厚生労働科学研究費補助金(エイズ対策研究事業)

分担研究報告書

# インド・パキスタンにおける非サブタイプB型HIV感染者の薬剤耐性に関する研究

分担研究者　磯村 思无 名古屋女子大学家政学部教授

研究要旨

インドにおいて今年度中に収集した治療中のサブタイプC型HIV感染者27検体を解析した結果、11検体(41%)に薬剤耐性関連遺伝子が見られ、うち1例はPR領域にも見られた(V82I)。この検体はRT領域に薬剤耐性遺伝子がみられていた。RT領域にみられた薬剤耐性遺伝子のうち、1例は従来のサブタイプB型には報告されていないV118C, L210S(サブタイプB型ではそれぞれV118I, L210W)がみられた。

パキスタンにおいて収集された未治療HIV感染者58例中、6例(10%)に薬剤耐性関連遺伝子が見られ，うち4例はPR領域に見られ、従来のサブタイプB型には報告されていないV82Iであった(サブタイプB型ではV82A/F/T/S)。RT領域には3例に薬剤耐性関連遺伝子が見られた(V108I, T69S, L100I)。このうち1例はPR領域にも薬剤耐性関連遺伝子が見られていた。

## A. 研究目的

インド・パキスタンにおける非サブタイプB型HIV感染者の薬剤耐性関連遺伝子(genotype)と感染性(phenotype)との関連を構築し、non-B subtypeのAID治療に重要な基礎的および臨床的データーを提供する事を目的とする。

## B. 研究方法

インドにおいてはサンジャイ・ガンジー医科学研究所微生物部教授のDr. Dhole、パキスタンについてはAWAN Hospital院長のDr. Rafiqの協力を得て、患者血清を採取し、逆転写酵素およびプロテアーゼ領域の変異部位を解析した。その結果と、関連する臨床的データーおよびサブタイプBを基にした薬剤耐性のデーターと比較検討し、新たに得られた逆転写酵素(RT)あるいはプロテアーゼ(PR)領域の変異部位におけるgenotypeを検討した。

(倫理面への配慮)

調査研究実施国の実情にあわせ、その国の方針を尊重しつつ、原則としてわが国の基準すなわち「ヒトゲノム・遺伝子解析研究に関する倫理指針(平成13年3月29日文部科学省・厚生労働省・経済産業省告示第1号)」を遵守して研究を遂行する。すなわち、検体収集にあたっては、現地側共同研究者によるインフォームドコンセントを確認後、被検者に遺伝子解析の研究目的であり、被検者のプライバシーの守秘義務に十分配慮する旨、説明し同意を得た上で血液を採取する。論文作成にあたっては被検者の氏名や年令等、個人が同定できるような記載を避け、個人情報は全て現地側共同研究者の管理とする。得られた検体は日本に持ち帰り、遺伝子解析で得られたデーターは全て現地側共同研究者に提供する。

## C. 研究結果

インドのサンジャイ・ガンジー医科学研究所において今年度中に収集した治療中のサブタイプC型HIV感

染者27検体を解析した結果、11 検体 (41%)に薬剤耐性関連遺伝子が見られ、うち 1 例は PR 領域にも見られた (V82I)。この検体はRT領域に薬剤耐性遺伝子がみられていた。RT 領域にみられた薬剤耐性遺伝子のうち、1例は従来のサブタイプ B 型には報告されていない V118C, L210S(サブタイプ B 型ではそれぞれ V118I, L210W)がみられた(表1)。

パキスタンの AWAN Hospital において収集された未治療HIV感染者58例中、6例(10%)に薬剤耐性関連遺伝子が見られ, うち4例はPR領域に見られ、従来のサブタイプB型には報告されていないV82Iであった(サブタイプB型ではV82A/F/T/S)。RT領域には3例に薬剤耐性関連遺伝子が見られた(V108I, T69S, L100I)。このうち1例はPR領域にも薬剤耐性関連遺伝子が見られていた(表2)。

D. 考察

インドにおいては治療中の HIV 感染者の41%と高率に薬剤耐性関連遺伝子が見られており、服薬指導を含めたアドヘレンスの悪さが推察される。一方、パキスタンの未治療HIV感染者においては、薬剤耐性関連遺伝子の陽性率は約10%で、当研究グループのケニアのスラム地区の陽性率(約20%)と比較して低率であった。

E. 結論

インドにおいて今年度中に収集した治療中のサブタイプ C 型HIV 感染者27検体を解析した結果、11 検体 (41%)に薬剤耐性関連遺伝子が見られ、うち 1 例は従来のサブタイプ B 型には報告されていない V118C, L210S(サブタイプ B 型ではそれぞれ V118I, L210W)がみられた。 パキスタンにおいて収集された未治療 HIV 感染者58 例中、6例(10%)に薬剤耐性関連遺伝子が見られ, うち4例は PR 領域に見られ、従来のサブタイプ B 型には報告されていない V82I であった(サブタイプ B 型では V82A/F/T/S)。

F. 健康危険情報

特になし

G. 研究発表

研究成果の刊行に関する一覧表参照

H. 知的所有権の出願・登録状況

特になし。

表1

Drug resistance mutations of subtype C HIV-1 with treatment in India

| LAB No | PR Mutation | RT Mutation | | |
|---|---|---|---|---|
| 1021 | | M184V | G190A | |
| 1048 | V82I | K103S | G190A | M184V |
| 1188 | | K103N | M184V | |
| 1280 | | V108I | | |
| 1360 | | K103N | Y181C | |
| 1361 | | K103N | | |
| 1365 | | M184V | | |
| 1366 | | Y181C | M184V | G190A |
| 1367 | | K103N | M184V | G190A |
| 1371 | | K103N | | |
| 1374 | | V118C | L210S | |

表2

Drug resistance mutations of treatment-naïve patients in Pakistan

| Sample No. | Subtype | PR Mutation | RT Mutation |
|---|---|---|---|
| 28 | A | | V108I |
| 32 | G | V82I | |
| 39 | G | V82I | |
| 50 | A | | T69S |
| 55 | G | V82I | |
| 58 | G | V82I | L100I |

厚生労働科学研究費補助金(エイズ対策研究事業)
分担研究報告書

# 非サブタイプB型HIVにおける薬剤耐性関連遺伝子に関する研究(名古屋地域)

分担研究者　金田 次弘 名古屋医療センター臨床研究センター 部長
研究協力者　伊部 史朗 名古屋医療センター臨床研究センター 研究員

研究要旨

今年度、当医療センターで解析できた非サブタイプB型HIV患者は6例で、サブタイプAEが4例、Aが1例、Cが1例であった。これらの症例ついて、逆転写酵素領域およびプロテアーゼ領域の薬剤耐性変異の部位を解析したところ、プロテアーゼ領域において、secondary mutationのL10Vがサブタイプ AE(1例)に、L10Iがサブタイプ AおよびAE(2例)に、K20Rがサブタイプ Aに、K20Iがサブタイプ AE(1例)に、M36Iがサブタイプ A, AE, C全例(6例)に見られた。primary mutationおよび逆転写酵素領域においては薬剤耐性変異はみられず、以上の6例共、非サブタイプB型に特有と思われる変異はみられなかった。

## A. 研究目的

近年, 国際化の中で日本国内においても、サブタイプB以外のHIVが多く流行している。多くは東南アジアからのサブタイプAEであるが、サブタイプAやCなどアフリカやインド、中国で多く流行しているものも散見されている。昨年度に引き続いて、名古屋医療センターに受診したHIV感染者のうち、非サブタイプBの患者の薬剤耐性関連遺伝子を解析する。

## B. 研究方法

国立名古屋病院に受診した非サブタイプB型HIV患者から患者血清を採取し、Env 領域、逆転写酵素およびプロテアーゼ領域の変異部位を解析する。その結果と、関連する臨床的データーおよびサブタイプ Bを基にした薬剤耐性のデーターと比較検討し、新たに得られた逆転写酵素あるいはプロテアーゼ領域の変異部位におけるgenotypeを解析した。

(倫理面への配慮)

調査研究実施国の実情にあわせ、その国の方針を尊重しつつ、原則としてわが国の基準すなわち「ヒトゲノム・遺伝子解析研究に関する倫理指針(平成13年3月29日文部科学省・厚生労働省・経済産業省告示第1号)」を遵守して研究を遂行した。

## C. 研究結果

国立名古屋病院したHIV感染者のうち、非サブタイプB型HIV患者は6例で、サブタイプ AEが4例、Aが1例、C が1例であった。これらの症例ついて、逆転写酵素領域およびプロテアーゼ領域の薬剤耐性変異の部位を解析したところ、プロテアーゼ領域において、secondary mutationのL10Vがサブタイプ AE(1例)に、L10Iがサブタイプ AおよびAE(2例)に、K20Rがサブタイプ Aに、K20Iがサブタイプ AE(1例)に、M36Iがサブタイプ A, AE, C 全例(6例)に見られた。primary mutation および逆転写酵素領域においては薬剤耐性変異はみられず、以上の6例共、非サブタイプ B 型に

特有と思われる変異はみられなかった。

D. 考察

当医療センターではサブタイプ B に比べて、非サブタイプ B の症例数が少なく、しかも今年度は、治療に抵抗を示した非サブタイプ B の症例はなく、新たな耐性変異は見られなかった。従って、今年度、耐性変異が解析できた6例はすべて未治療感染者であり、見られた変異はすべてサブタイプ B 型に見られる変異と同様なものであった。今後、さらに non-B サブタイプの症例を重ねて解析を行なう予定である。

E. 結論

今回、新たに解析した未治療非サブタイプ B 型 HIV 感染者6例においては、見られた薬剤耐性変異はすべてサブタイプ B 型に見られる変異と同様なものであった。

F. 健康危険情報

特になし

G. 研究発表

研究成果の刊行に関する一覧表参照

H. 知的所有権の出願・登録状況

特になし。

厚生労働科学研究費補助金(エイズ対策研究事業)
分担研究報告書

# 非サブタイプB型HIVにおける薬剤耐性関連遺伝子に関する研究(大阪地域)

分担研究者　大竹 徹 大阪府公衆衛生研究所、ウイルス課、課長
研究協力者　森 治代 大阪府公衆衛生研究所、ウイルス課、研究員

研究要旨

逆転写酵素阻害剤ddI, d4Tおよびプロテアーゼ阻害剤NFVの治療経過中、ウイルス量が上昇してきたサブタイプAEの症例について解析したところ、逆転写酵素領域において、M41L, T215Fが出現し、さらにサブタイプBには報告されていない新しい変異としてI135Tが見られた。、また、プロテアーゼ領域において、A71T, M36I, K20T(サブタイプBではK20M/R/I), V82I(サブタイプBではV82A/F/L/T/S)、さらにサブタイプBには報告されていない新しい変異としてL23IとM89Iが見られた。

## A. 研究目的

非サブタイプBのデータから導かれたジェノタイプ解析アルゴリズムの構築を目的とし、、非サブタイプBにおける薬剤耐性HIVの遺伝学的特徴(genotype)と感染性(phenotype)との関連を解析する。

## B. 研究方法

非サブタイプB型HIV患者から患者血清を採取し、Env領域、逆転写酵素およびプロテアーゼ領域の変異部位を解析する。その結果と、関連する臨床的データーおよびサブタイプBを基にした薬剤耐性のデーターと比較検討し、新たに得られた逆転写酵素あるいはプロテアーゼ領域の変異部位におけるgenotypeを解析した。

(倫理面への配慮)

調査研究実施国の実情にあわせ、その国の方針を尊重しつつ、原則としてわが国の基準すなわち「ヒトゲノム・遺伝子解析研究に関する倫理指針(平成13年3月29日文部科学省・厚生労働省・経済産業省告示第1号)」を遵守して研究を遂行した。

## C. 研究結果

逆転写酵素阻害剤ddI, d4Tおよびプロテアーゼ阻害剤NFVの治療経過中、ウイルス量が上昇してきたサブタイプAEの症例について解析したところ、逆転写酵素領域において、M41L, T215Fが出現し、さらにサブタイプBには報告されていない新しい変異としてI135Tが見られた。、また、プロテアーゼ領域において、A71T, M36I, K20T(サブタイプBではK20M/R/I), V82I(サブタイプBではV82A/F/L/T/S)、さらにサブタイプBには報告されていない新しい変異としてL23IとM89Iが見られた(表)。

## D. 考察

今年度、抗AIDS薬治療中のサブタイプAEの症例において、サブタイプBには報告されていない新しい薬剤耐性変異がいくつか見られたが、このうち、M89Iについては、phenotypeの解析によってサブタイプC, F, Gにおいて NFV 耐性に関与しているというが報告が

# Genotypic mutations of subtype AE HIV-1-infected patients failing antiretroviral therapy

| ID | Sampling | Viral Load | Treatment | Mutation | |
|---|---|---|---|---|---|
| | | | | RT | PR |
| 0-36-1 | 1997/10/3 | 250,000 | AZT, 3TC | none | L10I, M36I |
| 0-36-2 | 1998/5/21 | 2,000 | | | |
| 0-36-3 | 1998/6/16 | 7,900 | | M184V | |
| 0-36-4 | 1998/9/29 | <400 | ddI, d4T, NFV | | |
| 0-36-6 | 2000/2/22 | <50 | | | |
| 0-36-11 | 2005/4/8 | 900 | | | |
| 0-36-12 | 2005/7/7 | 1,300 | | M41L, T215F, I135T | K20T, M36I, A71T, L23I, V82I, M89I |

AIDS 19:1799-1806, 2005 に見られた。

E. 結論

抗AIDS薬治療中のサブタイプAEの症例において、逆転写酵素領域においてI135Tが、プロテアーゼ領域において、K20T(サブタイプBではK20M/R/I), V82I(サブタイプBではV82A/F/L/T/S)、さらに新しい部位として、L23IとM89Iが見られた。

F. 健康危険情報

特になし

G. 研究発表

研究成果の刊行に関する一覧表参照

H. 知的所有権の出願・登録状況

特になし。

## 研究成果の刊行に関する一覧表


1. Khamadi SA, Ichimura H, et al.: HIV-1 subtypes in circulation in Northern Kenya. AIDS Res. Hum. Retroviruses (2005), 21(9):810-4.
2. Agdamag DM, Ichimura H, et al.: Rapidly spreading HCV infections from limited sources simulating an AIDS outbreak in the Philippines. J Med Virol (2005), 77:221-226.
3. Kurbanov F, Ichimura H, et al.: A New Subtype (Subgenotype) Ac (A3) of Hepatitis B Virus and Recombination between Genotypes A and E in Cameroon. J Gen Virol (2005), 86:2047-2056.
4. Takemura T, Ichimura H, et al.: A novel SIV from black mangabey (Lophocebus aterrimus) in Democratic Republic of Congo. J Gen Virol (2005), 86:1967-1971.
5. Otake T, Kawahata T, Mori H, Kojima Y, Hayakawa K, Novel method of inactivation of human immunodeficiency virus type 1 by the freeze pressure generation method, Applied Microbiology and Biotechnology, **67**, 746-751, 2005
6. New estimation method for highly sensitive quantitatiion of human immunodeficiency virus type 1 DNA and its application. H. Nagai, K. Wada, T. Morishita, M. Utsumi, Y. Nishiyama and T. Kaneda J. Virol. Methods **124**, 157-165 (2005).
7. Conventional HPLC Method Used for Simultaneous Determination of the Seven HIV Protease Inhibitors and Nonnucleoside Reverse Transcription Inhibitor Efavirenz in Human Plasma. M. Takahashi, M. Yoshida, T. Oki, N. Okumura, T. Suzuki and T. Kaneda Biol. Pharm. Bull., **28**, 1286-1290 (2005).
8. PNA-In Situ Hybridization Method for Detection of HIV-1 DNA in Virus-Infected Cells and Subsequent Detection of Cellular and Viral Proteins. T. Hagiwara, J. Hattori and T. Kaneda. In Situ Hybridization Protocols 3rd edition (edited by I. A. Darby), Humana Press, NJ, pp139-149 (2005).
9. 山本　直彦　アジアのエイズ：インドーエイズ流行におけるレオリエント
日本エイズ学会誌　第８巻　第１号　ｐ７－１１、　（２００６年）
10. 大竹　徹，　ウイルスの高圧不活化と血液製剤への利用，
Foods Food Ingredients J Jpn,**210**,44-48,2005
11. ＨＰＬＣによるプロテアーゼ阻害剤アタザナビルの血中濃度測定法の開発。
高橋昌明、吉田昌生、大木　剛、奥村直哉、鈴木達男、金田次弘
日本病院薬剤師会雑誌、41，731-734（2005）.

12. カレトラ™投与外来HIV感染患者における脂質異常とロピナビル血中濃度の評価。
高橋昌明、吉田昌生、大木　剛、奥村直哉、鈴木達男、金田次弘
日本病院薬剤師会雑誌、41, 873-876 (2005).

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 21, Number 9, 2005, pp. 810–814


# Sequence Note

# HIV Type 1 Subtypes in Circulation in Northern Kenya

SAMOEL A. KHAMADI,[1] WASHINGTON OCHIENG,[1] RAPHAEL W. LIHANA,[1] JOYCELINE KINYUA,[1] JOSEPH MURIUKI,[1] JOSEPH MWANGI,[1] RAPHAEL LWEMBE,[1] MICHAEL KIPTOO,[1] SAIDA OSMAN,[1] NANCY LAGAT,[1] ROGER PELLE,[2] ANNE MUIGAI,[3] JANE Y. CARTER,[4] ISAO OISHI,[5] HIROSHI ICHIMURA,[5] D.L. MWANIKI,[1] FREDRICK A. OKOTH,[1] SOLOMON MPOKE,[1] and ELIJAH M. SONGOK[1]

## ABSTRACT

**The genetic subtypes of HIV-1 circulating in northern Kenya have not been characterized. Here we report the partial sequencing and analysis of samples collected in the years 2003 and 2004 from 72 HIV-1-positive patients in northern Kenya, which borders Ethiopia, Somalia, and Sudan. From the analysis of partial *env* sequences, it was determined that 50% were subtype A, 39% subtype C, and 11% subtype D. This shows that in the northern border region of Kenya subtypes A and C are the dominant HIV-1 subtypes in circulation. Ethiopia is dominated mainly by HIV-1 subtype C, which incidentally is the dominant subtype in the town of Moyale, which borders Ethiopia. These results show that cross-border movements play an important role in the circulation of subtypes in Northern Kenya.**

KENYA IS BORDERED IN THE NORTH by countries that have had political upheavals in the past leading to a lot of movement of populations across the borders into Kenya. These countries are Ethiopia, Somalia, and Sudan. In this region, not much is known about the circulating subtypes of HIV-1.

Work done between 1998 and 1999 shows that Sudan is dominated mainly by subtypes A, C, and D, with subtype D being the dominant circulating subtype.[1] In Ethiopia, the HIV-1 epidemic is dominated exclusively by HIV-1 C viruses[2] while in Somalia the circulating subtypes have not been clearly defined.

In this study to determine the circulating subtypes of HIV-1 in northern Kenya, HIV-1-positive patients and blood donors attending STD clinics and District hospitals in Mandera, Moyale, and Turkana District between August 2003 and April 2004 were recruited. The study subjects gave written informed consent and 3 ml of blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes. Peripheral blood mononuclear cells (PBMCs) were extracted and used for polymerase chain reaction (PCR) amplification. A nested strategy was used to amplify about 450 base pairs of the *env* gene (nt 7850–8310) i.e., the gp41 region.[3] The primers used in the PCR were gp40F1 (5′-TCTTAGGAGCAGCAGGAAGCACTATGGG-3′) and gp41R1 (5′-AACGACAAAGGTGAGTATCCCTGCCTAA-3′) for the first round of PCR and primers gp46F2 (5′-ACAATTATTGTCTGGTATAGTGCAACAGCA-3′) and gp47R2 (5′-TTAAACCTATCAAGCCTCCTACTATCATTA-3′ for the nested PCR. The PCR conditions included denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 60 sec, with a final extension at 72°C for 5 min. The resulting products were electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light to identify the amplified products. The PCR products were sequenced directly using the BigDye Terminator DNA sequencing kit from Applied Biosystems. Electrophoresis and data collection were accomplished with an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA). The CLUSTAL W method[4] was used to align the resulting 400–450 bp nucleotide sequences to-

[1]Kenya Medical Research Institute, [2]International Livestock Research Institute Kenya, [3]Jomo Kenyatta University of Agriculture and Technology, [4]African Medical and Research Foundation Kenya, and [5]KEMRI-JICA Project, Nairobi, Kenya.

gether with relevant reference sequences from the Los Alamos reference database.[5] Phylogenetic relationships were deduced using the neighbor-joining method.[6] The phylogenetic tree was drawn using the tree view program.[7]

Phylogenetic analysis of the *env* gp41 region of samples from 72 HIV-1-positive patients revealed that 50% (32 samples) of the samples were subtype A, 39% (28 samples) were subtype C, and 11% (8 samples) were subtype D. The results also showed a significant difference in the distribution of the HIV-1 subtypes in Turkana and Moyale. In Moyale a majority of the samples were subtype C (51%); 40% of the samples were subtype A and 9% were subtype D. Moyale contributed 82% of the total subtype C found in this region. This region borders Ethiopia where the dominant HIV-1 subtype is C. In Turkana, the dominant subtype in circulation is A (64%), while the rest is subtype C (20%) and D (16%). The number of samples from Mandera successfully analyzed was too few (2) to draw any significant conclusions. These samples were HIV-1 subtype A. The phylogeny of these viruses

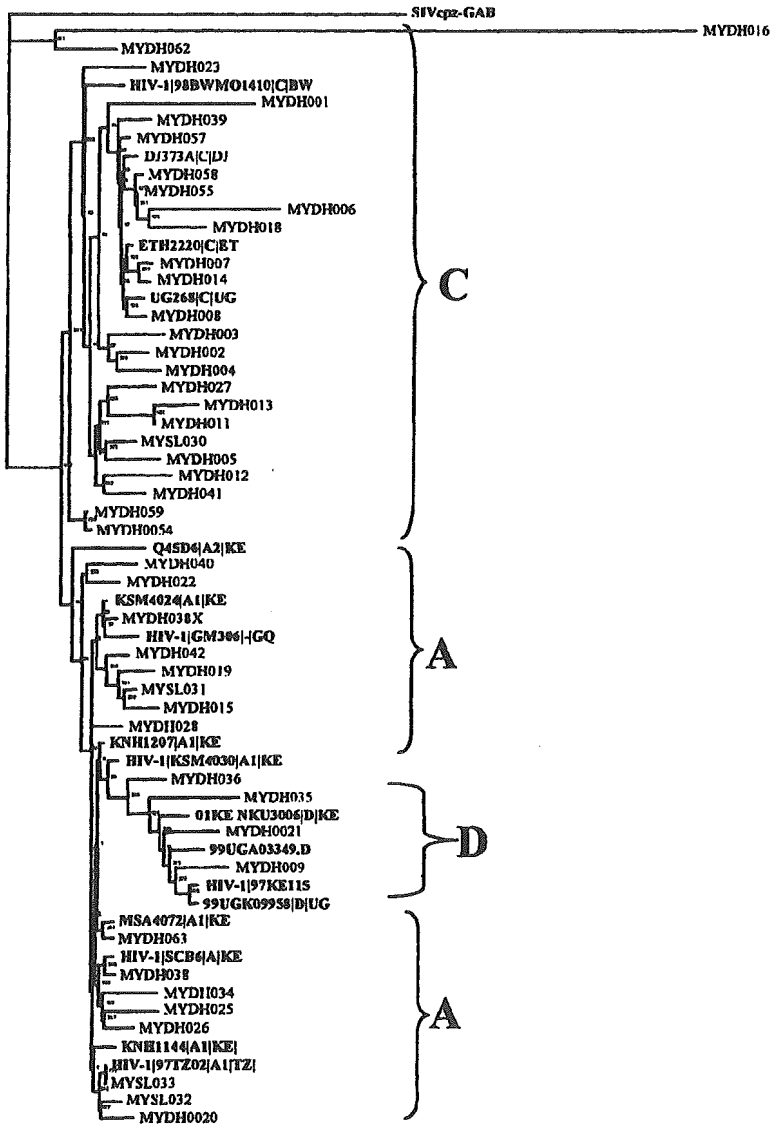


FIG. 1. Phylogenetic analysis of the gp41 *env* region of HIV-1 subtypes from Moyale in northern Kenya. The simian immunodeficiency virus $SIV_{cpzgab}$ was used as the outgroup. The sequences have been indicated by codes MYDH and MYSL denoting Moyale District Hospital and Moyale Sololo, respectively. The A subtypes clustered with references from Kenya, Gambia, and Tanzania; the C subtypes clustered together with references from Ethiopia, Uganda, Djibouti, and Botswana; and the D subtypes clustered with those from Uganda and Kenya.

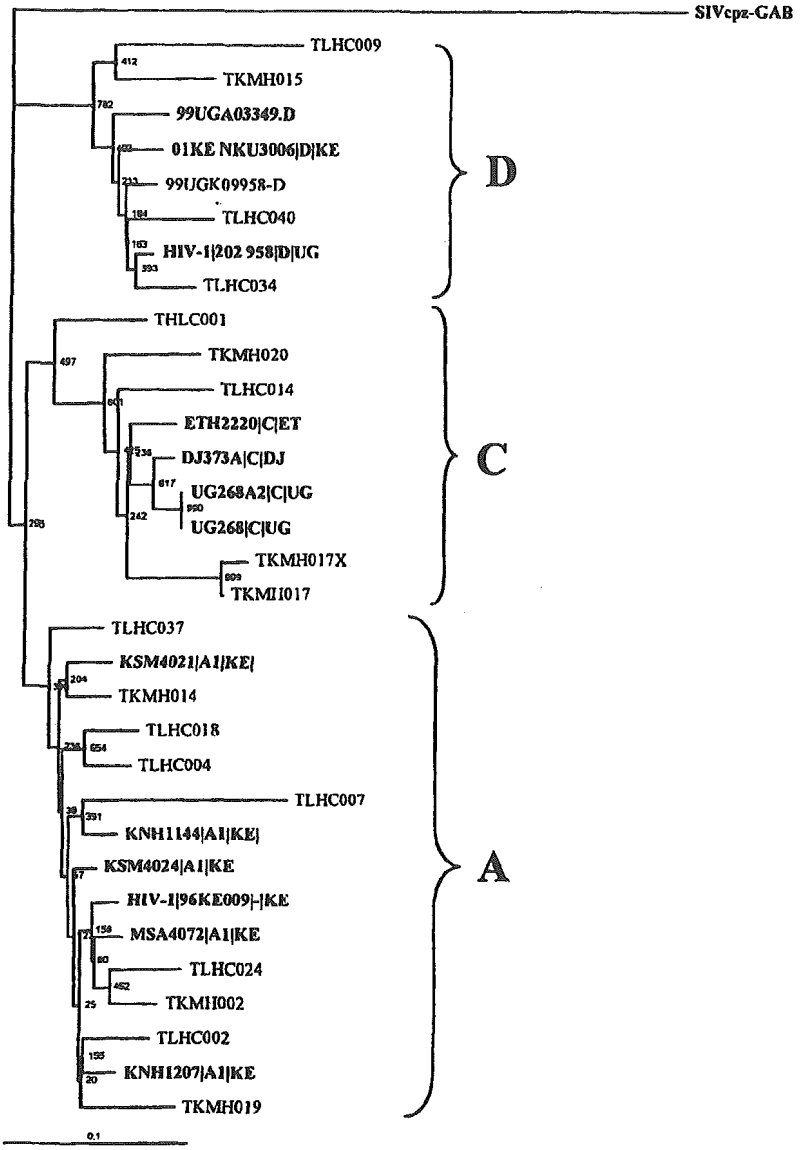


**FIG. 2.** Phylogenetic analysis of the gp41 *env* region HIV-1 subtypes from Turkana in northern Kenya. The sequences have been indicated by codes TLHC and TKMH denoting Turkana Lobiding Health Centre and Turkana Kakuma Mission Hospital, respectively. The A subtypes in this region clustered with references from Kenya; the C subtypes clustered with references from Ethiopia, Djibouti, and Uganda; and the D subtypes from the region clustered with references from Uganda and Kenya as indicated on the tree.

is displayed in Figs. 1–3. The information available about the study subjects is shown along with the subtype in Table 1. These results indicate a different picture of HIV-1 subtypes in circulation compared to other parts of Kenya where the dominant subtype in circulation is A (70%).

## SEQUENCE DATA

GenBank accession numbers (listed in Table 1) for the *env* gp41 sequences are AY697976–AY698021, AY694410–AY694411, AY693585–AY693603, and AY705732–AY705737.

## ACKNOWLEDGMENTS

The KEMRI-JICA program funded this project. The work was done in collaboration with the African Medical and Research Foundation. We wish to thank the staff in Northern Kenya, i.e., in Moyale, Mandera, Kakuma, and Lokichogio hospitals, who assisted in the collection and initial processing of the samples.

TABLE 1. INFORMATION ABOUT STUDY SUBJECTS

| *ID* | *Year* | *GenBank accession no.* | *Age (years)* | *Sex* | *Subtype (env)* |
|---|---|---|---|---|---|
| MYDH012 | 2003 | AY698019 | 32 | F | C |
| MYDH013 | 2003 | AY698011 | 2 | F | C |
| MYSL032 | 2003 | AY697991 | 50 | F | A1 |
| MYDH054 | 2003 | AY698010 | 34 | F | C |
| MYDH058 | 2003 | AY698002 | 34 | M | C |
| MYDH057 | 2003 | AY698003 | 20 | F | C |
| MYDH034 | 2003 | AY698021 | 23 | F | A1 |
| MYDH035 | 2003 | AY698004 | 25 | F | D |
| MYDH038 | 2003 | AY697981 | 28 | F | A |
| MYDH055 | 2003 | AY698009 | 32 | M | C |
| MYDH059 | 2003 | AY698008 | 34 | F | C |
| MYDH025 | 2003 | AY697978 | 21 | F | A1 |
| MYSL033 | 2003 | AY697984 | 30 | F | A1 |
| MYSL031 | 2003 | AY697986 | 44 | M | A |
| MYDH023 | 2003 | AY698018 | 7 | M | C |
| MYDH022 | 2003 | AY697988 | 50 | F | A1 |
| MYDH028 | 2003 | AY698020 | 26 | F | A1 |
| MYDH027 | 2003 | AY697980 | 32 | M | C |
| MYSL030 | 2003 | AY697985 | 40 | M | C |
| MYDH021 | 2003 | AY697979 | 38 | M | D |
| MYDH036 | 2003 | AY697987 | 32 | M | A |
| MYDH039 | 2003 | AY697976 | 20 | F | C |
| MYDH020 | 2003 | AY697983 | 30 | F | A1 |
| MYDH038 | 2003 | AY698005 | 28 | F | A1 |
| MYDH026 | 2003 | AY697982 | 30 | M | A1 |
| MYDH003 | 2003 | AY697992 | 35 | M | C |
| MYDH005 | 2003 | AY697989 | 40 | M | C |
| MYDH004 | 2003 | AY697990 | 45 | F | C |
| MYDH002 | 2003 | AY697993 | 45 | M | C |
| MYDH007 | 2003 | AY697995 | 20 | F | C |
| MYDH014 | 2003 | AY697996 | 25 | M | C |
| MYDH001 | 2003 | AY697997 | 25 | F | C |
| MYDH063 | 2004 | AY698007 | 35 | M | A1 |
| MYDH016 | 2003 | AY697998 | 46 | M | A |
| MYDH015 | 2003 | AY697994 | 41 | M | A1 |
| MYDH019 | 2003 | AY698017 | 47 | M | A1 |
| MYDH018 | 2003 | AY698016 | 42 | M | C |
| MYDH006 | 2003 | AY697999 | 32 | M | C |
| MYDH009 | 2003 | AY698001 | 32 | M | D |
| MYDH041 | 2003 | AY698014 | 56 | M | C |
| MYDH011 | 2003 | AY698015 | 30 | F | C |
| MYDH040 | 2003 | AY698013 | 27 | F | A |
| MYDH042 | 2003 | AY698012 | 32 | F | A1 |
| MYDH062 | 2004 | AY698005 | 38 | M | D |
| MYDH008 | 2003 | AY698000 | 50 | M | C |
| TLHC007 | 2003 | AY693585 | 28 | M | A1 |
| TLHC014 | 2003 | AY693588 | 33 | M | C |
| TLHC018 | 2003 | AY693587 | 39 | F | A |
| TLHC024 | 2003 | AY693589 | 24 | M | A1 |
| TLHC034 | 2003 | AY693597 | 39 | F | D |
| TLHC040 | 2003 | AY693596 | 34 | F | D |
| TLHC004 | 2003 | AY693591 | 28 | F | A1 |
| TLHC037 | 2003 | AY693595 | 24 | F | A1 |
| TLHC002 | 2003 | AY693586 | 16 | F | A1 |
| TLHC009 | 2003 | AY693590 | 21 | F | D |
| TLHC001 | 2003 | AY693592 | 30 | F | A |
| TLHC101 | 2004 | AY705733 | 24 | M | A |
| TLHC106 | 2004 | AY705737 | 35 | M | A1 |
| TLHC107 | 2004 | AY705732 | 42 | M | A |
| TLHC109 | 2004 | AY705734 | 45 | F | C |
| TLHC111 | 2004 | AY705735 | 46 | M | A |

TABLE 1. INFORMATION ABOUT STUDY SUBJECTS (CONT'D)

| *ID* | *Year* | *GenBank accession no.* | *Age (years)* | *Sex* | *Subtype (env)* |
|---|---|---|---|---|---|
| TLHC112 | 2004 | AY705736 | 50 | M | A1 |
| TKMH017X | 2004 | AY693601 | 30 | F | C |
| TKMH017 | 2003 | AY693602 | 40 | M | C |
| TKMH002 | 2003 | AY693598 | 20 | F | A |
| TKMH020 | 2003 | AY693599 | 24 | M | C |
| TKMH014 | 2003 | AY693600 | 30 | F | A1 |
| TKMH019 | 2003 | AY693593 | 35 | M | A |
| TKMH015 | 2003 | AY693594 | 40 | F | D |
| MADH005 | 2003 | AY694411 | 37 | F | A1 |
| MADH003 | 2003 | AY694410 | 20 | M | A |

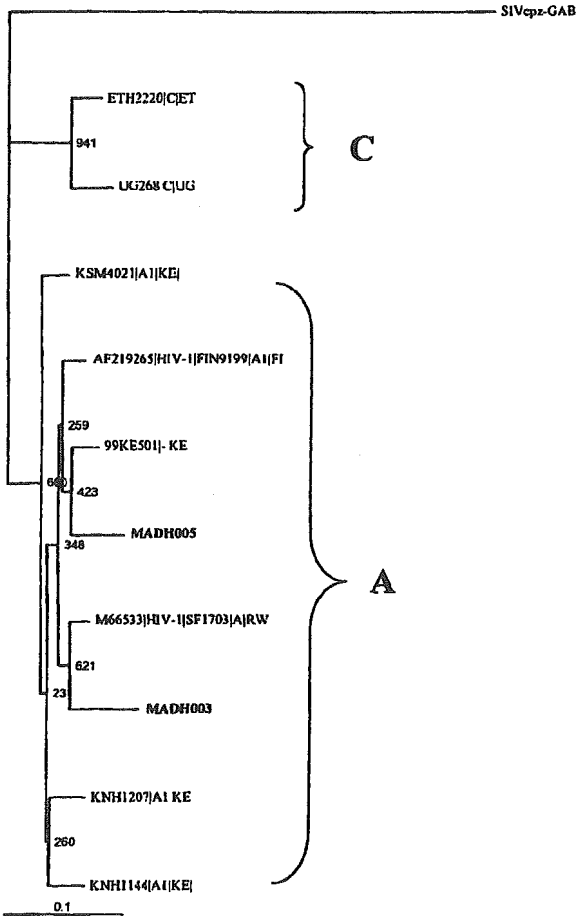


FIG. 3. Phylogenetic analysis of the gp41 *env* region HIV-1 subtypes from Mandera in northern Kenya. The sequences have been indicated by codes MADH denoting Mandera District Hospital. The two samples were all subtype A and clustered with subtypes from Rwanda and Kenya.

## REFERENCES

1. Hierholzer M, Graham RR, El Khidir I, Tasker S, Darwish M, Chapman GD, Fagbami AH, Soliman A, Birx DL, McCutchan F, and Carr JK: HIV type 1 strains from East and West Africa are intermixed in Sudan. AIDS Res Human Retroviruses 2002;18(15): 1163–1166.
2. Abebe A, Kuiken CL, Goudsmit J, Valk M, Messele T, Sahlu T, Yeneneh H, Fontanet A, De Wolf F, Rinke and De Wit TF: HIV type 1 subtype C in Addis Ababa, Ethiopia. AIDS Res Human Retroviruses 1997;13(12):1071–1075.
3. Yang C, Dash BC, Simon F, Groen G, Pieniazek D, Gao F, Hahn BH, and Lal RB: Detection of diverse variants of human immunodeficiency virus-1 groups M, N, and O and simian immunodeficiency viruses from chimpanzees by using generic *pol* and *env* primer pairs. J Infect Dis 2000;181:1791–1795.
4. Thompson JD, Higgins DG, and Gibson TJ: CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22: 4673–4680.
5. Kuiken C, Foley B, Hahn B, *et al.*: *HIV Sequence Compendium 2001*. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM.
6. Saitou N and Nei M: The neighbor joining method: A new method for reconstruction of phylogenetic trees. Mol Biol Evol 1987;4: 406–425.
7. Page RDM: TREEVIEW: An application to display phylogenetic trees on personal computers. Comput Appl Biosci 1996; 12:357–358.

Address reprint requests to:
*Samoel Ashimosi Khamadi*
*P.O. Box 54628-00200*
*Nairobi, Kenya*

*E-mail:* skhamadi@nairobi.mimcom.net

Journal of Medical Virology 77:221–226 (2005)

# Rapid Spread of Hepatitis C Virus Among Injecting-Drug Users in the Philippines: Implications for HIV Epidemics

**Dorothy M. Agdamag,[1,2] Seiji Kageyama,[2]* Evelyn T. Alesna,[3] Rontgene M. Solante,[1] Prisca S. Leaño,[1] Anna Marie L. Heredia,[3] Ilya P. Abellanosa-Tac-An,[4] Eutiquio T. Vibal,[5] Lourdes D. Jereza,[6] and Hiroshi Ichimura[2]**

[1]*STD AIDS Cooperative Central Laboratory, San Lazaro Hospital, Manila, Philippines*
[2]*Department of Viral Infection and International Health, Kanazawa University, Kanazawa, Japan*
[3]*Cebu Center for Infectious Diseases, Cebu City, Philippines*
[4]*Cebu City Health Office, Cebu City, Philippines*
[5]*Cebu Center for the Ultimate Rehabilitation of Drug Dependents, Cebu City, Philippines*
[6]*University of Southern Philippines, Cebu City, Philippines*

From the trends of human immunodeficiency virus (HIV) epidemics in South and Southeast Asia, it was postulated that an HIV epidemic would start as a blood-borne infection among injecting-drug users in the Philippines. In 2002, 560 individuals were recruited in Metro Cebu, Philippines and tested for HIV, hepatitis C virus (HCV), and hepatitis B virus (HBV) infections. The seroprevalence of anti-HCV among injecting-drug users (70.1%, 61/87) was significantly higher than those among inhalation drug users (16.3%, 7/43; $P=0.00$; OR = 12), sex workers (0%, 0/130; $P=0.00$; OR = $\infty$), antenatal clinic attendees (0%, 0/100; $P=0.00$; OR = $\infty$), and students/health care workers (2%, 4/200; $P=0.00$; OR = 115). The seroprevalence of HBsAg among injecting-drug users (10.3%, 9/87) was significantly higher than those among sex workers (2.3%, 3/130; $P=0.01$; OR = 4.9), and antenatal clinic attendees (3%, 3/100; $P=0.04$; OR = 3.7), but was not statistically different from those among inhalation drug users (9.3%, 4/43; $P=0.9$) and students/health care workers (4.5%, 9/200; $P=0.06$). None of the study population was reactive to anti-HIV antibody. The HCV strains obtained from the injecting-drug users belonged to either genotype 1a or 2b and the strains in each genotype clustered closely to each other. There was no dual infection with genotype 1a and 2b. These results suggest that the HCV infection in injecting-drug users may be emanating rapidly from limited number individuals in Metro Cebu, Philippines. ***J. Med. Virol. 77:221–226, 2005.*** © 2005 Wiley-Liss, Inc.

**KEY WORDS:** HCV epidemic; Injecting-drug users; genotype; source; HIV/AIDS outbreak; HIV prevalence



## INTRODUCTION

The Philippines is one of the low prevalence countries for human immunodeficiency virus (HIV). Based on the AIDS registry of the Department of Health in the Philippines, the total number of HIV cases has increased but remained at low level at a cumulative total of 2,107 as of June 2004. The main mode of HIV transmission has been reported to be heterosexual contact since 1984. Although HIV-positive cases have appeared sporadically among sexually active populations such as sex workers, no outbreak has occurred among them in this country. However, wide-range HIV strains have been introduced in the country, that is; five HIV-1 subtypes (A, B, C, D, and F), a circulating recombinant form (CRF01_AE) [Paladin et al., 1998; Santiago et al., 1998; Espantaleon et al., 2003], a recombinant strain (*gag*-A/*env*-B) [Espantaleon et al., 2003]. Even HIV-2 [Leano et al., 2003] has been identified. Among these, HIV-1 subtype B was the most predominant, followed by CRF01_AE [Paladin et al., 1998; Santiago et al., 2003]. The low prevalence and the variety of HIV strains in the Philippines indicate that HIV has been imported mainly from abroad and the gateway of HIV into the Philippines has been quite open.

Grant sponsor: Japan Society for the Promotion of Science; Grant numbers: 16406014, DOST-10417; Grant sponsor: The Nippon Foundation Fellowship for Asian Public Intellectuals.

*Correspondence to: Seiji Kageyama, Department of Viral Infection and International Health, Graduate School of Medical Science, Kanazawa University, 13-1, Takara-Machi, Kanazawa, 920-8640, Japan. E-mail: kageyama@med.kanazawa-u.ac.jp

Accepted 21 June 2005
DOI 10.1002/jmv.20439
Published online in Wiley InterScience (www.interscience.wiley.com)

Therefore, the migration sites and the subsequent circulation pathways of HIV have become one of the most important concerns for the prevention of an AIDS outbreak in the Philippines.

The past trend of HIV/AIDS outbreak in South and Southeast Asia reported by the World Health Organization (*WHO; HIV/AIDS in Asia and the Pacific Region* 2003) and others [Ruxrungtham et al., 2004] have implied that the Asian AIDS epidemic may start among injecting-drug users with secondary new infections become evident among sex workers. This is reasonable when considering the fact that the probability of HIV infection is 10-fold higher for transmission through contaminated needle sharing than that through sexual contact [Royce et al., 1997]. Therefore, it could be postulated that an HIV outbreak would start as a blood-borne infection among injecting-drug users in the low HIV-prevalence countries including the Philippines, and that the HIV outbreak could be preceded by other blood-borne infections, such as hepatitis C virus (HCV) and hepatitis B virus (HBV) infections.

HIV, HCV, and HBV are the major blood-borne pathogens, which spread among injecting-drug users via shared syringes and other injection devices [Lauer and Walker, 2001]. The seroprevalence of HCV antibody (anti-HCV) has been reported globally to be 65–90% among injecting-drug users [van den Hoek et al., 1990; Chamot et al., 1992; Crofts et al., 1993; Van Ameijden et al., 1993; Lauer and Walker, 2001; Soriano et al., 2002] and 82.9–100% among HIV-infected injecting-drug users [van Asten et al., 2004]. However, the reports on the prevalence and the characteristics of HCV and HBV have been limited in the Philippines. According to the available data, the positive rate for anti-HCV was 2.2% (9/392 tested) and the same rate was also noted for HBsAg among blood donors in 1990 [Arguillas et al., 1991], and anti-HCV was reported to be 4.6% (23/502 tested) among prison inmates [Katayama et al., 1996].

In this study, an HCV-epidemic site was identified in the Philippines and the genetic links of the HCV strains infecting injecting-drug users were analyzed to determine their migration site, circulation pathways, and the speed of transmission.

## MATERIALS AND METHODS

### Subjects

From June to August 2002, 560 individuals were recruited in Metro Cebu of the Philippines. Study population was categorized into five groups; injecting-drug users (n = 87), inhalation drug users (n = 43), sex workers (n = 130), antenatal clinic attendees (n = 100), and students and health care workers (n = 200). Characteristics of the study population are shown in Table I. Injecting-drug users were from two areas; an urban area where there was easy access to prohibited drugs and the drug rehabilitation centers. Injecting-drug users were identified by a pre-tested interview questionnaire conducted by trained staff. All of the 560 participants agreed to be part of the study after the researchers explained the objectives and the conduct of the study, and signified their intent to join the study by signing an informed consent form.

### Serological Testing

A total of 5-ml whole blood was collected from each participant. Plasma was separated and subjected to each test.

Determine HIV-1/2 (ABBOTT JAPAN, Tokyo, Japan) and Determine HBsAg (ABBOTT JAPAN) were used for the detection of anti-HIV antibody and hepatitis B surface antigen, respectively. HCV PHA (Abbott Laboratories HCV 2nd Generation) was kindly provided by Abbott, Japan, for research purpose and was used for the detection of anti-HCV in this study. All the systems were used according to the manufacturer's instructions.

### RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction (PCR)

HCV-RNA was extracted from 100 μl of plasma using SMITEST EX-R&D (Genome Science Laboratories, Fukushima, Japan), and reverse-transcribed according to First-Strand cDNA Synthesis protocol (Invitrogen, Carlsbad, CA) with antisense gene-specific primers, hep32 (5′-GCDGARTACCTGGTCATAGC-3′) for NS5B regions of HCV genome. A part of NS5B region of HCV gene was amplified by nested PCR with primers, hep31b (5′-TGGGSTTCTCDTATGAYACC-3′)/hep32 in the first round, and hep33b (5′-AYACCCGMTGYTTTGACTC-3′)/hep34b (5′-CCTCCGTGAAKRCTCKCAG-3′) in the second round. Nested PCR was performed with 20 μl reaction mixture containing 2.5 mM $MgCl_2$, 200 μM each dNTP, 0.5 μM primers, and one unit of Amplitaq Gold® (Applied Biosystems, Foster City, CA). First-round PCR was done with one cycle of 94°C for 10 min, and 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec with a final extension of 72°C for 10 min. Second-round PCR was done in the same condition except for the annealing temperature at 60°C. PCR amplification was confirmed by visualization with ethidium bromide staining of the gel [White et al., 2000].

TABLE I. Characteristics of Injecting- and Inhalation-Drug Users and Others

| Population | Tested (male/female) | Mean age (range) |
|---|---|---|
| Injecting-drug users | 87 (80/7) | 30 (13–46) |
| Inhalation-drug users | 43 (42/1) | 29 (11–53) |
| Sex workers | 130 (2/128) | 25 (18–46) |
| Antenatal clinic attendees | 100 (0/100) | 26 (17–42) |
| Students/health care workers | 200 (65/135) | 31 (6–61) |

### Genotyping

The PCR product was subjected to nucleotide sequence determination directly with the primers of hep33b and hep34b for NS5B region. Some of the PCR-products were cloned with TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced as described previously [Thompson et al., 1994]. At least 11 clones per sample were analyzed to investigate the possible co-existence of different HCV genotypes.

The sample sequences were aligned with HCV sequences from the database in STD AIDS Cooperative Central Laboratory (Manila, The Philippines) and HCV sequence database (http://gluttony.lanl.gov/content/hcv-db/combined_search/search) by ClustalW with subsequent inspection and manual modification [Thompson et al., 1994]. The frequency of nucleotide substitution in each base of the sequences was estimated by the Kimura two-parameter method. A phylogenetic tree was constructed by the neighbor-joining method, and its reliability was estimated by 1,000 bootstrap replications. The profile of the tree was visualized with the program of Njplot [Perriere and Gouy, 1996].

### Statistical Analysis

Prevalence data of HCV and HBV infection was analyzed by $\chi^2$-test and $P$-value less than 0.05 was considered to be significant.

## RESULTS

### Prevalence of HCV, HBV, and HIV Infections

Of the 87 injecting-drug users, 61 (70.1%) were positive for anti-HCV. Twenty-eight of the injecting-drug users were recruited from an area at the downtown of Metro Cebu, and all (100%, 28/28) had anti-HCV. Of the 43 inhalation drug users, only 7 (16.3%) had anti-HCV. No one was positive for anti-HCV in the 130 sex workers and the 100 antenatal clinic patients. Among the students/health care workers (n = 200), only 4 (2%) were positive for anti-HCV (Table II). Thus, the prevalence of anti-HCV was significantly higher among injecting-drug users than inhalation drug users ($P$ = 0.00; Odds ratio (OR) = 12, 95% Confidence interval (CI): 5–31), sex workers ($P$ = 0.00; OR = ∞), antenatal clinic patients ($P$ = 0.00; OR = ∞), and students/health care workers ($P$ = 0.00; OR = 115, 95% CI: 38–346), indicating that injecting-drug use is associated significantly with HCV infection.

The seroprevalence of HBsAg among injecting-drug users (10.3%, 9/87) was significantly higher than that among sex workers (2.3%, 3/130; $P$ = 0.01; OR = 5, 95% CI: 1–19) and antenatal clinic attendees (3.0%, 3/100; $P$ = 0.04; OR = 4, 95% CI: 1–14), but not than that among inhalation drug users (9.3%, 4/43; $P$ = 0.9) and students/health care workers (4.5%, 9/200; $P$ = 0.06) (Table II).

HIV antibody was not detected in any of these groups (Table II).

Seven (8%) of the 87 injecting-drug users were dually positive for HBsAg and anti-HCV. Among other population groups, there was no dual positive case.

### HCV Genotypes

Of the 61 injecting-drug users positive for anti-HCV (Table II), 52 samples were available for further analysis and 38 samples were positive by PCR with NS5B primers. Twenty-three of the PCR-positive samples were selected random and were subjected to nucleotide sequencing. The PCR products were directly sequenced and analyzed phylogenetically. A phylogenetic tree (Fig. 1) based on NS5B sequences (nucleotides, 7,975–8,196 [Choo et al., 1991]) showed two HCV genotypes, 1a and 2b. Of the 23 HCV strains examined, 15 clustered significantly with genotype 1a reference sequences (with bootstrap value 97%), and most of them sub-clustered together, while two strains (02dx02 and 02du98) did not. The remaining eight clustered significantly with genotype 2b reference sequences and formed a significant sub-cluster (with bootstrap value 96%), suggesting that the source of HCV 2b circulation among the injecting-drug users in Metro Cebu is limited and 02du49 could be a founder strain (Fig. 1).

### Heterogeneity of HCV Strains in an Injecting-Drug User

To investigate the possible co-existence of different HCV genotypes in injecting-drug users, the PCR products of randomly selected 9 strains (5 genotype 1a

TABLE II. Seroprevalence of Hepatitis B Virus, Hepatitis C Virus, and HIV Infections among Selected Population in Metro Cebu

| Population | Tested | Positive cases (%) for: HBsAg | Anti-HCV | Anti-HIV |
|---|---|---|---|---|
| Injecting-drug users | 87 | 9 (10%) | 61 (70) | 0 |
| Downtown of Metro Cebu[a] | 28 | 3 (11) | 28 (100) | 0 |
| Drug rehabilitation centers | 59 | 6 (10) | 33 (56) | 0 |
| Inhalation drug users[b] | 43 | 4 (9.3) | 7 (16) | 0 |
| Sex workers | 130 | 3 (2.3) | 0 | 0 |
| Antenatal clinic attendees | 100 | 3 (3.0) | 0 | 0 |
| Students/health care workers | 200 | 9 (4.5) | 4 (2.0) | 0 |

[a]Clients from the downtown of Metro Cebu (n = 28) were all injecting-drug users.
[b]All the inhalation-drug users were from drug rehabilitation centers.

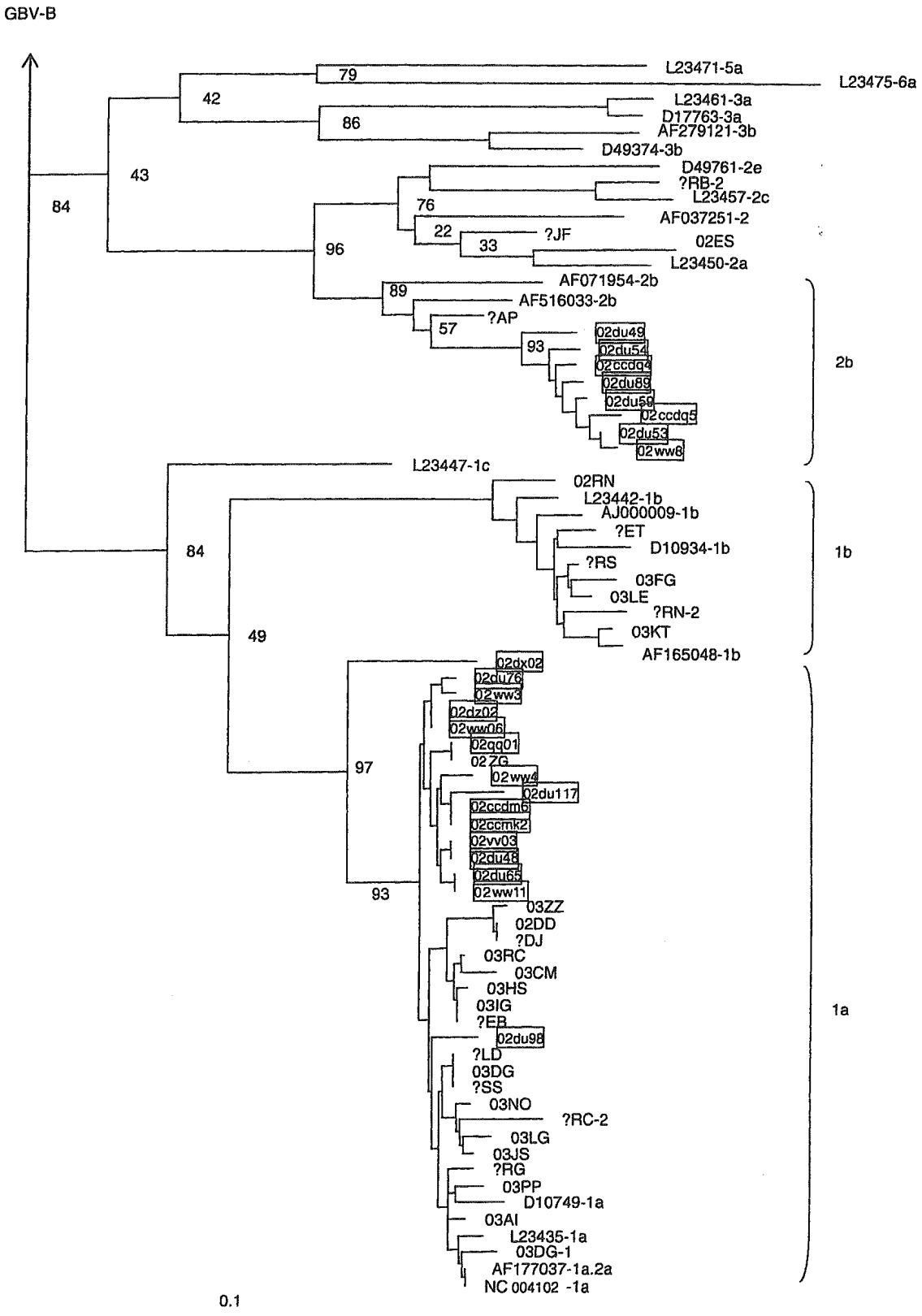


Fig. 1. Phylogenetic trees of 23 HCV strains (highlighted in the boxes) from injecting-drug users in Metro Cebu and 31 HCV strains from other area of the Philippines, performed on 227 nt within the NS5B region by the neighbor-joining method with GBV-B (accession no. NC 001655) as an outgroup. Analyzed samples were indicated with two digits of the collecting year at the head of the ID (e.g., 02ES). If the collecting year is unknown, IDs are shown with the symbol of "?" (e.g.,?JF). Accession numbers were used for the IDs of the genotype-known reference strains with two digits indicating genotypes at the end of the number (e.g., L23471-5a). Bootstrap values are given on the branches as percentage from 1,000 replicates.

strains: 02dz02, 02ccdm6, 02ccmk2, 02du98, and 02qq01; and 4 genotype 2b strains: 02ww8, 02ccdq4, 02ccdq5, and 02du49) were cloned. At least 11 clones per sample were sequenced in the regions of NS5B and analyzed phylogenetically. Phylogenetic trees based on NS5B sequences showed that nucleotide sequences of all the clones in each individual were homogeneous, and co-existence of genotype 1a and 2b were not observed.

## DISCUSSION

In the current study, it was found that an HCV infection was epidemic in Metro Cebu of the Philippines, where 70% of injecting-drug users were positive for anti-HCV. The prevalence of anti-HCV among injecting-drug users has been reported to be 65–90% globally [van den Hoek et al., 1990; Chamot et al., 1992; Crofts et al., 1993; Van Ameijden et al., 1993], and that of Metro Cebu in our study was consistent with previous reports. Despite the high prevalence of anti-HCV positive cases among the tested injecting-drug users, HIV infection was not observed.

Like most RNA viruses, HCV exhibits genetic heterogeneity [Bukh et al., 1995; Zuckerman and Zuckerman, 1995], which has been reported even within the same individual [Houghton et al., 1991; Okamoto et al., 1991; Chen et al., 1992; Martell et al., 1992; Higashi et al., 1993]. In our study, two HCV genotypes, 1a and 2b were circulating among injecting-drug users in Metro Cebu, and each injecting-drug user had homogeneous HCV population regardless of the genotypes. These results suggest that these HCV strains have been introduced recently into injecting-drug users in Metro Cebu and spread rapidly among them. However, the origins have not been specified yet and further investigation is required.

The rate of HBsAg was found to be from 2% to 10% among the different population groups in Metro Cebu. However, there was no significant difference in the seroprevalence of HBsAg between injecting-drug users and inhalation drug users ($P = 0.85$). This may be because newly acquired HBV results in acute infection, needle sharing among injecting-drug users may not contribute to the increase in the HBV chronic infection, and HBV antigen carrier state may mainly be induced by vertical infections. For the further discussion, the detection of anti-HBs antibody will be required.

The Philippines and Indonesia are both island countries and have similar distances from Thailand and Cambodia where HIV infection is most prevalent in Asia. By the year 1999, Indonesia had been considered to be one of the low and slow HIV prevalence countries like the Philippines. However, in late 2000, sharp increase in HIV prevalence among injecting-drug users (up to over 35% in Jakarta) was noted (HIV/AIDS in Asia and the Pacific Region 2001, WHO). This increasing trend of HIV prevalence was also noted among blood donors, thereafter, suggesting that the use of contaminated needle sharing (causing HCV infection) triggered an AIDS outbreak before the increase in the number of HIV-infections through sexual transmission. As seen in Indonesia, HIV spreads first among injecting-drug users, followed by sex workers in other Asian countries especially if drug users are the clients of sex workers [Ruxrungtham et al., 2004]. However, it seems that HIV has not yet spread extremely through the blood-borne pathway in the Philippines. As shown in this study, HIV infection was very rare even among HCV-positive injecting-drug users. However, convincing evidence will be required by the further analyses with increasing the number of subjects and in geographically different places in the Philippines. Although HIV is of low prevalence, the rapid spread of HCV infection indicates that the injecting-drug users can be at highest risk in causing an AIDS epidemic in this country.

In this study, it was demonstrated that the HCV infection clustered among injecting-drug users in Metro Cebu of the Philippines. HCV infection seemed to be spreading rapidly among injecting-drug users from limited sources. Further studies must be conducted to identify the migration site(s) and the subsequent circulation mode of HCV infection more precisely, which can serve as a model for probable migration sites of HIV infections at an early phase of a possible AIDS epidemic in the Philippines.

## ACKNOWLEDGMENTS

The authors are grateful to Mr. M. Villanobos for the technological assistance. Japan Society for the Promotion of Science (Grant-in-Aid for Scientific Research, 16406014 and Ronpaku Program, DOST-10417), Japan and The Nippon Foundation Fellowship for Asian Public Intellectuals, Indonesia, Japan, Malaysia, Philippines, and Thailand, supported this work.

## REFERENCES

Arguillas MO, Domingo EO, Tsuda F, Mayumi M, Suzuki H. 1991. Seroepidemiology of hepatitis C virus infection in the Philippines: A preliminary study and comparison with hepatitis B virus infection among blood donors, medical personnel, and patient groups in Davao, Philippines. Gastroenterol Jpn 26:170–175.

Bukh J, Miller RH, Purcell RH. 1995. Genetic heterogeneity of hepatitis C virus: Quasispecies and genotypes. Semin Liver Dis 15:41–63.

Chamot E, de Saussure P, Hirschel B, Deglon JJ, Perrin LH. 1992. Incidence of hepatitis C, hepatitis B and HIV infections among drug users in a methadone-maintenance programme. AIDS 6:430–431.

Chen PJ, Lin MH, Tai KF, Liu PC, Lin CJ, Chen DS. 1992. The Taiwanese hepatitis C virus genome: Sequence determination and mapping the 5′ termini of viral genomic and antigenomic RNA. Virology 188:102–113.

Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit D, Medina-Selby R, Barr PJ, et al. 1991. Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci USA 88:2451–2455.

Crofts N, Hopper JL, Bowden DS, Breschkin AM, Milner R, Locarnini SA. 1993. Hepatitis C virus infection among a cohort of Victorian injecting drug users. Med J Aust 159:237–241.

Espantaleon A, Kageyama S, Bernardo MT, Nakano T, Leano PS, Alban P, Abrenica R, Morimatsu S, Teraoka H, Agdamag DM. 2003. The influence of the expanding HIV genetic diversity on molecular diagnosis in the Philippines. Int J STD AIDS 14:125–131.

Higashi Y, Kakumu S, Yoshioka K, Wakita T, Mizokami M, Ohba K, Ito Y, Ishikawa T, Takayanagi M, Nagai Y. 1993. Dynamics of genome change in the E2/NS1 region of hepatitis C virus in vivo. Virology 197:659–668.