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Case Report

Genetic diagnosis of seronegative (HIV−) partner of female patient with AIDS in the context of HIV transmission

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ABSTRACT

Aim: Complex genetic diagnosis of seronegative (HIV−) long-term partner of female patient with AIDS C3 in the context of HIV transmission. Case report and literature review.
Background: HIV infection was excluded in 47-year-old man, a long-term sexual partner of female patient diagnosed with AIDS C3. The risk of HIV infection was estimated as high.
Objective: We focused on the genetic diagnosis of serodiscordant couple. We determined the presence of *CCR5-Δ32*, *CCR2-64I*, *HLA-B*, killer cell immunoglobulin-like receptor (*KIR*) and their ligand genes and human endogenous retroviruses K113 and K115.
Methods: Genotyping was performed using PCR methods.
Results: Analysis of partner's genotype revealed the presence of *CCR5-Δ32/Δ32* and *KIR* genes encoding activating receptors (*KIR2DS1*, *2DS5*, *3DS1*), features associated with reduced risk of HIV transmission. Similarities in patient's and her partner's *HLA* (*HLA-B*51*) and similar inhibitory *KIR* repertoire and their ligands (*KIR2DL1+HLA-C2*, *KIR2DL3+HLA-C1*, *KIR3DL1+HLA-B Bw4-80Ile*) could favor the transmission of the virus.
Conclusion: Genetic diagnosis is not routinely recommended, observation of exposed and uninfected individuals would allow to implement new knowledge into more effective care.

1. Introduction

The risk of HIV infection through unprotected heterosexual contact is estimated at 0.0001–0.014/intercourse [1,2]. The decisive role in the transmission of the virus is played by the viral load [2], but several research studies point also the impact of the age and sex of the infected person, type and frequency of sexual activity, circumcision, presence of other sexually transmitted diseases, and use of barrier methods of contraception [3]. We cannot forget about host genetic background of HIV infection, especially presence of mutation in HIV co-receptors (*CCR5*, *CCR2*), variability in histocompatibility antigens (*HLA*) and killer-cell immunoglobulin-like receptors (KIRs) [4,5]. Chemokine receptors are used as co-receptors for HIV, and it is known that some of their allelic versions may impact HIV infection. It is well documented that presence of *CCR5-Δ32* mutation in case of homozygous individuals is associated with blocking of R5 HIV strains into the cell [4,5]. *CCR2-64I* was found to have an anti-HIV effects, particularly slowing the progression toward AIDS, but also in the virus transmission context [6,7].

KIRs are receptors on NK cells and some CD8+ T cells. Among the KIR receptors we can distinguish the group of the receptors with long cytoplasmic domains, transmitting inhibitory signal to the NK cells (KIR2DL, KIR3DL) and the group of the activating receptors that have short cytoplasmic fragments (KIR2DS, KIR3DS) [8]. Interactions between KIR and HLA class I ligands seem to be critically involved in the immunosurveillance process. By interacting with MHC class I molecule on other cells, KIR control signal pathways and in consequence the activity of the NK cells. KIR genes are characterized by haplotype polymorphism, the presence of different numbers and types of genes in specific individuals, corresponding to the intensity and quality of the innate and acquired immune response. An individual can be a carrier of 6–16 different *KIR* genes, but even the same panel of genes in different individuals does not guarantee the same repertoire of NK receptors on the surface because they undergo individually differentiated

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expression [5]. Immune response to HIV takes on a different shape depending on the set of KIR receptors and their ligands (HLA epitopes), although observations are sometimes contradictory in this area.

Some authors consider the possible connection between human endogenous retroviruses (HERVs) and HIV infection (9–11). Endogenous retroviruses are probably the remnants of ancient infection of exogenous retroviruses into human genome during evolution. Most of them accumulated many mutations that are transcriptionally inactive but some are still expressed (e.g. HERV-K113). In the context of HIV replication, especially interesting are sequences belonging to the HERV-K family, encoded functional homolog of HIV regulatory genes *rev*. It was suggested that HIV upregulated HERV-K genes and the level of HERV-K protein was correlated with HIV viremia [9,12].

2. The case

In this study we analyzed selected genes of repeatedly exposed to HIV but seronegative subject (male, A.M) and his HIV positive partner (female, E.M). 47-year-old woman was admitted to the neurology department due to continuing month-long sensory disturbances in the face and right upper limb and transient, multi-day events of dysarthria and right lower extremity weakness. The patient lost 15 kg of weight in 6 months. Additional tests revealed thrombocytopenia, mildly deranged liver enzymes, biochemical parameters of generalized inflammation, and serological features of infection with *Toxoplasma gondii*. CSF examination and imaging studies of central nervous system formed the basis of diagnosis of cerebral toxoplasmosis in the course of HIV infection. The patient was diagnosed with AIDS, stage C3, with profound immune system impairment (number of CD4 <100 cells/μL, HIV RNA 4435 copies/ml). Toxoplasmosis treatment and antiretroviral therapy were started. Following the delayed diagnosis of HIV/AIDS in the patient, HIV testing was performed in the current sexual partner (husband, A.M., 47, who declared monogamous relationship with the patient), and in two children. The presence of HIV antibodies was not confirmed in any of the subjects. The route of HIV transmission was not established in the course of medical history of the patient (possible exposure via intercourse or intravenous drug using). Due to the natural history of HIV infection (i.e. typical progression time of 8–12 years from initial infection to the development of AIDS), and continuous sexual activity evidenced both by the patient and her partner, the risk of HIV transmission was determined as long-term and significant. Lack of HIV seroconversion in the partner instigated the expansion of the diagnostic process with genetic testing.

We performed the detection of genetic factors that could influence on HIV transmission.

CCR5-Δ32 and *CCR2-64I* alleles were determined as we described previously [13]. The detection of HERV-K113 was executed according to our previous study [11]. *KIR* genotyping was performed using PCR-SSP according to the previous works [14]. *HLA-B* genotyping was performed using HLA-Ready Gene Kit (InnoTrain Diagnostic GmbH) according to the manufacturer's protocol. Analysis was performed using HLA-B-Ready Gene program (InnoTrain Diagnostic GmbH). *KIR* ligands were detected using Olerup SSP KIR HLA Ligand Kit according to the manufacturer's protocol. The results of the patient and her partner are shown in Table 1.

Table 1
Results of genetic tests for patient (E.M.) and her partner (A.M.).

Allele/gene/sequences	Partner (A.M.)	Patient (E.M.)
CCR5/Δ32	*CCR5-Δ32/Δ32*	*CCR5+/Δ32*
CCR2-64I	*CCR2-64V/64V*	*CCR2-64V/64V*
KIR2DL1	+	+
KIR2DL2	–	–
KIR2DL3	+	+
KIR2DL5	+	–
KIR3DL1	+	+
KIR2DS1	+	–
KIR2DS2	–	–
KIR2DS3	–	–
KIR2DS4	+ (allele v)	+ (allele v)
KIR2DS5	+	–
KIR3DS1	+	–
HLA	*B*08/B*51*	*B*35/B*51*
	HLA-B Bw6(+)	*HLA-B Bw6(+)*
	HLA-B Bw4 80Ile (+)	*HLA-B Bw4 80Ile (+)*
	HLA-C1(80Asp)(+)	*HLA-C1(80Asp)(+)*
	HLA-C2(80Lys)(+)	*HLA-C2(80Lys)(+)*
	HLA-A Bw4+ (–)	*HLA-A Bw4+* (–)
HERV-K113	–	–
HERV-K115	–	–

3. Discussion

The genotyping for the *Δ32* mutation in both *CCR5* alleles in patient's partner revealed homozygous Δ32 mutation (*CCR5-Δ32/Δ32*). CCR5 is the chemokine receptor located on the surface of T lymphocytes, macrophages, immature dendritic cells and microglial cells in the central nervous system. It also serves as main co-receptor for HIV. *CCR5-Δ32* mutation results in a truncated protein which does not function as a chemokine receptor or as HIV co-receptor. It provides almost complete protection against HIV infection in the case of homozygous individuals [4,13], and in heterozygous significantly reduces the risk of infection during heterosexual exposure [13]. This was confirmed by the present case, where patient A.M., *CCR5-Δ32/32*, was not infected with HIV despite many years of heterosexual exposure.

We also studied *CCR2* genotype for presence of *CCR2-64I* mutation. A single nucleotide polymorphism (SNP) in the *CCR2* gene results in the substitution of valine by isoleucine (V64I) in the transmembrane region of the protein. The mutation in *CCR2* may account for the protection against HIV infection [15]. It has been suggested that *CCR2-64I* product binds CCR5 proteins in the cytoplasm, thereby preventing their expression on the cell surface and potentially hindering the transmission of HIV [16]. Both, the patient and her partner were found to possess the genotype *CCR2-64 V/64 V* and it suggests no effect on susceptibility to HIV infection.

In the case of *HLA* class I, patient's partner A.M. was found to carry *HLA-B*08* and *B*51* alleles with *Bw6* and *Bw4-80Ile* epitopes, while patient E.M. had *HLA-B*35* and *B*51* alleles, also with the same epitopes. The course of HIV infection is mainly attributed to HLA class I and it is believed that their greater diversity (heterozygosity), may reduce HIV transmission [17]. Both the patient and her partner have an allele *HLA-B*51*, which may facilitate infection (because of *HLA* compatibility). There is little information available concerning the relationship between *HLA-B*08* allele carried by patient's partner and the susceptibility to HIV infection. However, according to some researchers (also our group, unpublished data), allele *HLA-B*08* belongs to the group of factors prolonging survival in patients with AIDS or improving CD4T cell count restoration during antiretroviral therapy [18].

Both, patient and her partner, were found to carry *HLA-B Bw4-80Ile* which associated with decreased risk of HIV infection. HLA-B molecules associated with a slow progression of HIV infection such as HLA-B27, B57, and B51, carry mostly Bw4 epitopes [17].

The studied patient E.M. possessed poorer repertoire of KIR genes than her partner A.M. (haplotype A, with one *KIR2DS4*