High-Rate of Human T Lymphotropic Virus Type IIa Infection in HIV Type 1-Infected Intravenous Drug Abusers in Ireland

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ABSTRACT

Serological and molecular analyses of a cohort of HIV-1-infected intravenous drug ah users (IVDAs) (*n* = 103) in Dublin, Ireland have demonstrated that 15 of 103 (14.6%) were infected with HTLV-II, which is the highest infection rate yet recorded for any European country. Restriction fragment length polymorphism (RFLP) analysis of the *env* region of the pro virus demonstrated that the infection involved only the HTLV-IIa subtype; the HTLV-IIb subtype was not detected. Phylogenetic analysis of the nucleotide sequences of the long terminal repeat (LTR) confirmed infection with the HTLV-IIa subtype, and demonstrated that the viruses clustered closely with HTLV-IIa isolates from North American IVDAs. Previous observations that IVDAs in southern Europe, specifically Spain and Italy, appear to be infected predominantly with the HTLV-IIb subtype, along with the present report and evidence that IVDAs in Sweden arc infected with the HTLV-IIa subtype, suggest different origins of HTLV-II infection in Europe.

INTRODUCTION

HUMAN T LVMPHOTROPIC VIRUSES TYPE I (HTLV-I) and type II (HTLV-II) arc closely related retroviruses that have a tropism for T lymphocytes.[1,2] HTLV-I infection is endemic in southwestern Japan, the Caribbean basin, and in parts of South America and Africa.[3-8] In endemic areas infection has been associated with an aggressive $CD4^+$ T cell malignancy, adult T cell leukemia (ATL)[9,10]; a chronic neurological disorder, HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[11,12]; and a characteristic uveitis, HTLV-I uveitis (HUV).[13,14] HTLV-II infection has been shown lo be endemic in a number of native American populations[15-17] and high rates of infection have been documented in intravenous drug abusers (IVDAs) in North America, pans of western Europe, and in Southeast Asia.[18-21] In contrast to HTLV-I, there are no definite associations between HTLV-II infection and clinical disorders; however, there is growing evidence that infection may be associated with a variety of neurological disorders, some of which closely resemble HAM/TSP.[20-27]

Molecular characterization of HTLV-II isolates from North America and Europe has, demonstrated the existence of at least two major molecular subtypes, designated HTLV-IIa and HTLV-IIb.[20] Studies of IVDAs in North America have suggested that HTLV-IIa is the predominant infection in this population.[20] While studies of infection in European IVDAs have been somewhat limited, it has been suggested that in southern-Europe (specifically Spain and Italy) this involves predominanlly, and in many instances exclusively, the HTLV-IIb subtype.[18,28-38] In contrast, a single report from Sweden has suggested that HTLV-IIa predominates in that country.[39].To appreciate better the extent of HTLV-II infections within Europe, we have now investigated HTLV-I1 infections in a cohort of HIV-l-infected IVDAs in the Republic of Ireland. The study

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has demonstrated that this population has the highest rate of HTLV-II infection so far documented in Europe, und is equivalent to that seen in urban areas of the United Stales.[20] Moreover, molecular analysis has indicated that this involves exclusively the HTLV-IIa subtype, suggesting that infections in northern and southern Europe almost certainly have different origins and have probably been introduced al different limes.

MATERIALS AND METHODS

Study population and serological studies

Venous blood samples were obtained from 103 intravenous drug abusers (IVDAs) attending a sexually transmitted disease (STD) clinic and/or a drug rehabilitation unit in central Dublin. The subjects included 71 males (median age. 34.25 years) and 32 females (median age. 32.85 years). All of the subjects were of Irish descent and all were seropositive for H1V-1. Sera were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) (HTLV I/1I antibody detection assay; Murex. Kent, U.K.) and repeatedly reactive samples were confirmed by Western blot (HTLV blot 2.4; Genelabs Diagnostics, Singapore).

Polymerase chain reaction and restriction fragment length polymorphism analysis

DNA for polymerase chain reaction (PCR) studies was extracted from the peripheral blood mononuclear cells (PBMCs), using the Puregene DNA isolation kit (Centra Systems, Minneapolis, MN).

pX *region.* To differentiate HTLV-I and HTLV-II infections, restriction fragment length polymorphism (RFLP) analysis of an amplified product of a region of *pX* was performed using methods previously reported.[40] Reactions were carried out in a Perkin-Elmer Cetus (Emeryville. CA) 9600 DNA thermal cycler and amplified products were digested with *Taq*I restriction endonuclease (Stratagene, La Jolla, CA) in accordance with the manufacturer guidelines, and analyzed by agarose gel elec-trophoresis.

Envelope region. To differentiate HTLV-IIa and HTLV-IIb, RFLP analysis of the *env* region was carried out, Specifically, nested PCR was used to amplify a region encoding the envelope gp21 transmembrane protein. Primers employed in the first round were E2 (5' CTGCAGAAGCTAGCAGGTCTA 3') and E5 (5' AGCCAAGTGTCCCTTCGACTA 3'), corresponding to nucleuses 6661-6641 and 5603-5623 of the HTLV-II isolate Mo-T. The nested primers were E2 and El (5' CTGCAA-CAACTCCATTATCCT 3'J. corresponding to nucleotides 6031-6051 of HTLV-II Mo-T. Amplification was performed in 100.0-jul reaction mixtures containing 2 *μg* of DNA, a 200 *μM* concentration of each dNTP, 40 pmol of each primer, 10 mM Tris-HCi (pH 8.3), 50 mM KC1, 0.1 mM $MgCl_2$. and 2.5 U of *Taq* polymerase. Five microliters of the amplified product from the first round of PCR was used as a template in the nested PCR. The same cycling conditions were used in both the initial and nested PCRs and consisted of an initial denaturation al 94°C for 5 min, followed by 35 cycles at 94°C for 40 sec, 53°C for 30 sec, and 72°C for 40 sec. with a final additional 10-min extension at 72°C The amplified product was then analysed following digestion with *Xho*I restriction endonuclease (Strat-ngence) as described previously.[41]

Long terminal repeal region. For nucleotide sequencing and phylogenetic studies a 623-nucleolide region of the long terminal repeat (LTR) region was amplified from five randomly chosen *pX* and *env* PCR-positive samples, using nested PCR. First-round PCK primers were F II LTRNru (5' TCGCGATGACAATGGCGAC-TAGCCTC 3'), corresponding to nucleotides 1-20 of the HTLV-II Mo-T isolate plus a 6-base Nru restriction site at the 5' end, and Long *Gag* (5' GGGGGCTTTGGGTATTGGAGTTGGG 3'), corresponding to nucleotides 854-83 1 of HTLV-II Mo-T. Nested PCR primers were MoI6 (5' GCCTCCCAAGCCAGCCAC 3'P and MSW-*Gag* (5' GGGAAAGCCCGTGGATTTGCCCCAT 3'), corresponding to nucleotides 16-31 and 831-807. respectively, of HTLV-II Mo-T. Amplifications were performed in 100.0-*μ*1 reaction mixtures containing 2 *μ*g of DNA, a 200 *μ*M concentration of each dNTP, 40 pmol of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KC1, 0.1 mM $MgCl_2$ and 2.5 U of *Taq* polymerase. Ten microliters of the first-round PCR product was used as a template for the nested PCR. Thermal cycling conditions used for both the first-round and nested PCRs included an initial denaturation at 94°C for 10 min, and subsequently 35 cycles al 94°C for 40 sec, 57.6°C for 30 sec, and 72°C for 40 sec, both of which were followed by a 10-min extension at 72°C. All PCR products and restriction digests were analyzed on 2% agarose gels followed by ethidium bromide staining.

Cloning, nucleotide sequencing, and phylogenetic analysis

PCR products amplified from the LTR region were excised from agarose gels, purified using the Promega (Madison, WI) Wizard PCR prep system, ligated to plasmid PCR 2.1 (Original TA cloning kit; Invitrogcn. San Diego, CA) and used to transform competent *Escherichia coli* (INVaF') under conditions recommended by the manufacturers. Purified plasmid DNAs from recombinant clones were analyzed using Taq FS dye terminator cycle sequencing on an ABI Prism 373 DNA stretch sequencer (Perkin-Elmer Cetus). Three primers — two universal, M13R (5' GGAAACAGCTATGACCATG 3') and P7 (5' CGCCAGGGTTTTCCCAGTCACGAC 3'), and MSW-LTR (5' TGACGATTACCCCCCTGCCCATAAA 3'), corresponding to nucleotides 231-254 of HTLV-II Mo-T — were employed. Raw sequence data were aligned and edited using DNAsis-Mac version 2.0 (Hitachi Software Engineering, San Bruno, CA). Sequence alignment for phylogenetic analysis was performed using Geneworks software (IntelliGenetics, San Jose, CA). Editing of the alignments was done using MacClade .05 (Sinauer Associates, Sunderlaind, MA) and MacClade was also employed in the determination of the empirical average transition/transversion ratio of the aligned sequences. Phyloge-netic trees were constructed and evaluated using programs from PHYLIP version 3.572 (Felsenstein, 1995). Three independent methods were used to construct the trees: neighbor joining (NJ), Fitch and Wagner parsimony (pars), and maximum likelihood (ML). Fifty-seven HTLV-II LTR sequences were included in the phylogenetic analysis, and in all of the methods an LTR sequence from a primate T cell lymphotropic virus (PTLV) isolated from *Pan paniscus* was used as an oulgroup.[42] The empirically

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determined transition/transversion ratio was used for NJ and ML methods. The NJ and pars trees were evaluated using 1000 bootstrap replicates.

Nucleotide sequences used in the phylogenetic analyses

Fifty-seven HTLV-IIa and HTLV-IIb LTR sequences were used in the construction of the phylogenetic trees. Isolates and corresponding GenBank accession numbers were as follows. The HTLV-IIa sequences included eight european IVDAs (NORN2N, accession number U10258: DUB095, AF0329S9; DUB500. AF032990; DUB408, AF032991; DUB496, Af032992; DUB991, AF032993: SMH 1. Y09147; SMH 2. Y09148), four American IVDAs (ATLI8, U10252; LAS, U10256; SFIDU ao 5-5, U73010; SFIDU a2 6-2, U73022), four Brazilian Indians (SPWV; Braz.A21, U10253; KAY73, L42509: KAY139. L42508J, two North American Indians (PUEB.RB, U10262; NAV.DS, U10257), a Mexican prostitute (Mcxyl7, L42510), and the prototype HTLV-IIa isolate from the United Stales (Mo, M1060). HTLV-IIb sequences were from 22 European IVDAs (Gu, X89270; ITA47A, UI0254; ITA50A, U10255: SPAN 129, U10256; SPAN 130, UI0266: I-AM, Y09I49; I-EC Y09150; I-IT, Y09151; I-EA, Y09152; I-GI, Y09153; I-OG, Y09I54; I-OV, Y09149; RC, L77241; 130, L77242; 324, L77243; RVP, L77244), 5 American IVDAs (JG, L06857; NY185, U10259; PENN7A, U10260; SFIDU bx 4-10, U73016; SFIDU bx 6-4, U73018), 3 Colombian Indians (G12, LI 1456: WYUI, U12792; WYU2, U12794), and a Cameroon pygmy (PYGCAM1, Z46888). The prototype HTLV-IIb sequence NRA (120734) was also included. An LTR sequence from a primate T cell lymphotropic virus (PTLV) isolated from a pygmy chimpanzee, *Pan paniscus,* was used as an outgroup in all three tree-construction methods (PP1664, Z46344).[42] Also included was an LTR sequence form an Efe pygmy from the Democratic Republic of Congo/Zaire (Efe2, Y14365), which has been designated as a new subtype, HTLV-IId.[43]

RESULTS

Serological, PCR, and KI-'LP analysis

All 103 sera were initially analyzed using an enzyme-linked immunosorbent assay (ELISA) and repeatedly reactive samples were confirmed by Western blotting (WB). Sixteen samples (15.5%) (4 female, 12 male) were reactive on ELISA. WB confirmed that 12 of these (11.7%) were seropositive with the 4 remaining ELISA-positive samples displaying indeterminate patterns (4 of 103, 3.88%) (Table 1). Eleven of the 12 con-finned samples had WB patterns consistent with HTLV-II infection (10.7%), The remaining sample had a pattern consistent with dual HTLV-I and HTLV-II infection, in that there was reactivity to both HTLV-I and -II recombinant gp46 proteins. The Western blot reactivities of me four individuals with indeterminate patterns arc outlined in Table 2, where it can be seen that there was no consistent pattern of reactivity in this group.

DNA was extracted from PBMCs of seven HTLV-II-seropositive individuals and the four with indeterminate patterns, and PCR with RFLP analysis of the *tax* region was used to confirm and differentiate HTLV-I and HTLV-II infection.[40] Amplification produced a product of the expected size (159 bp) and RFLP analysis based on *Taq*I restriction endonuclease digestion produced three fragments of 85, 53, and 21 nucleotides, confirming that all of the II samples including the indetermi-nates involved HTLV-II infection.[40]

RFLP analysis of the envelope region encoding the trans-membrane protein gp21 was used to differentiate the HTLV-IIa and HTLV-IIb subtypes. Amplification of all 11 samples resulted in the expected 63 1-nucleotide product. *Xho*I digestion resulted in two DNA fragments of 178 and 453 nucleotides, indicating that all samples, also including the WB indeterminate samples, were infected with the HTLV-IIa subtype. The HTLV-IIb subtype was not detected. Unfortunately, whole blood samples could not be obtained from the individual who appeared to be seropositive for

TABLE 1. SEROLOGICAL AND MOLECULAR ANALYSIS OF HTLV-II INFECTIONS[a]

Patient number	*Sex*	*Age (years)*	*ELISA*	*Western blot*	*PCR/RFLP*	
					pX	env
DUB095	M	38	Pos	HTLV-II	HTLV-II	HTLV-IIa
DUB 991	M	33	Pos	Ind	HTLV-II	HTLV-IIa
DUB408	M	36	Pos	HTLV-II	HTLV-II	HTLV-IIa
DUB496	M	28	Pos	HTLV-II	HTLV-II	HTLV-IIa
DUB500	F	34	Pos	HTLV-II	HTLV-II	HTLV-IIa
DUB501	M	35	Pos	HTLV-U	HTLV-II	HTLV-IIa
DUB611	M	33	Pos	Ind	HTLV-II	HTLV-IIa
DUB616	M	36	Pos	HTLV-II	HTLV-JI	HTLV-IIa
DUB717	M	42	Pos	Ind	HTLV-JI	HTLV-IIa
DUB 805	M	38	Pos	HTLV-II	HTLV-II	HTLV-IIa
DUB812	F	31	Pos	HTLV-II	HTLV-II	HTLV-IIa
DLIB215	F	41	Pos	HTLV-II	—	—
DUB376	M	36	Pos	HTLV-II	—	—
DUB472	M	42	Pos	HTLV-II	—	—
DUB497	M	32	Pos	HTLV-I/II	—	—
DUB3173	F	42	Pos	HTLV-II	—	—

Abbreviations: Ind, Indeterminate Western blot; —, samples not analyzed.

[a] Samples initially seroreactive on ELISA (Pos) were further analyzed by Western blot and RFLP analysis to differentiate HTLV-I and HTLV-II infections *(pX)* and the HTLV-IIa and HTLV-IIb subtypes *(env).*

TABLE 2. WESTERN BLOT PROFILES OF SAMPLES WITH INDETERMINATE SEROLOGY THAT WERE POSITIVE FOR HTLV-II INFECTION BY PCR

Patient number	*Western blot band*		
	Gag	*Env*	*Other*
DUB991	None	GD21	None
DUB611	None	GD21, rgp46-II	None
DUB717	p24	GD21	None
DUB812	p24	None	None

both HTLV-I and HTLV-II (Table 1), and as such we were unable to confirm that this in fact represented dual infection.

Nucleotide sequence and phylogenetic analysis

Five samples were chosen randomly for nucleotide sequence analysis of the LTR region. An 831-nuclcotide product was amplified from the LTR and sequenced, and of this. 623 nu-cleotides corresponding to nucleotides 86-707 of the prototype isolate HTLV-!la Mo-T were used in the phylogenctic analysis. Absolute nucleotide sequence variation between the five Dublin samples and the HTLV-IIa Mo-T isolate ranged from 1.7 to 2.1%. The five Dublin sequences alone displayed divergence of 0.16 to 0.32%. Phylogenetic trees were constructed and evaluated by three different methods: neighbor joining (NJ), parsimony (pars), and maximum likelihood. NJ and pars trees were statistically evaluated using 1000 molecular bootstrap replicates. All of the methods resulted in trees of similar topology with well-documented separation of the HTLV-IIa and HTLV-IIb subtypes (Fig. 1). The five Dublin samples clearly clustered in the HTLV-IIa group by all three methods and the bootstrap values for the NJ and pars trees clearly supported the inclusion of the Dublin sequences in the HTLV-IIa clade. Using the NJ method with 1000 bootstraps, the calculated value for the HTLV-IIa subtypes was 1000/1000 and for the HTLV-IIb subtype it was 600/1000. In the pars analysis the HTLV-IIa subtype also had a value of 1000/1000 and for the HTLV-IIb subtype, it was 941/1000. For the ML method the clustering of the HTLV-IIa and -lib subtypes displayed a *p*; value of <0.01.

In each of the trees the Dublin samples clustered with the other 1VDA strains of the HTLV-IIa subtype, including four isolates from the United States: Los Angeles (LASA). Atlanta (ATL18), and San Francisco (SFIDU a0 5-5, SF1DU a2 6-2J[44]; three isolates from Europe, which included two from the United Kingdom (SMH1 SMH2)[45] and one from Norway (NOR2N); and with a Pueblo Indian isolate (PUEB.RB) (Fig. 1). Using the NJ method the bootstrap value for this group was 816/1000, and using the pars analysis it was 549/1000. The ML method showed that this was highly significant, with a *p* value of <0.01. These values demonstrate that a distinct HTLV-IIa IVDA clade was well supported by all three methods.

DISCUSSION

In the present study we have employed serological and molecular methods to characterize human T lymphotropic virus (HTLV) infections in a cohort of intravenous drug abusers (IV-DAs) in Dublin. Ireland. All individuals studied were of Irish descent and were attending a drug rehabilitation and/or a sexually transmitted disease clinic. Initial screening of 103 .sera using a commercial ELISA demonstrated immunoreactivity in 16 (15.5%): Western blotting confirmed infection in 12, with the remaining 4 having indeterminate patterns. Of the former, 11 (10.7%) had patterns consistent with HTLV-II infection and 1 had a pattern suggestive of dual HTLV-I and HTLV-II infection. PCR with RFLP analysis of the *tax* region of the provirus was used to confirm infection in seven of the HTLV-II-seropositive samples and to analyze further the four WB indeterminate samples. All of the former were confirmed as having HTLV-H infection and, in addition, all of the indeterminate samples were also shown to be HTLV-II infected by this method. The latter observation supports previous reports that certain individuals with HTLV-II infection can be sero-indeterminate or even seronegalive.[20] As noted, one individual appeared to be coinfected with HTLV-I and HTLV-II: however, venous blood samples were not available and PCR analysis could not be used to confirm this. Of the 15 individuals with single HTLV-I1 infection (11 Western blot positive and 4 Western blot indeterminate), 4 were female (12.5%) and 11 were male (15.5%), and the average age was 36.1 years. Unfortunately, because of unreliable and inconsistent histories we were unable to ascertain if there was a correlation between HTLV-II infection and total years of intravenous drug abuse.

To identify the HTLV-II molecular subtypes and to characterize further the viruses, additional RFLP and nucleotide sequence analyses were employed. RFLP analysis of the *env* gene region demonstrated that all samples were infected with the HTLV-IIa subtype. This finding contrasts with a number of studies from southern Europe and specifically in regions of Italy[28-30,32] and Spain,[33-37] where the HTLV-IIb subtype predominates and in some instances is the exclusive infection. A single report has suggested that, similar to Ireland, the HTLV-IIa subtype appears to predominate in an IVDA population in Stockholm, Sweden.[39] In comparison with other studies, where prevalence rates have been reported to range between 0 and 6.5%, the Irish infection rate is the highest yet reported for any European country,[18,20,28-39,46-48] and on the basis of PCR analysis this was 14.6%. of the cohort studied. It should be noted that all of the Irish patients tested were coinfectcd with human immunodeficiency type 1 virus (HIV-1). In other studies it has also been found that the prevalence of HTLV infection, and particularly HTLV-II, is much higher in HIV-positive populations. In one Swedish study it was found that of 1158 IVDAs tested for HTLV-I/II, 2.4% were seropositive, whereas if only the HIV-infected IVDA patients in this cohort were examined the prevalence rose to 11.4%.[47] In a Spanish HTLV-II-positive cohort of IVDAs it was found that 84% of their patients were coinfected with HIV-1.[35] It is unclear if the high rate of infection observed in this Irish cohort is representative of all IVDAs, and not just those who are HIV-1 infected. Unfortunately, we have up to now been unable to recruit significant numbers of non-HIV-1-in-fected IVDAs in our studies. However, we are currently attempting to establish such a cohort to determine if the rates of infection differ between the two populations. The finding of the two subtypes of HTLV-I1 in geographically different parts of Europe would indicate that infection probably has been introduced to Europe at different times.

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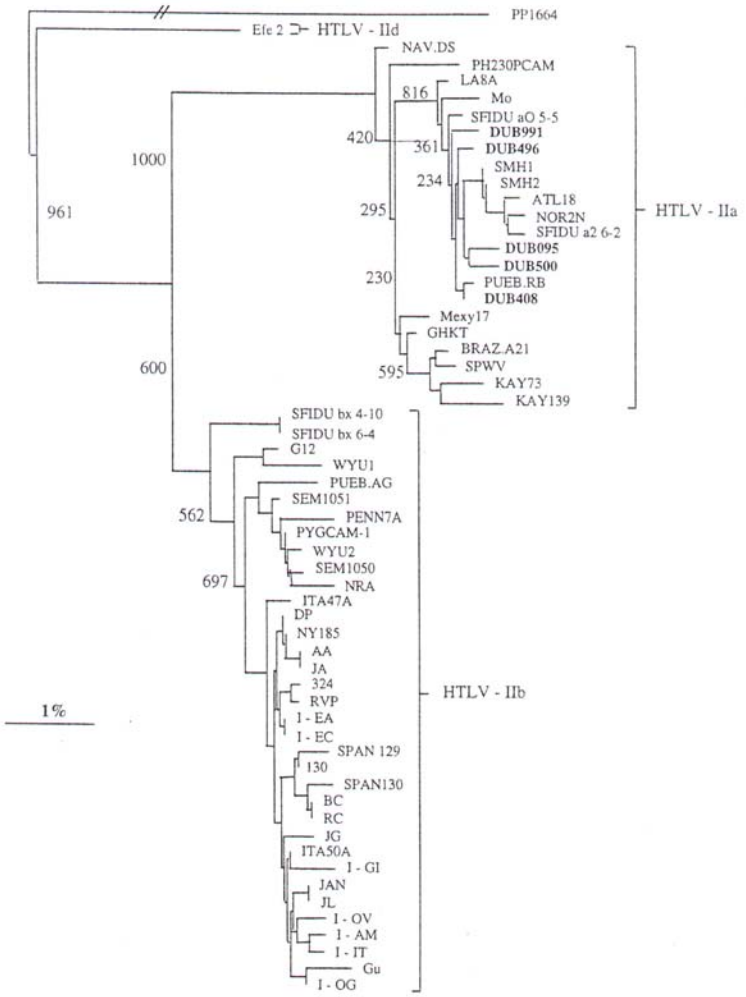

FIG. 1. Phylogenetic analysis of the LTR sequences (corresponding to nucleotides 86-707 of the prototype HTLV-IIa Mo-T isolate) constructed using the neighbor-joining method. Included in the analysis were HTLV-IIa strains from the five Irish IVDA strains (DUB095. DUB500, DUB408. DUB496, and DUB991), in bold face print, IVDAs from the United Stales and Europe (ATLI8, LAS, SF a0 5-5, SF a2 6-2, Norn2, SMH I, and SMH 2). Brazilian Indians (SPWV; Braz.A21, KAY73, and KAY139), North American Indians (PUEB.RR and NAV.DS), and a Mexican child prostitute (Mexy 17). HTLV-IIb sequences came from American and European IVDAs (Nra. JG, NY 185, PENN7A, Gu, JTA47A, 1TA50A, SPANI29, SPAN130, I-AM, I-EC, I-IT, I-EA, I-GI, I-OG, I-OV, RC, BF, DP, AA, JA, JL, JAN, 130, 324, RVP, SF bx 4-10, and SF bx 6-4), North American Indians (PUEB-AG, SEM1050, and SEM1051). Colombian Indians (G12, WYUI, and WYU2) and a Cameroon pygmy (PYGCAM I). An HTLV-IId LTR sequence from an Efc pygmy from the Democratic Republic of Congo/Zaire (Efe2) was also included in the analysis. The tree was rooted with a primate T cell lymphotropic virus (PTLV) LTR sequence (PP1664) isolated from a pygmy chimpanzee, *Pan paniscus.* Details of the different strains used are given in Malenals and Methods. Statistical evaluation was done with 1000 bootstrap replicates whose values are shown on the branches. Horizontal branch lengths are drawn to scale; the bar represents 1% divergence. The tree clearly shows the clustering of the HTLV-IIa and -lib subtypes, with the five Irish IVDA strains grouped in the IIa clade with the HTLV-IIa American and European IVDAs, a Pueblo Indian, and the Mo-T isolate (Mo).

of HTLV-IIa infection to Ireland may have occurred recently, as has been suggested in a Swedish study.[47] However, screening of a limited number of archival serological samples from IVDAs in Dublin, which were obtained more than a decade ago, have suggested that few of these were infected with HTLV-II (our unpublished data, 1998) and would support the view that infection has also been recently introduced.

A total of 623 nucleotides of the LTR region from five of the Dublin samples and all other available HTLV-II isolates was used to construct phylogenctic trees using neighbor joining (NJ),

Fitch and Wagner parsimony (pars), and maximum likelihood (ML) methods. The grouping of the Dublin simples in the HTLV-IIa clade could be clearly demonstrated by each method. The bootstrap values for the clade from the NJ and pars analyses (1000/1000 for each) supported this classification, as did the probability values in the ML method (p value < 0.01). The phy-logenetic analysis also demonstrated the close relationship of (he HTLV-IIa in the Dublin IVDAs with that found in four IVDAs from the United States (Atlanta, Los Angeles, and San Francisco) and three from Europe (two from the United Kingdom and one from Norway). Unfortunately, nucleotide sequence data from the Swedish HTLV-II isolates are not available and the relationship of these isolates to those in Ireland cannot be determined. It has been reported that infection with the HTLV-IIa subtype is the predominant infection among IVDAs in the United Slates, and it could be speculated that this might be the origin of infection in Dublin. Further studies are now necessary to determine if there has been any transmission of the virus to IVDAs in other parts of Ireland and to other high-risk groups. At present the role of HTLV-II in human disease is poorly understood. Evaluation of the clinical features in our population, which has such a high rate of infection, may allow a better understanding of this and of possible clinical sequelae resulting from interactions of concomitant HIV-1 and HTLV-II infections.

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REFERENCES

1. Hall WW, Kubo T, Ijichi S, Takahashi T, and Zhu SW: Human T-cell leukemia/lymphoma virus, type II (HTLV-II): Emergence of an important newly recognised pathogen. Semin Virol 1994;5:165-178.
2. Hollsberg P and Hafler DA: Seminars in medicine of the Beth Israel Hospital, Boston. Pathogenesis of diseases induced by human lym-photropic virus type-I infection. N Engl J Med 1993;328:1173-1182.
3. Hinuma Y, Komoda H, Chosa T, Kondo T, Kohakura M, Take-naka T, Kikuchi M, Ichimaru M, Yunoki K, Sato I, Mtsuo R, Tak-iuchi Y, Uchino H, and Hanoka M: Antibodies lo adult T cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: A nation-wide sero-epidemiological study. Int J Cancer 1982;29:631-635.
4. Kajiyama W, Kashiwagi S, Monura H, Ikematsu H, Hayashi J, and Ikematsu W: Seroepidemiological study of antibody to adult T cell leukemia virus in Okinawa, Japan. Am J Epidemiol 1986:123:41-17.
5. Blattner WA, Saxinger C, Riedel D, Hull B, Taylor G, Cleghorn H, Gallo R, Blumberg B, and Bartholomew C: A study of HTLV-I and its associated risk factors in Trinidad and Tobago. J AIDS 1990;3:1102-1108.
6. Murphy EL, Figueroa JP, Gibbs WN, Holding-Cobham M, Cranston B, Malley K, Bodner AJ, Alexander SS, and Blattner WA: Human T lymphotropic virus type-I (HTLV-I) seroprevalence in Jamaica. I. Demographic determinants. Am J Epidemiol 1991;133:1114-1124.
7. Saxinger W, Blattner WA, Levine PH, Biggar R, Huh M, Moghissi J, Jacobs P, Wilson L, Jacobson R, Crookes R, Strong M, Ansari AA, Dean AC, Nkrumah FK, Mourali N, and Gallo RC: Human T-leukemia virus (HTLV-I) antibodies in Africa. Science 1984 ;225:1473-1476.
8. Verdier M, Denis F, Sangare A, Barin F, Gershy-Damet G, Rey JL, Soro B, Leonard G, Mounier M, and Hugon J: Prevalence of antibody lo human T cell leukemia virus type-1 (HTLV-I) in populations of Ivory Coast. J Infect Dis 1989:160:363-370.
9. Yoshida M. Miyoshi I, and Hinuma Y: Isolation and characterization of retrovirus from cell lines of human T cell leukemia and its implications in the disease. Proc Nail Acad Sci USA 1982:79:2031-2035.
10. Takatsuki K: Adult T cell leukemia. Intern Med 1995;34:947-952.
11. Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, and de Thé G: Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 1985:2:407-410.
12. Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Mat-sumoto M, and Tara M: HTLV-I associated myelopathy, a new clinical entity. Lancet 1986;1:1031-1032.
13. Mochizuki M, Watanabe T, Yamaguchi K, Takatsuki K, Yoshimura K, Shiaro M, Nakashima S, Mori S, Araki S, and Miyata N: HTLV-I uveitis: A distinct clinical entity caused by HTLV-I. Jpn J Cancer 1992:83:236-239.
14. Nakao K and Ohba N: Clinical features of HTLV-I associated uveitis. Br J Ophthalmol 1993:77:274-279.
15. Hjelle B, Scalf R, and Swenson S: High frequency of human T cell leukemia-lymphoma virus type-II in New Mexico blood donors: Determination by sequence-specific oligonucleotide hybridization. Blood 1990;76:450-454.
16. Levin PH, Jacobson S, Elliot R, Cavallero A, Colclough G, Dorry C, Stephenson C, Knigge RM, Drummond J, Nishimura M, Taylor ME, Wiktor S, and Shaw GM: HTLV-II infection in Florida Indians. AIDS Res Hum Retroviruses 1993;9:123-127.
17. Maloney EM, Biggar RJ, Neel JV, Taylor ME, Hahn BH, Shaw GM, and Blattner WA: Endemic human T cell lymphotropic virus type-11 infection among isolated Brazilian Amerindians. J Infect Dis 1992:166:100-107.
18. The HTLV European Research Group: Seroepidemiology of the human T cell leukemia-lymphoma viruses in Europe. J Acquir Immune Defic Syndr Hum Retrovirol 1996;13:68-77.
19. Fukushima Y, Takahashi H, Hall WW, Nakasone T, Nakata S, Song P, Due DD, Hien B, Quang NX, Trinh TH, Nishioka K, Kitamura K, Komuro K, Vahlne A, and Honda M: Extraordinary high rate of HTLV type II seropositivity in intravenous drug abusers in South Vietnam. AIDS Res Hum Retroviruses 1995;11:637-644.
20. Hall WW, Ishak R, Zhu SW, Novoa P, Eriaku N, Takahashi H, da Costa Ferreira M, Azevedo V, Ishak MOG, da Costa Ferreira O, Monken C, and Kurata T: Human T lymphotropic virus type-II (HTLV-II): Epidemiology, molecular properties, and clinical features of infection. J Acquir Immune Defic Syndr Hum Retrovirol 1996;13(Suppl. 1):S204-S214.
21. Hjelle B, Appenzeller O, Mills R, Alexander S, Torrez-Martinez N, Jahnke R, and Ross G: Chronic neurodegenerative disease associated with HTLV-II infection. Lancet 1992;339:645-646.
22. Berger JR, Svenningsson A, Raffanti S, and Resnick L: Tropical spastic paraparesis-like illness occurring in a patient dually infected with HIV-I and HTLV-II. Neurology 1991;41(1):85-87.
23. Harrington WJ Jr, Sheremata W, Hjelle B, Dube DK, Bradshaw P, Foung SK. Snodgrass S, Toedter G, Cabral L, and Poiesz, B: Spastic ataxia associated with human T-cell lymphotropic virus type II infection. Ann Neural 1993;33(4):411-414.
24. Sheremata WA, Harrington WJ Jr, Bradshaw PA, Foung SK, Raf-fanti SP, Berger JR, Snodgrass S, Resnick L, and Poiesz BJ: Association of "(tropical) ataxic neuropathy" with HTLV-II. Virus Res 1993;29(l):71-77.
25. Jacobson S, Lehky T, Nishimura M, Robinson S, McFarlin DE, and Dhib-Jalbut S: Isolation of HTLV-II from a patient with chronic, progressive neurological disease clinically indistinguishable from HTLV-I-associated myelopathy /tropical spastic paraparesis. Ann Neurol 1993;33(4):392-396.
26. Murphy EL, Engstrom JW, Miller K, Sacher RA, Busch MP, and Hollingsworth CG: HTLV-II associated myelopathy in 43-year-old woman. Lancet 1993;341(8847):757-758.

27. Murphy EL, Fridey J, Smith JW, Engstrom J, Sacher RA. Miller K, Gibble J, Stevens J, Thomson R, Hansma D, Kaplan J, Khabbaz R, and Nemo G: HTLV-associated myelopathy in a cohort of HTLV-I and HTLV-II-infected blood donors. Neurology 1997;48 (2):315-320.
28. Calabro ML, Luparello M, Grottola A, Del Mistro A, Fiore JR, Angarano G, and Chieco-Bianchi L: Detection of human T lymphotropic virus type IIb in human immunodeficiency virus type 1-coinfectcd persons in southeastern Italy. J Infect Dis 1993;168:1273-1277.
29. Salemi M, Cattaneo E, Casoli C, and Bertazzoni U: Identification of IIa and IIb molecular subtypes of human T-cell lymphotropic virus type II among Italian injecting drug users. J Acquir Immune Defic Syndr Hum Retrovirol 1995;8(5):516-520.
30. Ferrante P, Mancuso R, Zuffolato R, Puricelli S, Mannella E, Ro-mano L, Zanetti A, Cattaneo E, and Corrao V: Molecular analysis of HTLV-I and HTLV-II isolates from Italian blood donors, intravenous drug users and prisoners. New Microbiol 1997;20(2):93-104.
31. Zella D, Cavicchini A, Salemi M, Casoli C, Lori F, Achilli G, Cattaneo E, Landini V, and Bertazzoni U: Molecular characterization of two isolates of human T cell leukaemia virus type II from Italian drug abusers and comparison of genome structure with other isolates. J Gen Virol 1993;74(3):437-444.
32. Chironna M, Calabro ML, Quarto M, Germinario C, Fiore JR, Favero A, Potenza D, Chieco-Bianchi L, and Barbuti S: HTLV-I and HTLV-II infections in subjects at risk for HIV-I infection from southeastern Italy (Apuilia region). Int J Cancer 1996;65(6):746-750.
33. Vallejo A and Garcia-Saiz A: HTLV-II subtype b among injecting drug users in Spain. Vox Sang 1994:67:81-82.
34. Henrard DR, Soriano V, Robertson E, Gutierrez M, Stephens J, Dronda F, Miles F, Pujol E, Buytendorp M, and Castro A: Prevalence of human T-cell lymphotropic virus type-I (HTLV-I) and HTLV-2 infection among Spanish drug users measured by HTLV-I assay and HTLV-I and 2 assay. HTLV-I and HTLV-2 Spanish Study Group. J Clin Microbiol 1995;33:1735-1738.
35. Soriano V, Vallejo A, Gutierrez M, Tuset C, Cilia G, Martines-Zapico K, Dronda F, Caballero E, Calderon E, Aguilera A, Martin AM, Llibre J, del Romero J, Ortiz de Lejarazu R, Ulloa F, Eiros J and Gonzalez-Lahoz J: Epidemiology of human T-lymphotropic virus type II (HTLV-II) infection in Spain, HTLV Spanish Study Group. Eur J Kpidcmiol 1996:12:625-629.
36. Soriano V, Gutierrez M, Vallejo A, Tuset C, Dronda F, and Pujol E: HTLV-II infection in Spain, Analysis of 113 cases identified until November, 1994. Spanish Group for the Study of HTLV/I/II Med Clin (Barcelona) 1995;105:25l-254.
37. Vallejo A, Casado C, Canto C, Varela JM, Barge T, Rabella N, Herrera MI, and Garcia Saiz A: Isolation, sequencing and ultra-structure of HTLV-I and HTLV-II. Presence of the HTLV-II subtype b among Spanish intravenous drug addicts. Med Clin (Barcelona) 1994; 103:730-736.
38. Vallejo A, Ferrante P, Soriano V, Calabro ML, Mancuso K, Here-dia A, Mannella E, Favero A, Garcia-Saiz A, Chieco-Bianchi L, Gonzalez-Lahoz J, and Hewlett IK: Nucleotide sequence and restriction fragment-length polymorphism analysis of human T-cell lymphotropic virus type II (HTLV-II) in southern Europe: Evidence for the HTLV-IIa and HTLV-IIb subtypes. J Acquir Immune Defic Syndr Hum Retrovirol 1996;13:384-391.
39. Anderson S, Ahmed R, Bredberg-Raden U, Albert J, Krook A. Kall K, Biberfield G, and Popovic M: HTLV-II infected Swedish intravenous drug abusers carry subtype. A. J Acquir Immune Defic Syndr Hum Retrovirol 1995;10:280.
40. Lee H, Swanson P, Shorty VS, Zack JA, Rosenblatt JD, and Chen ISY: High rate of HTLV-II infection in Seropositive IV drug abusers in New Orleans. Science 1989;244:471-475.
41. Hall WW, Takahashi H, Liu C, Kaplan MH, Scheewind O, Ijichi S, Nagashima K, and Gallo RC: Multiple isolates and characteristics of human T-cell leukemia type-II. J Virol 1992;66(4):2456-2463.
42. Vandamme AM, Liu HF, Van Brussel M, De Meurichy W, Desmyter J, and Goubau P: The presence of a divergent T-lymphotropic virus in a wild-caught pygmy chimpanzee *(Pan paniscus)* supports an African origin for the human T-lymphotropic/simian T-lymphotropic group of viruses. J Gen Virol 1996;77:1089-1099.
43. Vandamme AM, Salemi M, Van Brussel M, Liu HP, Van Laethem K, Van Ranst M, Michels L, Desmyter J, and Goubau P: African origin of human T-cell lymphotropic virus type 2 (HTLV-2) supported by a potential new HTLV-2d subtypes in Congolese Bambuti Efe pygmy. J Virol 1998:72(5):4327-4340.
44. Murphy EL, Mahieux R, de The G, Tekaia F, Ameti D, Horton J, and Gessain A: Molecular epidemiology of HTLV-II among United Stales blood donors and intravenous drug users: An age-cohort effect for HTLV-II RFLP type aO. Virology 1998;242(2):425-434.
45. Salemi M, Vandamme AM, Gradozzi c, Van Laethem K, Cattaneo E, Taylor G, Casoli C, Goubau P, Desmyter J, and Bertazzoni U: Evolutionary rate and genetic heterogeneity of human T-cell lymphotropic virus type II (HTLV-II) using isolates from European injecting drug users. J Mol Evol 1998;46(5):602-611.
46. Blomberg J, Moestrup T, Frimand J, Hansson BG, Krogsgaard K, Grillner L, and Nordenfelt E: HTLV-I and HTLV-II in intravenous drug users from Sweden and Denmark. Scand J Infect Dis 1994;26(l):23-26.
47. Andersson S, Krook A, Kall K, Julander I, Thorstensson R, and Biberfeld G: HTLV infections among Swedish intravenous drug users in 1992. Scand J Infect Dis 1995;27:547-550.
48. Krook A and Blomberg J; HTLV-II among injecting drug users in Stockholm. Scand J Infect Dis 1994;26:129-132.

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