

Relationship between Polymerase Gamma (POLG) Polymorphisms and Antiretroviral Therapy-Induced Lipodystrophy in HIV-Infected Patients: a Case-Control Study

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ABSTRACT N-124

Background. Antiretroviral drugs used in the treatment of HIV-1 inhibit replication of mitochondrial DNA (mtDNA) by inhibition of polymerase gamma (POLG), which may contribute to severe mitochondrial toxicity leading to lipodystrophy. Polymorphisms of *POLG* could explain the variability of mitochondrial toxicity in HIV-1-infected patients. We explored the relationship between selected polymorphisms of *POLG* and lipodystrophy related to anti-retroviral therapy.

Methods. We studied polymorphic changes at three highly conserved amino acid residues (R1142, E1143 and R1146) and the CAG repeats of *POLG* in a case-control study including HIV-1 treated patients with lipodystrophy (n=69) and 2 controls (without lipodystrophy) per case matched by age, race and sex (n=138). Treatments (types + duration) and biological data (31 parameters including lactatemia, glycemia, mtDNA, AST) were recorded. Matched univariate odd ratio (OR) with 95% confidence interval (CI) was calculated by conditional logistic regression and the relative risk (RR) determinates. A MANOVA test was performed ($\alpha=5\%$) to determine which parameters that were checked in the study were linked to the development of the lipodystrophy. We used Wilks' lambda distribution, probability distribution, to test multivariate hypothesis.

Results. Compared with matched controls, case patients had higher levels of triglycerides, alkaline phosphatase and also a longer history of HIV-1 infection and treatment. Only polymorphism in E1143 was significantly more frequent in case patients with lipodystrophy (15% versus 3.6%, OR=4.5, 95% CI [1.32-17.44], p=0.008, RR=4), and this was associated with a significant decrease of mtDNA in PBMC. In addition, among 31 parameters tested, MANOVA test showed that the lipodystrophy syndrome has a strong link with E1143 polymorphisms (p=0.043). The HIV-1 infected patients with lipodystrophy had also received significantly more protease inhibitors.

Conclusion. We confirm the role of several traditional risk factors already reported in the development of lipodystrophy. Patients harbouring changes of E1143 in the catalytic site of POLG exhibit a 4-fold increased risk to develop lipodystrophy than HIV-1 treated patients who do not have changes in E1143, and this could be due to decreased content of mtDNA in PBMC in these patients. Therefore, the toxicity of HAART (highly active antiretroviral therapy) leading to lipodystrophy in some HIV-1 infected patients could be explained in part by the occurrence of POLG polymorphisms.

AIM

The aim of this study was to investigate the possibility that *POLG* gene polymorphisms located in the active site of the enzyme, are associated with development of lipodystrophy in HIV patients receiving antiretroviral (HAART) treatment through the development of the mitochondrial toxicity.

METHODOLOGY

Patients

"Matched-control" was generated by randomly selecting 2 controls per case matched by age (± 5 years), race and sex (Table 1). They were selected between the period from May 2002 and September-October 2004. The lipodystrophy is defined as peripheral lipodystrophy, central fat accumulation and lipomatosis [Carr A. Lancet (361) 2003].

Biochemistry (glycemia, lactatemia, triglyceridemia, cholesterolemia), hematology (CD4+, CD8+, ratio CD4+/CD8+), and treatments (type and duration) were recorded for each patient (31 parameters) (Table 1).

	Matched control without lipodystrophy n=138	Cases with lipodystrophy n=69	†p=	‡p=
Age, years, mean (SD)	42.4 (8.6)	42.4 (8.0)		
Gender				
Female (%)	52 (37.7)	26 (37.7)		
Male (%)	86 (62.3)	43 (62.3)		
Race				
African (%)	10 (7.2)	5 (7.2)		
Caucasian (%)	128 (92.8)	64 (92.8)		
Alkaline phosphatase (IU/L)	80 ± 40	97 ± 38	0.003	<0.001
γ-GT (IU/L)	58 ± 70	71 ± 69	0.211	0.032
Triglyceridemia (mmol/L)	1.87 ± 1.68	2.62 ± 2.75	0.05	0.066
Total duration of HIV-1 seropositivity (months)	109.3 ± 67.0	134.4 ± 57.4	0.007	0.120
Total duration of therapy (months)	56.2 ± 44.5	79.0 ± 38.0	0.0003	0.344
Number of line of treatment	4.2 ± 3.7	6.8 ± 4.7	0.001	0.103
Total duration of NRTI therapy (months)	17.7 ± 21.1	34.9 ± 20.9	0.001	0.003
DDI cumulative (months)	11.4 ± 16.0	17.5 ± 20.0	0.04	0.074
D4T cumulative (months)	17.7 ± 21.1	34.9 ± 20.9	0.001	0.003
Number of patients received PI therapy (yes/no)	49/87	36/32	0.0279*	0.986

Table 1. Demographic data, biochemistry, treatment status of HIV-1 infected patients with (cases) and without (control) lipodystrophy. DDC: dideoxycytidine; DDI: didanosine; D4T: stavudine; γ-GT: gamma glutamine transferase; The results are expressed as mean ± standard deviation. † Means were compared using Student *t*-test ($\alpha=0.05$) and a multivariate analysis of variance (‡ MANOVA test). * χ^2 -squared test ($\alpha=0.05$). See below for statistical analysis.

- **Quantitative-PCR:** quantification of the mtDNA in the peripheral blood mononuclear cells (PBMC) was quantified by Q-PCR (TaqMan®. Applied Biosystems).

- **PCR sequencing:** polymorphisms (SNP) R1142 (arginine), E1143 (glutamine acid) and R1145 (arginine), (CAG)_n repeat region (Figure 1) were studied by sequencing (ABI-PRISM 310, Applied Biosystems).



Figure 1: Organization of the subunit A of POLG enzyme. Localization of the (CAG)_n repeat region and the SNPs in the different domains of this protein.

- **Statistical analysis** was performed using the R software version 2.5.0. Results are expressed as mean (m) and standard deviation (SD). To compare the means of two groups we used the Student's *t*-test. To assess qualitative descriptor, chi-square (χ^2) test was used. Matched univariate odd ratio (OR) with 95% confidence interval (CI) was calculated by conditional logistic regression and the relative risk (RR) determinates. Type one error was set at 5%. To test among the parameters checked in this study, which are linked with the development of the lipodystrophy, MANOVA test was used ($\alpha=5\%$) and we used Wilks' lambda distribution, probability distribution to test multivariate hypothesis.

RESULTS

- Among 31 parameters tested, MANOVA test showed that the lipodystrophy syndrome has a strong link with E1143 polymorphisms (p=0.043) (Table2).

- The logistic regression shows that only polymorphism E1143 was significantly more frequent in case patients with lipodystrophy (15% versus 3.6%, OR=4.5, 95% CI [1.32-17.44], p=0.008, RR=4).

Patients	Matched control without lipodystrophy	Cases with lipodystrophy	*p=	‡p=
Ratio mtDNA/β-actin	0.28 ± 0.44	0.16 ± 0.17	0.006 †	0.1350
POLG's polymorphisms				
Repeat of CAG (n=)				
10/10	110	50	0.844	0.087
10/11	21	16		
Others	7	3		
R ₁₁₄₂				
R ₁₁₄₂ /R ₁₁₄₂	136	67	0.858	0.192
R ₁₁₄₂ /L ₁₁₄₂	2	2		
E ₁₁₄₃				
E ₁₁₄₃ /E ₁₁₄₃	133	59	0.010	0.043
E ₁₁₄₃ /D ₁₁₄₃	0	2		
E ₁₁₄₃ /C ₁₁₄₃	5	8		
R ₁₁₄₅	138	69		

Table 2. Molecular characterization (polymorphisms) of HIV-1 infected patients with (cases) and without (control) lipodystrophy. † Means were compared using Student *t*-test ($\alpha=0.05$) and a multivariate analysis of variance (‡ MANOVA test). * Chi-squared test ($\alpha=0.05$).

- Polymorphism E1143 was associated with a significant decrease of mtDNA in PBMC (Table3).

Relative mtDNA to β-actin content		
E ₁₁₄₃ single nucleotide polymorphism (SNP)	Matched control without lipodystrophy	Cases with lipodystrophy
E ₁₁₄₃ /E ₁₁₄₃	0.28 ± 0.33	0.21 ± 0.36
E ₁₁₄₃ /D ₁₁₄₃ and E ₁₁₄₃ /C ₁₁₄₃ *	0.16 ± 0.14	0.13 ± 0.12

Table 3. Amount of mtDNA in the HIV-1 infected patients with (Cases) and without lipodystrophy (Controls) according to the E1143 single nucleotide polymorphism (SNP). * ANOVA test, p=0.0019.

CONCLUSION-HYPOTHESIS

We confirmed the importance of several previously reported risk factors for the development of lipodystrophy (type of treatment = D4T and DDI, total duration of therapy). Patients harbouring changes in E1143 in the catalytic site of POLG exhibit a 4-fold increased risk of developing lipodystrophy than HIV-1 treated patients who do not have these changes. This could be due to the decreased content of mtDNA in PBMC in these patients. Therefore, the toxicity of HAART leading to lipodystrophy in some HIV-1 infected patients could be explained in part by the occurrence of POLG polymorphisms