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HIV-1 Infection in Persons Homozygous for *CCR5-Δ32* Allele: The Next Case and the Review

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Abstract

CC-chemokine receptor 5 serves as the coreceptor for the HIV-1 R5 strains, which are responsible for the majority of HIV transmissions. A deletion of 32 nucleotides in the gene encoding this receptor (termed* CCR5-Δ32*) leads to the suppression of CC-chemokine receptor 5 presentation at the cell surface, thus impeding process of HIV entry into the cell. Individuals homozygous for the* CCR5-Δ32 *allele are resistant to infection with HIV-1 R5 strains, and are extremely rare among HIV-1-infected individuals. We have described a person homozygous for* CCR5-Δ32*, who was infected with subtype B HIV-1. Based on examination of proviral V3 sequences obtained from the first clinical blood sample within less than five months after seroconversion, the CXC-chemokine receptor 4-using strains (X4 or R5/X4) were detected. Data on HIV-1-infected patients homozygous for the* CCR5-Δ32 *allele, course of HIV-1 infection in these cases, and the infecting viral strains from current and all former reports on HIV-1 infection in* CCR5-Δ32 *homozygotes were gathered and compared.

Identification of HIV-1-infected persons homozygous for* CCR5-Δ32 *supports the evidence that the lack of functional CC-chemokine receptor 5 at the cell surface does not confer absolute protection against HIV-1 infection, which should be considered when designing future HIV pre-exposure prophylaxis schemes based on CC-chemokine receptor 5 blocking drugs. **(AIDS Rev. 2017;19:219-30)**

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Key words

Homozygous* CCR5-Δ32 *mutation. HIV-1 transmission. Coreceptor. CCR5 blocker.

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Introduction

The HIV-1 R5 strains are responsible for the majority of HIV transmissions and are important in early infection[1]. They infect host immune cells by binding to the cell surface CD4 molecule along with the CC-chemokine receptor 5 (CCR5) chemokine receptor, which serves as a coreceptor during viral entry. A frame-shifting deletion of 32 nucleotides in the gene encoding CCR5 receptor (called *CCR5-Δ32)* is a well described functional mutation, which results in a lack of the last three transmembrane domains of the CCR5 protein. Truncated CCR5 molecule is not presented at the cell surface, thus hampering entry of HIV-1 R5 strains into the cell[2]. The *CCR5-Δ32* allele is the most prevalent among Europeans (average frequency 10%), while it is very rare or absent in the indigenous populations of the Americas, Africa, and Eastern Asia[3].

As demonstrated in recent meta-analyses, heterozygous *CCR5-Δ32* genotype has no effect on protecting from HIV-1 infection[4], whereas among HIV-1-infected individuals it is linked to delayed disease progression[5]. On the other hand, homozygotes for the *CCR5-Δ32* allele are highly protected from HIV-1 infection and completely resistant to infection with HIV-1 strains using exclusively CCR5 for cell entry. The frequency of homozygous *CCR5-Δ32* genotype is elevated among highly HIV-1-exposed but uninfected individuals[6,7], while among HIV-1-positive individuals, only 17 cases with this genotype has been reported so far, although tens of thousands were tested[8-23]. Data on disease progression among such rare HIV-1-infected homozygotes, as well as the information about infecting viral strains, are to some extent inconsistent, although most of the described patients experienced rapid progression in terms of decline of $CD4^+$ T-cell count[8-23].

As the absence of the functional CCR5 protein in *CCR5-Δ32* homozygotes was not associated with any apparent clinically unfavorable conditions, blocking of CCR5 was considered to be a safe therapeutic option[2]. Maraviroc is currently the only antiretroviral drug targeted at CCR5. It is indicated for treatment-experienced patients infected with R5 strains of HIV-1, and the possible use of this compound in HIV pre-exposure prophylaxis is also examined[24,25]. Along with the report on the "Berlin patient" (diagnosed with acute myeloid leukemia), who was the first, and at present the only, case regarded as cured of HIV infection after receiving hematopoietic stem cell transplantation from a *CCR5-Δ32* homozygous donor, other novel strategies, including gene-editing technologies, aimed at eliminating CCR5 from a cell surface are being increasingly developed and are a promising approach, with a HIV curative potential[26,27]. Given efforts to develop such HIV curative therapies designed to modify or disrupt CCR5 cell expression, cases of HIV-infected individuals with the homozygous *CCR5-Δ32* genotype deserve attention.

New HIV-1-infected person homozygous for the *CCR5-Δ32* allele

Here we report on another person who is HIV-1-positive despite being homozygous for the *CCR5-Δ32*. Detection of 32 base-pair deletion was performed in a patient's genomic DNA isolated from peripheral blood with the use of QIAamp® DNA Blood Mini Kit (QIAGEN GmbH). Polymerase chain reaction (PCR) allowing for the amplification of the *CCR5* gene fragment was done as described before[6]. The resulting PCR products, of 183 nt for wild-type alleles and 151 nt for alleles with the *CCR5-Δ32* polymorphism, were visualized by electrophoresis in 2% agarose gel containing 0.5 μg/ml ethidium bromide. The homozygous deletion of 32 nucleotides in *CCR5* gene was confirmed by bidirectional sequencing of *CCR5* fragment encompassing the deletion, using ABI Prism Big Dye Terminator v3.1 cycle sequencing kit and 96-capillary 3730xl DNA Analyzer (Applied Biosystems).

The studied patient was a white man of Polish origin (Table 1) who was diagnosed as HIV-1-positive in the Outpatient Clinic for AIDS Diagnostics and Therapy (Specialist Hospital in Chorzow, Chorzow, Poland). The first confirmatory Western Blot test (MPD HIV1/2 BLOT 2.2, MP Biomedicals) positive for antibodies directed against HIV-1 p17, p24, p55, p31, gp41, gp120, and gp160 antigens, was performed in February 2013. At the time of diagnosis the patient was 21 years old. He reported homosexual contacts as the route of HIV-1 transmission, with no history of injecting drugs use or nosocomial exposure to HIV infection. During his first clinical presentation, in March 2013, neither symptoms of acute retroviral disease nor AIDS-defining diseases were observed; however, the patient notified non-specific cardiac disorders dating from December 2012.

The patient was recruited to the EuroCoord study, in which persons with recent and long-term HIV-1 infections were differentiated with the use of immunoenzymatic assay (Sedia™ HIV-1 Limiting Antigen [LAg]-Avidity EIA, Sedia BioSciences Corporation). Blood samples for this immunoenzymatic assay and for the

Table 1. Information on HIV-1-infected homozygotes for the *CCR5-Δ32* allele

Patient no.	Reference; year and country of survey/cohort*	Sex	Race/ origin	Route of infection	HIV-1 diagnosis date	Seroconversion date	Course of infection in the absence of ART	ART introduction/response
#1	[8]; 1997 [9]; 2002 Australia, Sydney	Male	European descent	Homosexual intercourse	March 1992	March 1992	No accelerated disease progression; initial CD4 = 960/µl (March 1992) and 320/µl (February 1997), VL = 19,000 c/ml (June 1996) and 26,000 (February 1997)	February 1997-April 2001, ART (AZT, 3TC), good response: CD4 rise from 320/µl to 440/µl, VL decrease to < 50 c/ml; no clinical symptoms
#2	[10]; 1997 [11]; 1998 USA, Multicenter Haemophilia Cohort study	Male	White	Hemophilia A treatment with iv. coagulation factor VIII	1985	January 1982	Rapid decline of CD4 to < 200/µl (4.4 years) in the absence of ART	1989, AZT monotherapy; oral hairy leukoplakia (1993), AIDS – esophageal candidiasis (1995), time to AIDS: 13.7 years; death from liver failure (1996)
#3	[12]; 1997 France, SEROCO Cohort	Male	White	Homosexual intercourse	October 1989	October 1989	Rapid disease course; initial CD4 "normal", afterward rapid and persistent decline to < 150/µl (time not specified) despite "very low" VL; no information on clinical symptoms	No information on ART
#4	[13]; 1997 Italy, Clinic of Infectious Diseases, University of Milan	Male	White (Italian)	Homosexual intercourse	1994	Before 1988	NS, initial CD4 = 87/µl (1994), and VL = 14,664 c/ml (1994); no information on further disease progression and clinical symptoms	No information on ART
#5	[14]; 1999 Australia, Sydney	Male	Caucasian	Homosexual intercourse	April 1995	Between May 1994 and April 1995	Rapid CD4 decline; initial CD4 "low", < 200/µl (November 1996), VL = 93,050 c/ml (1 year after diagnosis)	During 20 months of ART, CD4 remained at 240/µl with "low" VL; no clinical symptoms
#6	[15]; 1999 Germany, Hanover Medical School	Male	Caucasian (German)	Heterosexual intercourse	NS	NS (before 1999)	Rapid CD4 decline (criteria not specified); initial CD4 "normal"	2 years after diagnosis, ART started, resulted in VL decrease from 20,000 c/ml to < 50 c/ml; no clinical symptoms

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Table 1. Information on HIV-1-infected homozygotes for the *CCR5*-Δ*32* allele *(continued)*

Patient no.	Reference; year and country of survey/cohort*	Sex	Race/ origin	Route of infection	HIV-1 diagnosis date	Seroconversion date	Course of infection in the absence of ART	ART introduction/response
#7	[16]; 2002 USA, HIVNET Vaccine Preparedness Study	Male	Latino	Homosexual intercourse	November 1992	Between January 1992 and November 1992	Rapid CD4 decline in the absence of ART; initial CD4 = 280/μl (November 1992), over the next 5 years CD4 decreased to < 100/μl (May 1997)	February 1996-March 1997; ART (3TC, d4T, IDV); on ART: persistently low CD4 with VL < 400 c/ml; no clinical symptoms
#8	[16]; 2002 USA, HIVNET Vaccine Preparedness Study	Male	White	Homosexual intercourse	October 1995	Between May 1995 and October 1995, primary HIV-1 infection: May 1995	Rapid CD4 decline in the absence of ART; initial CD4 = 250/μl and VL = 90,000 c/ml (October 1995)	4 months after diagnosis, AZT monotherapy; 16-32 months after diagnosis, ART (AZT, 3TC, SQV, RTV): CD4 in a range of 200-400/μl and VL < 50 c/ml; no significant clinical symptoms
#9	[17]; 2002 [18]; 2006 USA, Multicenter AIDS Cohort Study	Male	White	Homosexual intercourse	September 1998	Primary HIV-1 infection: March 1998	Rapid CD4 decline in the absence of ART; initial CD4 = 1,648/μl (March 1998) and 498/μl (September 1999)	February 2000 (16 months after diagnosis), ART (AZT, 3TC, NVP) started at CD4 = 295/μl and VL = 73 c/ml, during 11 months of ART: rise in CD4, and VL < 50 c/ml; no clinical symptoms
#10	[19]; 2003 Denmark, Sweden - patients with hemophilia A, hemophilia B, Willebrand disease	Male	White (Danish)	Hemophilia A treatment with iv coagulation factor VIII	1985	Primary HIV-1 infection: 1982 (putatively)	Rapid disease course in the absence of ART; initial CD4 = 100/μl (1986), no increase afterwards; AIDS diagnosis: *Pneumocystis carinii* pneumonia (1986)	1987-1988, AZT monotherapy for 17 months, terminated due to leukopenia, next trials of treatment terminated due to adverse effects on bone marrow, no rise in CD4, death within 8 years of infection (1990)
#11	[18]; 2006 Australia	Male	NS	Homosexual intercourse or injection drug use	September 1991	NS (before 1991)	NS, no information on disease progression	NS, no information on ART

(Continue)

Table 1. Information on HIV-1-infected homozygotes for the *CCR5-Δ32* allele *(continued)*

Patient no.	Reference; year and country of survey/cohort*	Sex	Race/ origin	Route of infection	HIV-1 diagnosis date	Seroconversion date	Course of infection in the absence of ART	ART introduction/response
#12	[20]; 2007 France, USA, China; SEROCO Cohort	Male	NS	Homosexual intercourse	NS	NS	NS, no information on disease progression	NS, no information on ART
#13	[20]; 2007 France, USA, China; SEROCO Cohort	Male	NS	Homosexual intercourse	NS	NS	NS, no information on disease progression	NS, no information on ART
#14	[21]; 2008 Germany, German HIV-1 Seroconverter Study	Male	Caucasian (German)	Homosexual intercourse	June 2002	June 2002	Rapid disease progression in the absence of ART, initial CD4 = 344/μl and VL = 2,323,000 c/ml (June 2002); during 10 months of ART interruption, CD4 dropped from 924 to 275/μl with VL = 22,400 c/ml	8 days after diagnosis for 8 months (d4T, EFV, LPVr), and 18 months after diagnosis for > 4 years (AZT, 3TC, EFV), good response to ART: CD4 rise, VL suppressed; clinical symptoms on ART: *Chlamydia*-associated proctitis and *Shigella sonnei* gastroenteritis
#15	[22]; 2015 International HIV Controller Study	Male	NS	Sexual intercourse	2007 first sample available	NS (before/in 2007)	Slow disease progression in the absence of ART, preserved CD4 > 400/μl, and VL < 4,000 c/ml for 3.3 years, afterward decreasing CD4 and VL > 10,000 c/ml	ART-naive during follow-up
#16	[22]; 2015 International HIV Controller Study	Male	NS	Sexual intercourse	2006, first sample available	NS (before/in 2006)	Slow disease progression in the absence of ART, preserved CD4 and VL < 4,000 c/ml for 4.6 years, afterward decreasing CD4 and VL > 10,000 c/ml	January 2012, ART started, effects not known due to short time of observation

(Continue)

Table 1. Information on HIV-1-infected homozygotes for the *CCR5-Δ32* allele *(continued)*

Patient no.	Reference; year and country of survey/cohort*	Sex	Race/ origin	Route of infection	HIV-1 diagnosis date	Seroconversion date	Course of infection in the absence of ART	ART introduction/response
#17	[23]; 2015 Canada, Vancouver Injection Drug Users Study	NS	NS	Injection drug use	NS	August 2001	No accelerated disease progression; initial CD4 > 400/μl (January 2002), nadir CD4 = 270/μl, with subsequent rebound to ~ 400/μl (12 months post-infection), initial VL = 50,118 c/ml (January 2002), small decreases in VL (within 12 months post-infection), no data on clinical symptoms	ART-naive during follow-up
#18	This report; Poland, EuroCoord Study	Male	White (Polish)	Homosexual intercourse	February 2013	Between November 2012 and February 2013	Rapid disease progression, initial CD4 = 651/μl, CD4 < 350/μl (19 months after diagnosis), VL rise from initial 5,475 to 13,163 c/ml (9 months after diagnosis), no clinical symptoms	ART-naive during follow-up

*Data presented in the table are according to the source articles.
3TC: lamivudine; ART: antiretroviral therapy; AZT: azidothymidine; CD4: $CD4^+$ T-cell count; d4T: stavudine; EFV: efavirenz; IDV: indinavir; LPVr: lopinavir-ritonavir; NS: not specified; NVP: nevirapine; RTV: ritonavir; SQV; saquinavir; VL: HIV viral load.

genetic analyses were obtained at first clinical presentation (March 2013). According to the results of LAg-Avidity EIA (normalized optical density = 0.835 in the initial testing, and median normalized optical density = 0.760 in the confirmatory, triplicate testing), accompanied by the viral load of > 1000 HIV-1 RNA copies/ml, the patient was deemed to have a recent HIV-1 infection of duration less than 130 days. Together with the positive Western Blot in February 2013, this means that seroconversion occurred between November 2012 and February 2013.

The patient's first $CD4^+$ T-cell count was 651/μl (March 2013), and subsequently a rapid decline was noted, with 423/μl in November 2013, and 340/μl in September 2014. The loss of $CD4^+$ T-cells was linked to the increase in viral load from initial 5 475 HIV-1 RNA copies/ml to 13 163 copies/ml in November 2013. From HIV-1 diagnosis to September 2014, the patient did not receive any antiretroviral treatment. At the last clinical consultation, in September 2014, due to advanced immunosuppression, introduction of antiretroviral treatment was discussed with the patient. Unfortunately, the patient was lost to follow-up in the outpatient clinic, and further attempts to contact the patient were unsuccessful.

Tropism, subtype, and drug susceptibility of the transmitted virus were evaluated based on genotypic analyses of proviral DNA amplified from the patient's genomic DNA isolated from the blood sample obtained at first clinical presentation. To assess coreceptor usage, gp120 proviral sequences containing V3-loop coding region were amplified in triplicate with nested-PCR, and population-based sequencing was performed. Proviral V3 sequences were interpreted with the geno2pheno coreceptor usage prediction algorithm (http://coreceptor.bioinf.mpi-inf.mpg.de/index.php). Additional coreceptor usage predictions were performed with the PhenoSeq (https://www.burnet.edu.au/facilities/9_phenoseq), $WebPSSM_{SINSI}$, $WebPSSM_{X4R5}$ (https://indra.mullins.microbiol.washington.edu/webpssm), 11/25 and the net charge rules. Based on the geno2pheno analysis of all three replicates, virus infecting the patient with homozygous *CCR5-Δ32* mutation was considered to be able to use CXCR4 chemokine receptor as a coreceptor (X4 or R5/X4 variant), with a false positive rate (FPR) of classifying a R5-strain falsely as X4 of 1.7% (Table 2). Geno2pheno results were in accordance with the coreceptor usage predictions performed with the PhenoSeq, $WebPSSM_{SINSI}$, $WebPSSM_{X4R5}$, and the 11/25 rule. Examination of four nested-PCR amplified and sequenced proviral DNA fragments, coding for the entire protease (nucleotides according to the numbering positions of HIV-1 HXB2: 2253-2549, GenBank accession no: KT324378), part of the reverse transcriptase (nucleotides: 2565-3299, GenBank accession no: KT324538), part of the gp120 (nucleotides: 6583-7314, GenBank accession no: KT778163), and part of the gp41 (nucleotides: 7817-8345 GenBank accession no: KT324422) showed that the patient was infected with HIV-1 subtype B. No major drug resistance related mutations were found in the protease, reverse transcriptase, and gp41 sequences.

The research was approved by the National Institute of Public Health, National Institute of Hygiene Bioethics Committee, Poland (no. 3/2007). Prior to collection of samples for LAg-Avidity EIA and all genetic analyses, written informed consent was obtained from the study participants. Data on the patient's infection course were retrieved from medical records.

What do we know about HIV-1 infection in patients homozygous for the *CCR5-Δ32* allele?

Combining data from current and previous reports on HIV-1-infected homozygotes for the *CCR5-Δ32* allele (Table 1), as many as 17 of 18 were men (in one study sex was not specified), and those for whom the race was indicated (12) were most commonly white people of European origin[8-23]. The HIV-1 infection in these patients could be acquired by different routes, but most of them (12/18; 66.7%) were infected through homosexual contacts[8,12-14,16-18,20,21], for one person the route of infection was heterosexual intercourse[15], and for a further two men sexual transmission was indicated without specifying the type of sexual contacts[22]. Another two persons were hemophiliacs who contracted infection through injections of contaminated coagulation factor VIII in the early epidemics (putatively in 1982)[10,19]. For one person, injecting drug use was presumed to be the transmission route[23] and this route also could not be excluded in case of one homosexual man[18]. Thus, it appears that *CCR5-Δ32* homozygotes may become infected regardless of the route of transmission, although the homosexual men may presumably be more vulnerable to such infections. The predominating homosexual route of HIV-1 infection among *CCR5-Δ32* homozygotes may however reflect the general epidemiology of HIV-1 subtype B transmission in Europe, North America, and Australia where the studies were carried out, rather than association of HIV-1 transmission in *CCR5-Δ32* homozygotes with the type

Table 2. Information on HIV-1 strains infecting homozygotes for the *CCR5*-Δ*32* allele

Patient no.	Reference	V3 containing sequence GenBank accession	Sample	Time of sample collection	Subtype	Coreceptor usage/viral phenotype based on V3 sequence analysis*	Analysis of available V3 sequences with†,‡						Coreceptor usage/ viral phenotype based on cell infection/ phenotypic assay
							g2p FPR§	Pheno-Seq	Web-PSSM$_{SINSI}$#	Web-PSSM$_{X4R5}$#	11/25 rule¶	Net charge rule**	
#1	[8,9]	AF146728.1	Proviral DNA	4 years after seroconversion	B	CXCR4/SI	**0.2%**	**CXCR4-using**	**4.04**	**–1.00**	**G/K**	+4	CXCR4/SI
#2	[10,11]	AF034375.1-AF034385.1	Viral RNA	> 3 years after seroconversion	B	SI	**0.1%**	**CXCR4-using**	**10.96**	**8.44**	**R/Q**	**+7**	CXCR4/SI
#3	[12]	Not published	Proviral DNA	30 months after seroconversion	B	SI	–	–	–	–	–	–	Not determined
#4	[13]	U92491.1	Proviral DNA	> 6 years after seroconversion	B	SI	**0.2%**	**CXCR4-using**	**6.05**	**–1.98**	**R/T**	**+5**	SI
#5	[14]	Not published	NS	NS	B	SI	–	–	–	–	–	–	Not determined
#6	[15]	Not published	NS	NS	B	SI	–	–	–	–	–	–	Not determined
#7	[16]	No information on infecting virus due to lack of suitable samples for virus isolation/sequence analysis											
#8	[16]	Not published	Viral RNA	Primary infection, ≥ 5 months later	NS	SI	–	–	–	–	–	–	CXCR4/SI
#9	[17,18]	DQ356577.1-DQ356580.1	Proviral DNA	1 year after primary infection	B	Suggested CCR5	**6.6%**	**CXCR4-using**	–2.93	–7.54	S/E	+3	CXCR4/CCR5
#10	[19]	AY150664.1-AY150672.1	Viral RNA	NS (at least 3 years from primary infection)	B	CXCR4/SI	**2.6%**	**CXCR4-using**	**1.21**	–4.71	S/Q	**+5**	Not determined
#11	[18]	DQ356581.1-DQ356584.1	Proviral DNA	> 12 years after seroconversion	B	Suggested CCR5	**1.8%**	**CXCR4-using**	**–0.89**	–4.88	S/–	**+5**	CXCR4/CCR5
#12	[20]	No information on coreceptor usage											
#13	[20]	No information on coreceptor usage											

(Continue)

Table 2. Information on HIV-1 strains infecting homozygotes for the *CCR5-Δ32* allele *(continued)*

Patient no.	Reference	V3 containing sequence GenBank accession	Sample	Time of sample collection	Subtype	Coreceptor usage/viral phenotype based on V3 sequence analysis*	Analysis of available V3 sequences with†,‡						Coreceptor usage/ viral phenotype based on cell infection/ phenotypic assay
							g2p FPR§	Pheno-Seq	Web-PSSM$_{SINSI}$#	Web-PSSM$_{X4R5}$#	11/25 rule¶	Net charge rule**	
#14	[21]	Not published	Proviral DNA, viral RNA	NS	NS	CXCR4	–	–	–	–	–	–	Not determined
#15	[22]	Published in text	Viral RNA	≥ 2 years after seroconversion	B	CXCR4	**0.2%**	**CXCR4-using**	**1.89**	**–3.45**	**R/D**	+4	CXCR4
#16	[22]	Published in text	Viral RNA	≥ 2 years after seroconversion	B	CXCR4	**1.3%**	**CXCR4-using**	**–0.28**	–7.29	**R/E**	+2	CXCR6/CCR5
#17	[23]	Published in text	Viral RNA	5 months after infection	B	CXCR4	**2.6%**	**CXCR4-using**	**–2.91**	**–5.29**	**G/R**	**+7**	Not determined
#18	This report	KT778163	Proviral DNA	≤ 5 months from seroconversion	B	CXCR4	**1.7%**	**CXCR4-using**	**–2.15**	**–6.13**	**R/D**	+4	Not determined

*According to the source article; †Data presented with bold, underlined font indicate CXCR4-using strain; ‡For multiple HIV-1 V3 sequences obtained from one individual consensus of first available samples was considered; §g2g FPR – geno2pheno with false positive rate (probability of classifying an R5-virus falsely as X4) significance level of 10%; #WebPSSM$_{SINSI,}$ WebPSSM$_{X4R5}$ – the V3 with PSSM scores (obtained using SINSI or X4R5 matrix, respectively) of < –6.96 were considered R5, sequences with scores > –2.88 – CXCR4-using, intermediate values were interpreted based on the 11/25 rule; ¶11/25 rule – the presence of positively-charged amino acid, arginine (R) or lysine (K), in 11 and/or 25 position of V3 loop is predicative for CXCR4-tropism; **Net charge rule – total V3 charge calculated as the number of positively-charged amino acid residues (R, K) minus the number of negatively-charged (aspartic acid – D, glutamic acid – E) amino acid residues of ≥ +5 is predicative for CXCR4-tropism, NS: not specified; SI: syncytium inducing phenotype.

of exposure. On the other hand, the HIV-1 infection in individuals homozygous for *CCR5-Δ32* allele seems to be associated with heavy exposure[10,16,17].

The HIV-1 infection course in the absence of antiretroviral drugs was described for 14 patients with homozygous *CCR5-Δ32* genotype, and in 10 (71.4%) of them rapid decline of CD4+ T-cell count was noticed[10,12,14-19,21]. The fast decline of CD4+ T-cell count in the majority of individuals with homozygous *CCR5-Δ32* genotype, who cannot be infected with HIV-1 strains exclusively using CCR5 coreceptor is in line with the accelerated CD4+ T-cell decrease observed after the appearance of CXCR4-using variants that is linked to the greater pool of CD4+ T-cells expressing CXCR4 than CCR5[5]. However, a more complex interplay between viral and host factors is suggested to be involved in the accelerated disease progression in persons initially infected with CXCR4-using strains. Previously it was shown that high replicative capacity of the infecting, dual tropic viruses together with impaired hosts' humoral and cellular HIV-1-specific immune responses may contribute to fast clinical disease progression, with the development of AIDS-defining symptoms during the acute HIV-1 infection[28]. For the remaining four out of 14 persons with described HIV infection course, disease progression was not accelerated[8,23] or was defined as slow, with preserved CD4+ T-cell counts and viral load < 4,000 RNA copies/ml[22]. Three of them were deemed to be infected with CXCR4-using virus, while in the case of one patient with slow disease progression (#16), infection with the virus able to use CXCR6 and CCR5 coreceptors was recognized according to the phenotypic assay (Table 2)[22]. The occurrence of HIV-1 strain using alternative coreceptors in addition to CXCR4 and CCR5 as a result of changes in the V3 region has been previously demonstrated in an asymptomatic person with slow disease progression to AIDS[29].

For 10 of 18 HIV-1-infected *CCR5-Δ32* homozygotes, antiretroviral drug use was reported[8-10,14-17,19,21,22]. In three of these cases, two hemophiliacs experienced opportunistic infections or adverse effects under azidothymidine monotherapy[10,19], and in one subject therapy was initiated too recently to evaluate its outcome[22]. Among the remaining seven out of 10 persons receiving antiretroviral drugs, therapy was associated with a significant suppression of viral load[8,9,14-17,21]; additionally, in six of these seven therapy responders, no significant clinical symptoms were noticed, and only one individual under therapy had symptomatic infections with *Chlamydia* and *Shigella sonnei*[21]. This generally good virological and clinical response was not always associated with a rise in CD4+ T-cell count. In fact, for three persons, advantageous therapy outcome was observed despite persistently low CD4+ T-cell levels[14,16]. However, any conclusion concerning the outcome of the antiretroviral therapy in *CCR5-Δ32* homozygotes is uncertain, given the small number of patients on treatment and the differences in therapy regimens as well as the duration and stage of infection at therapy introduction.

For 15 *CCR5-Δ32* homozygotes, the HIV-1 strains were characterized by sequence analysis (Table 2). Thirteen of these 15 viruses were of subtype B, while for two strains subtype was not indicated and sequences were not published, thus not permitting the determination of the viral genotype. The prevalence of subtype B among HIV-1-infected homozygotes for the *CCR5-Δ32* allele is consistent with its predominance among populations in Europe, Australia, and North America, where all HIV-1-infected *CCR5-Δ32* homozygotes were identified.

The V3 sequences, crucial for genotypic prediction of HIV-1 coreceptor usage, were available for 10 of 18 HIV-1-infected *CCR5-Δ32* homozygotes (Table 2). For seven cases, sequences were published in the GenBank database, and for three subjects, directly in the manuscript. Our examination of all these sequences with the geno2pheno resulted in obtaining FPR indicative for CXCR4-using strains. In most cases (9/10) FPR were < 3%, and in one case of a person (#9) infected with virus using both coreceptors according to the cell infection assay, FPR was in the range of 4.4-7.1% (6.6% for consensus sequence) (Table 2). For all V3 sequences, geno2pheno results were supported by the PhenoSeq analysis. The other genotypic methods for coreceptor usage prediction, namely $WebPSSM_{SINSI}$, $WebPSSM_{X4R5}$, 11/25 and net charge rule, indicated CXCR4-using strains in nine, six, seven, and five individuals, respectively. Of note, discordant results of geno2pheno and other methods were obtained for two viruses, which were recognized to use both coreceptors for the cell entry (in patients #9 and #11)[17,18], and for one with CXCR6-tropism in the cell infection assay (in patient #16)[22].

Conclusion

In the face of developing new drugs and curative strategies directed against CCR5, keeping in mind the rare cases of individuals initially infected with non-CCR5-using strains is important. The use of

CCR5-blocking compounds as the novel HIV-1 pre-exposure prophylaxis in combinations with drugs targeted at other proteins rather than alone should be considered.

Declaration of interest

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Appendix

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