の 4 製剤がある。 これまで、当該 SD 処理 4 製剤が疑われた感染症報告は受けていない。 また、当該4製剤のSD処理工程では、SD試薬添加前に薬液の清澄化を目的としてろ過または遠心分離を行っており、工程ではpH、たん白濃度、SD試薬の濃度、温度および処理時間を管理している。またこれらの工程はGMPの下で管理しており、当然のことながら全ての作業は担当者と確認者のダブルチェックのもと行っているために、本報告のような工程管理の不備による感染事故は起こらないと考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリンーIH の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

Vox Sanguinis (2007) 92, 113–120
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DOI: 10.1111/j.1423-0410.2006.00866.x

ORIGINAL PAPER

Molecular epidemiologic study of a human immunodeficiency virus 1 outbreak in haemophiliacs B infected through clotting factor 9 after 1990

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Vox Sanguinis

Background and Objectives Twenty haemophiliacs were diagnosed as infected with human immunodeficiency virus 1 (HIV-1), 1 to 2 years after exposure to clotting factor 9 manufactured in Korea, beginning in early 1990. This study assessed the genetic relationships between viruses found in plasma donors and haemophiliacs.

Materials and Methods Sequencing of the *nef* and *pol* genes of viruses from infected haemophiliacs, plasma donors whose plasma was used in domestic clotting factor manufacture, haemophiliacs infected outside Korea, and local controls were determined by nested polymerase chain reactions and direct DNA sequencing. Phylogenetic analysis was used to investigate the relationships among the sequences.

Results Both plasma donors and the haemophiliacs were infected with a subclade of subtype B that is a founder effect lineage in Korea.

Conclusion Our data indicate that HIV-1 transmission to 20 haemophiliacs occurred through intravenous injection of Korean-made clotting factor.

Summary A clotting factor made in Korea from blood from cash-paid donors infected at least 20 haemophiliacs with HIV-1 subtype B.

Key words: domestic clotting factor 9, haemophiliacs, HIV-1, *nef* gene, phylogenetic analysis, plasma, *pol* gene.

Received: 5 June 2006,
revised 10 October 2006,
accepted 13 October 2006,
published online 27 November 2006

Introduction

One consequence of the rapid rate of human immunodeficiency virus 1 (HIV-1) mutation is that phylogenetic analysis of HIV-1 DNA sequences is a powerful tool for identifying closely related viral strains and allowing the inference of transmission between individuals. Many previous studies have used phylogenetic methods to examine suspected or known viral transmission events [1–5]. There are also geographic variations in HIV-1 sequences that may be of scientific use. By comparison to the worldwide sequences, it was found that Korean subtype B (KSB) HIV-1 sequences are quite distinct [6–11], indicating a strong founder effect, with the founding event(s) occurring in the late 1980s.

Prior to 1990, Korean patients suffering from haemophilia B (HP B) were treated with imported clotting factor 9 and other blood products. Domestic clotting factor 9 (DCF), produced by a non-heat inactivation processing, was supplied to almost all Korean HP B beginning in early 1990. Before exposure to DCF through intravenous injection, 18 of the 23 HPs were screened and found to be seronegative for anti-HIV-1

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Y.C. and H.S. participated in designing and performing the research; Y.C., B.T. and J.K. controlled and analysed the data; Y.C., H.S and B.T. wrote the paper; and all authors were involved in preparation and subsequent version of the paper.

This work was partially presented in abstract form at the second International AIDS Society conference on HIV pathogenesis and treatment, Paris, 2003, and XV International AIDS Conference in Bangkok, 2004.

antibodies. Except HP-21 who were diagnosed in 1987, between 1990 and 1994, 22 Korean HPs (19 HP B and 3 HP A) were diagnosed with HIV-1 infection after exposure to DCF. At the time of these diagnoses, there were 122 HP B registered at the Korea Haemophilia Clinic (seropositive rate 15·6%).

In this study, we performed further investigations of the 20 HIV-1-infected HP B and 3 HIV-1-infected HP A patients mentioned above, including a single HP B diagnosed in 1987 prior to receiving DCF, and two HP B who received clotting factors manufactured outside Korea. Considering the very low prevalence of HIV-1 in Korea (251 cases out of a population size of 47 million as of December 1992), the increased prevalence of HIV-1 cases occurring after 1990 within the 122 HP B was very unusual, especially compared to the very low prevalence of seropositives (less than 15 per million) among local blood donors [12]. This large cluster of HIV-1 cases within the haemophilia population prompted the Center for AIDS Research, Korean National Institute of Health, to form an investigation committee to examine this issue.

Before the first anti-HIV-1 antibody testing of HPs registering at the Korea Haemophilia Clinic, 16 of those patients had received transfusions (> 785 units in total, in Table 1). Despite multiple transfusions, they were free of HIV-1 infection until they began to inject DCF, after which time they were rarely transfused (a total 16 units in two HPs). Thus, it seems unlikely that these patients were infected with HIV-1 through transfusion. Interestingly, the only other similar outbreak was seen from 1989 to 1990 in Germany, where 9 of 48 HPs (18·8%) treated with a single contaminated batch of clotting factor 9 were infected with HIV-1 [13].

Here, we investigated whether there is a genetic association between the Korean HIV-1-infected HPs and HIV-1-infected plasma donors who are known to have seroconverted a short time after donating plasma that was used to make DCF.

Patients and methods

Twenty-three HIV-1-infected haemophiliacs

HPs 1–20 were diagnosed with HIV-1 infection between 1990 and 1994. HP 21 was infected with HIV-1 by imported factor 9 prior to 1987, and was diagnosed in 1987. Two other HPs (nos 22 and 23) were diagnosed in 1991 and 1994, but they had lived outside Korea for a prolonged period (Table 1). Two other HIV-1-infected Korean HPs were not included from this study, as one was infected with HIV-1 abroad prior to 1987, and the other was infected through mother-to-child transmission. Informed written consent was obtained from all living study participants.

Cash-paid plasma donors

In the late 1980s, cash-paid plasma collection occurred at domestic plasma centres run by Company X, which manufactures various blood products, including clotting factors. At these centres, four HIV-1-seropositive homosexual donors O, P, Q and R were detected during primary infection (Table 2). The last four plasma units (drawn on 30 November and 8, 20 and 23 December 1989) from donor O were withdrawn, but the prior 79 units had already been used for manufacturing various blood products. Because of the window period between infection and seroconversion, at least four units (drawn on 4, 10, 13 and 16 October 1989) were not safe. Between 3 January 1990 and 26 March 1990, 21 units of plasma were taken from donor P. Three units of plasma (taken on 20 and 23 January and 2 March 1990) from donor P were used to produce DCF according to the manufacturer's report. Two units of plasma were taken from donor Q on 29 January and 6 February 1992. Plasma from donor R was taken nine times between 6 April 1992 and 8 June 1992. Except for last two units, seven units were used for manufacturing blood products. In addition, donors O and R were also infected with hepatitis C virus (HCV) on at least 13 March 1991 and 16 March 1993, respectively.

Sequencing of the *nef* and *pol* genes

Peripheral blood mononuclear cells (PBMC) from 23 HPs and three donors (O, P and Q) were obtained between 1991 and 2003. To amplify proviral *nef* sequences from the PBMC, nested polymerase chain reactions (PCR) were used as previously described [7]. In cases that were negative for the nested PCR, a second set of nested primers was applied. The primers used were as follows: first PCR, CE21 (forward) and CE22 (reverse); second PCR, primer Nef5'5 and LTR3' [14]. The PCR products were purified and used for direct sequencing. We determined the *nef* gene sequence at least two different times in each HP sample except HP 15. We ruled out PCR contamination by physical separation of each PCR procedure, inclusion of negative controls, BLAST search, and phylogenetic analysis. To amplify partial *pol* sequences from PBMC, a nested PCR was employed as described in our previous studies [11,15].

nef and *pol* sequence data

The GenBank accession numbers for the *nef* sequences from the donors and HPs are AY121450, AY221654, AY121451, AY260770, AY584756–AY584759, AY899339 and AY899340 from donor O; AF063929, AY363309 and AY363310 from donor P; AF063918 from donor Q; AF063923 and AY363311 from HP 1; AF063928 and Z98020 from HP 2; AY221675–AY221715 and AY260771–AY260791 from the other HPs. The local *nef* sequences from non-HPs, which were used for comparison purposes, could be found under the following accession

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Table 1 Summary on 23 HIV-1 infected Korean haemophiliacs

Haemophiliacs	Age at HIV diagnosis	Deficient factor	Negative for anti-HIV antibody	Dx of HIV infection	Total number of blood transfusion[a] (unit)	Time on the first use of DCF	CD4+ T cell (the earliest)
20 haemophiliacs infected with Korean subclade B of HIV-1 subtype B							
1	19	9	April 1990	26 November 1990	Not available (death in 1994)	Early 1990	555 in 1991
2	14	9	No data	21 February 1991	33 from February 1986 to March 1989	May 1990	629 in 1991
3	11	9	21 February 1991	1 April 1991	3 before 1983 and 93 from 1988 to 1990	May 1990	576 in 1991
4	6	8	No data	24 August 1991	1 in August 1987	Not available	826 in 1994
5	11	9	28 February 1991	13 January 1992	155 (127 from 1981 to 1988 and 28 in 1989)	February 1991	554 in 1992
6	4	9	9 May 1989/25 February 1991	20 January 1992	90 from September 1988 to 6 December 1990 (death in 1998)	14 March 1991	771 in 1992
7	7	9	27 February 1991	22 January 1992	265 from 1 August 1987 to 23 January 1991	1 May 1990	804 in 1992
8	19	9	10 June 1989/9 April 1991	15 February 1992	16 from June 1989 to September 1990	April 1991	241 in 1992
9	23	9	27 February 1991	25 February 1992	5 in Mar 1989 and 3 in 6 April 1991	7 March 1991	589 in 1993
10	23	9	No data	26 March 1992	56 from March 1990 to September 1990 and 4 in March 1992	September 1990	336 in 1993
11	5	9	28 February 1990/14 March 1991	29 February 1992	4 from March 1989 to October 1989	16 April 1991	966 in 1992
12	29	9	6 March 1991	25 February 1992	4 in 1987 and 4 in 1989	March 1991	767 in 1992
13	11	9	25 February 1991	29 February 1992	3 in March 1987	7 March 1987/11 January 1991	446 in 1992
14	35	9	20 October 1989/22 February 1991	19 February 1992	24 in October 24 to October 28, 1989	February 1991	420 in 1992
15	16	9	21 February 1991	16 September 1992	1 in 1989	March 1991	507 in 1996
16	22	9	14 March 1991	5 December 1992	30 from September 1988 to October 1988	14 March 1991	315 in 1992
17	4	9	27 February 1991	26 February 1993	30 to January 1991	16 February 1991	612 in 1993
18	35	9	1 July 1991	2 March 1993	Many before February 1988, 2 in March 1988, and 12 in November 1991	2 April 1988/10 September 1989	173 in 1993
19	13	9	5 March 1991/2 March 1992	26 July 1993	0	5 March 1991	425 in 1996
20	39	8	27 February 1991	4 August 1994	0 (use of six vials of DCF instead of factor 8)	25 February 1991	234 in 1994
3 haemophiliacs infected with non-Korean subclade B of HIV-1 subtype B							
21	9	9	No data	3 August 1987	Used clotting factors imported from USA	1990	627 in 1987
22	35	9	No data	27 March 1991	4 in January 1987, lived in USA in 1988	1990	527 in 1991
23	10	8	No data	13 October 1994	Lived in Iran for 10 years since birth	October 1994	561 in 1996

[a]Blood transfusion includes fresh frozen plasma, cryoprecipitates, and so on. Haemophiliacs 13 and 18 were also exposed to DCF which was manufactured in 1987. Dx, diagnosis; DCF, domestic clotting factor 9.

Table 2 Summary on the four HIV-1 infected cash-paid plasma donors and domestic clotting factor (DCF)

Plasma donor	Age at HIV diagnosis	Period of plasma donation at Company X	Unit of plasma donation	Last negative for anti-HIV antibody	Diagnosis of HIV infection
O	27	5 January 1988 to 23 December 1989	83	16 October 1989	30 November 1989
P	30	3 January 1990 to 26 March 1990	19	15 March 1990	30 March 1990
DCF has been manufactured from late 1989 in Company X					
Q	32	29 January 1992 to 6 February 1992	2	29 January 1992	6 February 1992
R	22	6 April 1992 to 8 June 1992	9	30 May 1992	3 June 1992

numbers: AF462701–AF462767, AY121449–AY121471, AY363309–AY363369 and AY584754–AY584808.

The GenBank accession numbers for the 532 *pol* sequences from the donors, HPs and local sequences from non-HPs are as follows: AY585687 and AF407364 from donor O, AY347694 from donor P, AY347690 from HP 1, AY166460–AY166503, AY219009–AY219031, AY347683–AY347709, AY392099–AY392125 and AY731184–AY731229 for other HPs and local controls.

Phylogenetic analysis

The sequences described in the current study were aligned to the HIV-1 subtype reference set from the HIV Sequence Database (http://hiv-web.lanl.gov/content/hiv-db/Subtype_REF/align.html) and phylogenetic trees were built using the PHYLIP DNADIST (F84 model, Ts : Tv 1·7) and NEIGHBOUR programs. Trees built using the maximum parsimony method in PAUP produced identical trees with regard to which sequences fell within KSB within HIV-1 subtype B.

Results

Characteristics of haemophiliacs

Seventeen of 20 HPs (excluding nos 2, 4 and 10) screened negative for HIV-1 antibody just before they were administered (Table 1). The test was mainly performed by the Korea Haemophilia Clinic using an internationally marketed ELISA kit manufactured by Company X. In addition, four of the HPs (nos 6, 8, 11 and 14) tested negative for HIV-1 antibody at other university hospitals 1–2 years before using DCF. This group was mainly treated with imported factors prior to the inception of domestic DCF production (officially 12 December 1989, although 154 vials were produced in 1986).

Molecular epidemiologic data from the *nef* gene

Phylogenetic analysis revealed that 20 of the HPs (nos 1–20) and the three plasma donors (O, P and Q) were infected with KSB HIV-1, whereas the three HPs (nos 21–23) infected abroad were infected with non-KSB HIV-1. HPs 1–4 were the first cases among 20 HPs infected with the KSB (Fig. 1). As expected from the time period between seroconversion and sampling for DNA sequencing for this study, intrapatient DNA sequence identity among the HPs who had progressed to AIDS (HPs 3, 5, 16 and 21) was less than 95%. In contrast, in patients whose CD4+ T cell count maintained a steady state, such as HPs 7 (98·7%), 8 (95·1%), 13 (97·8%), 14 (96·6%), 19 (96·4%) and 22 (96·2%), sequence identities were > 95% after 7–9 years of infection.

Molecular epidemiologic data from the *pol* gene

We determined *pol* sequences from donor O (957 bp: AY585687), donor P (543 bp: AY347694, derived from September 1991), and 292 local control sequences from 107 local patients not known to have sold plasma for DCF production. In a phylogenetic tree including 23 domestic *pol* sequences (> 957 bp), sequences from eight HPs strongly clustered around those of donor O without the embedding of other local sequences (Fig. 2), and sequences from 10 HPs also strongly clustered. Although we could not include *pol* sequences from donor P in this phylogenetic tree or for comparison of sequence identities due to small fragment size of 543 bp, sequences from 10 HPs were clustered without an embedding of other local sequences in the lower cluster.

In regard to the frequency of the specific amino acid reverse transcriptase (RT) codons in KSB sequences, I135V, I202V, and R211K were detected in four, six and six of 45 local control patients (including two cash-paid plasma donors), respectively (Table 3). In contrast, the 20 HPs showed significantly higher frequencies of these three codons, in a manner consistent with their occurrence in the two cash-paid plasma donors. Specifically, two RT sequences from donor O showed I202V but not I135V and R211K, whereas those of donor P showed I135V (sequences after codon 190 were not determined). The frequencies of I135V, I202V and R211K (9, 9 and 12, respectively, based on the earliest sequences from each patient) were significantly higher in the 20 HPs than in the 45 local control patients ($P < 0·01$ by Student's *t*-test for all three codons) (Table 3). This finding also supports the epidemiological linkage for the transmission of HIV-1 from two plasma donors to at least 17 HPs.

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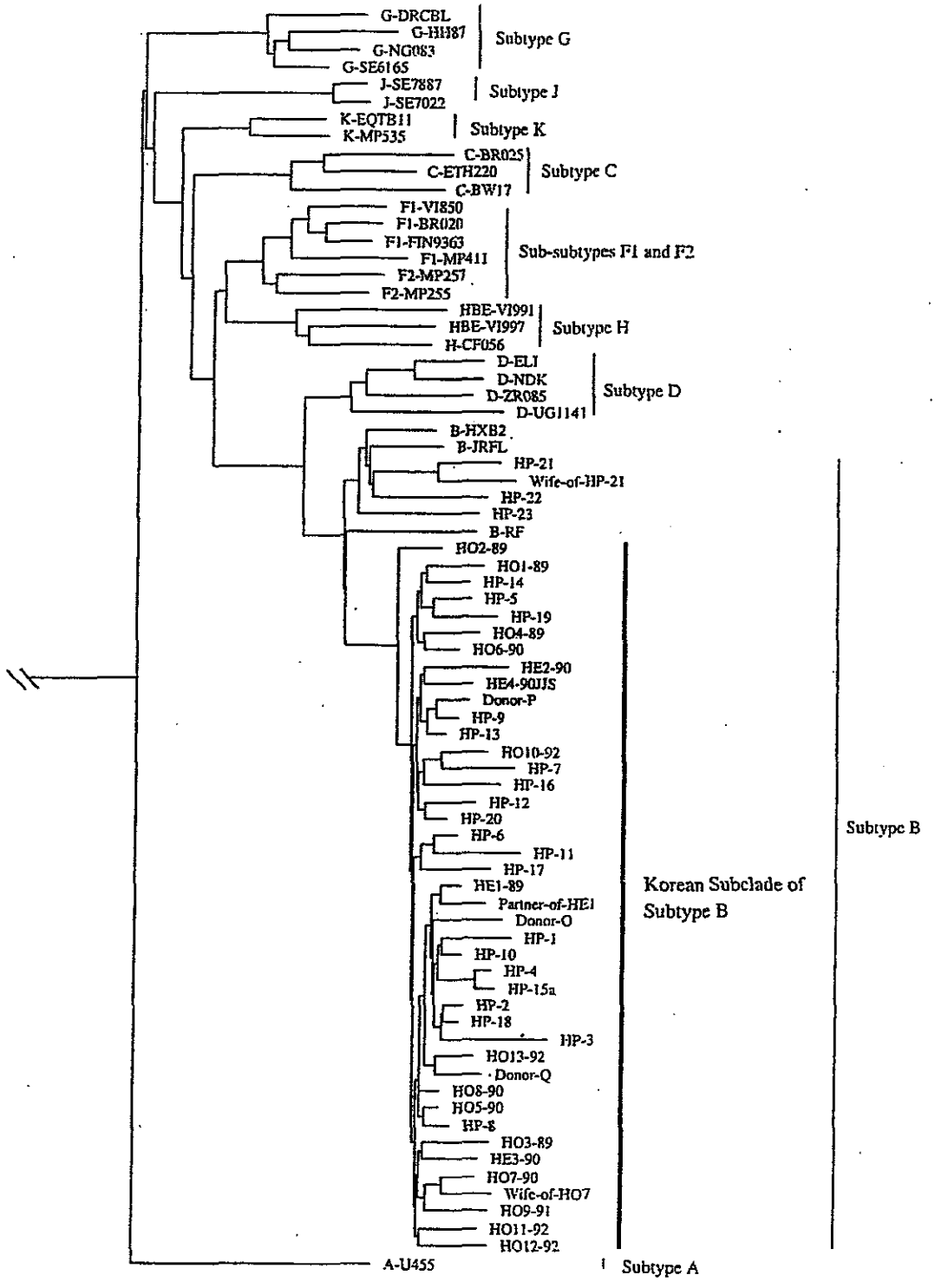

Fig. 1 Phylogenetic tree of *nef* sequences of 23 haemophiliacs (HP), plasma donors (O, P and Q) and 19 database *nef* sequences that showed the highest BLAST score with donors O and P and the HIV Database subtype reference set (HO means sequences obtained from homosexuals and HE, heterosexuals). The sequences described in the current study were aligned to the HIV-1 subtype reference set from the HIV Sequence Database (http://www.hiv.lanl.gov/content/hiv-db/SUBTYPE_REF/align.html) and sequences scoring highly in BLAST, and a phylogenetic tree was built using the PHYLIP DNADIST (F84 model, Ts : Tv 1·7) and NEIGHBOUR programs.

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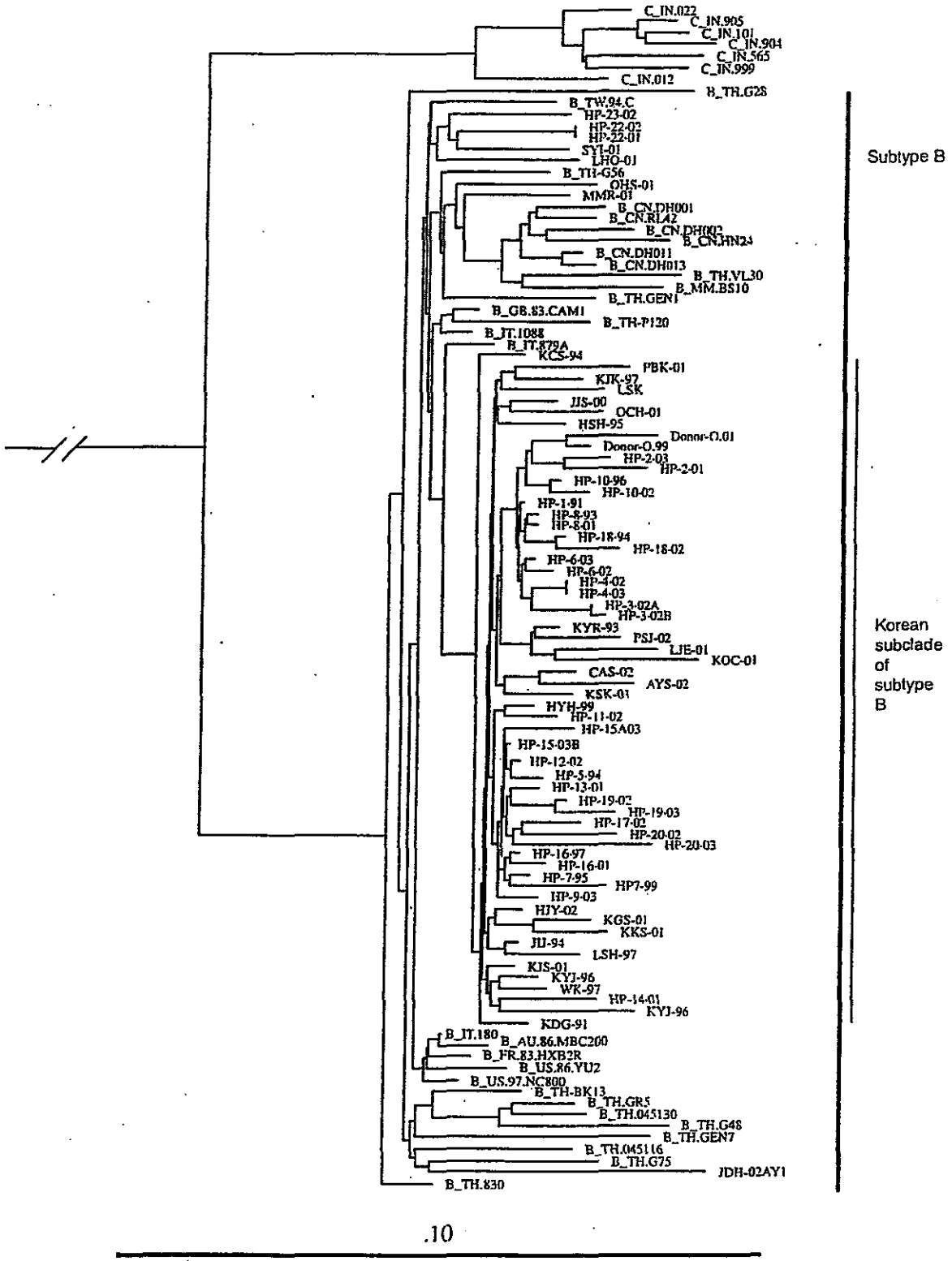

Fig. 2 *Phylogenetic tree of pol sequences of 23 haemophiliacs (HP), plasma donor O and 23 local sequences. Twenty-three local pol sequences = 957 bp were selected by the highest BLAST score with the two plasma donors. We did not include the pol sequences from donor P because of the shorter length (543 bp) sequenced from this sample. There are two strong clusterings with 18 HPs; sequences from eight HPs strongly clustered around those of donor O and sequences from 10 HPs also strongly clustered.*

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Table 3 Frequency of specific amino acids in reverse transcriptase (RT) of haemophiliacs is statistically significantly higher than those in 45 local HIV-1 with Korean subtype B

	Reverse transcriptase codons (consensus)			
Haemophiliacs	Codon 135 Ile	Codon 202 Ile	Codon 211 Arg	GenBank no.
Donor P	Val	No data	No data	AY347694
5	Val_2	·/Val_1	Lys_2	AF273194
12	Val_2	·$_2$	Lys_2	AY219017
13	Val_1	·/Val_1	·/Lys_1	AY166480
15	Val_3	·$_3$	Lys_3	AY347701
17	Val_3	·$_3$	Lys_3	AY219021
19	Val_3	·$_3$	Lys_3	AY219023
20	Val_3	·$_3$	Lys_3	AY219024
14	Val_2/.	·$_3$	Lys_3	AF273196
16	Val/.	·$_2$	Lys_2	AY392120
7	·$_7$	·$_7$	Lys_7	AF448137
11	·$_2$	·$_2$	Lys_2	AY219020
9	Leu_4	·$_4$	Lys_4	AY347699
Donor O	·$_2$	Val_2	·$_2$	AF273180
1	·$_1$	Val_1	·$_1$	AY347699
2	·$_4$	Val_4	·$_4$	AF448135
3	·$_3$	·$_1$/Val_2	·$_2$/Lys_1	AF273186
4	·$_2$	Val_2	·$_2$	AY219015
6	·$_2$	Val_2	·$_2$	AY219016
8	·$_3$	Val_3	·$_3$	AF481437
10	·$_2$	Val_3	·$_3$	AY219012
18	·$_9$	Val_{17}	·$_{17}$	AY166489

Number in subscript means times sequenced in each patient. A dot '·' indicates identity to the consensus for this codon.

Discussion

In this study, we found that 20 of 23 Korean HPs were infected with KSB HIV-1. Our investigation of molecular epidemiology rules out foreign imported factor 9 as the cause of infection in the 20 HPs and indicates that DCF is the most probable causative factor for this HIV-1 outbreak. This is further supported by medical record evidence that shows that all 20 HIV-1-infected HP with KSB were exposed to the same DCF. This was manufactured from plasma including donations of plasma from donors O and P collected a relatively short time prior to their documented seroconversion dates.

Eighteen of the 23 HIV-1-infected HPs had tested HIV-1 seronegative at the beginning of DCF therapy, but became seropositive within 2 years of using DCF. Second, more than 50% of the HPs were sexually inactive at the time of diagnosis. Third, in a case–control study taken as one arm, there were statistical associations between six lots of DCF and HIV-1 infection in HPs described in a report from an investigative committee (April 2004). Fourth, the prevalence of HIV-1 infection in the general population was very low at the time of the outbreak. A similar overseas study [6] also showed that only a portion of HPs exposed to contaminated clotting factor 9 developed HIV-1 infection.

Despite the 7–10 years time lag between the outbreak in 1991–1992 and our sampling in 1998–2002, our data show that the sequences from 20 HPs were most closely related with those from two plasma donors. Taken together, both the epidemiological data and this molecular data support the conclusion that HIV-1 transmission to most of the infected Korean HPs occurred from a common source, which is the intravenous injection of DCF, rather than transfusions or imported clotting factors.

DNA sequences and sequence analysis can indicate that virus transmission is likely to have occurred between one person and another. However, other relevant epidemiological information related to such cases must always be used in concert with the molecular epidemiology data. In this case, it is the combination of records providing the dates of cash-paid plasma donations, plus records providing the seroconversion dates of the donors and HPs, together with the molecular data establish the near certainty that the HPs were infected via the clotting factor. Even taken together, these data cannot prove that any one or more of these donors was the cause of the HP infections. It is possible that each HP received some virus from both donors, and also possible that donor Q or other, as yet unidentified donors also contributed to this problem. The only strong linkage is between the DCF product and this group of HPs. The individual cases, such as some HPs having sequences slightly closer to sequences from donor O and others having sequences slightly closer to donor P, are not highly significant. We do not have enough information about pooling of plasma donations and production sizes of batches of DCF to know if it is likely or possible that each of the HPs would have been exposed to virus from only one donor or to a combination of viruses from more than one donor.

Regarding the DCF manufacturing technique, Company X employed a solvent/detergent (tri-*n*-butylphosphate/Tween 80) technique adapted from the New York Blood Center (NYBC), with no heat treatment. After reporting a series of HIV-1 cases in US HPs, several improvements were implemented according to an investigator from NYBC. During an in-house audit of the Company X's manufacturing facility and processing as part of the activity of the investigation committee, a specialist from the NYBC noted that 'several improvements in processing were instituted in mid-1991, including use of a double signature addition, filtration just prior to solvent/detergent addition, and dilution to preset protein concentration' (Horowitz B: solvent/detergent usage by the Company X-summary of technology transfer and 2-day site visit. *Report* signed on 1 June 1993). This mid-1991 time point was after the detection of the first cases of infection in this cohort (in HPs 1, 2, 3 and 4). Still no heat

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treatment that has been shown to inactivate retroviruses [16,17] was used.

Korean subtype B sequences were first detected in homosexual Korean HIV-1 patients diagnosed in 1989 [7–11]. Our previous studies [7–11] showed that the KSB sequences represent a subclade of global subtype B, indicating a founder effect. All KSB sequences were found among domestic-residing homosexuals who did not have sexual contact with foreigners. In contrast, Korean patients infected with non-KSB HIV-1 had sexual contact with US army soldiers in Korea in the late 1980s or had visited the USA, whereas overseas sailors who had visited foreign countries and their spouses showed various subtypes according to their epidemiological history [7–11]. A total of 128 HIV-1-infected patients (1 in 1985, 4 in 1986, 9 in 1987, 22 in 1988, 37 in 1989 and 54 in 1990) were diagnosed in Korea before 1991. Based on epidemiological data, 37 of those were presumed to be infected with KSB and 91 with non-KSB including 59 overseas sailors, five of their wives, and 10 prostitutes who had worked next to US military camps in Korea.

In conclusion, the *nef* and *pol* sequences from donors O and P showed higher DNA sequence identities with those from the tested HPs than with local sequences from Korean seropositive individuals including homosexuals diagnosed with KSB HIV-1 infection before 1991. These data coincide with the clinical and medical records, and together indicate that HIV-1 transmission to 20 HPs occurred through IV injection of DCF rather than transfusions or imported clotting factors.

Acknowledgements

This study was supported in part by the grants of Korea Health 21 R & D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ9-PG3-20500-0001) (Y.C.), by the Korea Ginseng Corporation via Korean Society of Ginseng, and in part by the faculty research fund of Konkuk University in 2003.

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識別番号・報告回数			報告日	第一報入手日 2006 年 12 月 14 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②③人血清アルブミン ④乾燥濃縮人血液凝固第Ⅷ因子 ⑤乾燥濃縮人血液凝固第Ⅸ因子	研究報告の公表状況	Science 2006; 313: 1781-1784	公表国 ドイツ		
販売名 (企業名)	①献血アルブミン-Wf（ベネシス） ②献血アルブミン(5%)-Wf（ベネシス） ③アルブミン-Wf（ベネシス） ④コンコエイト－HT（ベネシス） ⑤クリスマシン－M（ベネシス）					

研究報告の概要

タンパク質の凝集はアルツハイマー病の発病機序として確立されてはいるが、そのプロセスが in vivo でどのように開始されるのかという点についてはほとんど明らかとなっていない。アルツハイマー病患者、またはβ-アミロイド前駆体タンパク質(APP)を発現しているトランスジェニックマウスから得たアミロイド-β(Aβ)を含んでいる脳抽出物の希釈したものを APP トランスジェニックマウスの大脳内に注射すると、時間と濃度に依存した大脳内のβ-アミロイドーシスとそれに伴う病変を誘導した。APP23 宿主マウスを Beta-1 抗体で受動免疫させると誘導される病変の進行が阻害された。さらに、70％蟻酸での 1 時間処理によりアミロイド誘導活性を完全に消失させたが、95℃、5 分間の熱処理はアミロイド誘導を消失させなかった。
脳抽出物のシーディング(seeding)活性は、Aβ免疫除去、タンパク変性、またはAβを宿主に免疫することによって、低下または消失する。外因性に誘導したアミロイドーシスの表現型は、宿主と誘導因子のソースの双方に依存して変わり、そのことは、プリオン系統を思わせるような、生物活性の異なる多型性Aβ系統の存在を示唆している。プリオン病はさまざまな伝播効率で種々の経路を介して野生型の宿主へ伝播することができ、プリオンの取り込みと分布に全身性の細胞機作が関与しているが、現在のところ、β-アミロイドーシス(特に AD)が、プリオン病と同じ意味で伝播性であるという証拠はない。しかし、異常Aβアセンブリーの誘因と増殖に関与する機作を理解することによって、特発性アルツハイマー病の起源を解明しうることとなろう。

使用上の注意記載状況・その他参考事項等

代表として献血アルブミン-Wf の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している.更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アルブミンを精製し、アルブミン濃度 5w/v％に調整した製剤であり、ウイルス不活化を目的として、製造工程において 60℃、10 時間の液状加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
アルツハイマー病患者のアミロイド-βが異常プリオン蛋白質と同様の感染性を持っている可能性が示唆されるとの報告である。 アルツハイマー病患者由来のアミロイド-βの感染性を示唆する情報は、トランスジェニックマウスへの脳内接種による感染実験のみであり、アルツハイマー病が実際に感染･伝播したとの事例は報告されていない。そのため、アルツハイマー病患者のアミロイド-βが異常プリオン蛋白質と同様の感染性を持っていると結論付けるには情報が乏しいと考える。	アルツハイマー病に関連する情報については、今後も注視することとする。

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