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Single Nucleotide Polymorphisms associated with alimentary fatty liver disease are not genetic risk factors for treatment-associated hepatic steatosis in HIV patients on HAART.

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Hepatic steatosis may occur with any type of HAART. Recently genome wide association studies have identified 5 single nucleotide polymorphisms (SNPs) predisposing to fatty liver disease in nutritional abnormalities. Using a non-invasive method termed "controlled attenuation parameter" we assessed liver fat in HAART-treated HIV-patients and correlated hepatic steatosis to genotype distribution of the 5 SNPs. Unlike alimentary fatty liver our data do not support a role of these SNPs for fatty liver disease on HAART.

Highly active antiretroviral therapy has dramatically reduced death rates from opportunistic diseases but is frequently complicated by dyslipidaemia and fatty liver disease (1). Although certain reverse transcriptase inhibitory nucleotides such as the "D" drugs didanosine and stavudine carry a particularly high risk of hepatic steatosis, fatty liver disease has been observed with any type of antiretroviral therapy. Recently two genome wide association studies have revealed that single nucleotide polymorphisms in or near the five genes *PNPLA3* (rs738409), *CSPG3/NCAN* (rs2228603), *GCKR* (rs780094), *PPP1R3B* (rs4240624) and *LYPLAL1* (rs12137855) are associated with fatty liver disease as well as distinct patterns of serum lipids and glycaemic traits (2-4). Indeed, carriers of the genetic risk variants have been observed significantly more frequently among patients with severe steatohepatitis, liver cirrhosis and liver cancer associated with alcoholic consumption and non-alcoholic liver disease (5-9). Using a novel non-invasive method termed "controlled attenuation parameter, CAP", we performed a pilot trial to check if any of the 5 genetic variants was also differentially distributed among HIV-infected patients who had developed hepatic steatosis on highly active antiretroviral therapy (HAART).

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Running title: Genetic polymorphisms and fatty liver disease under HAART

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Table 1: Distribution of *PNPLA3* (rs738409), *CSPG3/NCAN* (rs2228603), *GCKR* (rs780094), *PPP1R3B* (rs4240624) and *LYPLAL1* (rs12137855) polymorphisms and the frequencies of minor alleles in healthy controls and the HIV patients stratified with respect to their hepatic steatosis. No significant differences in SNP´s distribution were found between all HIV-infected patients with a fatty liver and those patients without hepatic steatosis, nor to healthy controls.

Single Nucleotide Polymorphisms and Fatty Liver Disease Genotype and Allele Distribution are not associated with Hepatic Steatosis in HIV Patients on HAART				
		Genotype		Frequency of the minor allele
***PNPLA3* (rs738409)**	**GG**	**GC**	**CC**	
Healthy Controls	87	55	10	24.7%
	57%	36%	7%	
HIV-Patients (all)	34	21	2	21.9%
	60%	37%	4%	
HIV-Patients without fatty liver disease(<238dB/m)	22	11	2	21.4%
	63%	31%	6%	
HIV-Patients with fatty liver disease (≥238 dB/m)	12	10	0	22.7%
	55%	45%	0%	
HIV-Patients with severe fatty liver disease (>260 dB/m)	6	6	0	25.0%
	50%	50%	0%	
***CSPG3/NCAN* (rs2228603)**	**CC**	**CT**	**TT**	
Healthy Controls	128	23	1	8.2%
	84%	15%	1%	
HIV-Patients (all)	46	11	0	9.6%
	81%	19%	0%	
HIV-Patients without fatty liver disease(<238dB/m)	30	5	0	7.1%
	86%	14%	0%	
HIV-Patients with fatty liver disease (≥238 dB/m)	16	6	0	13.6%
	73%	27%	0%	
HIV-Patients with severe fatty liver disease (>260 dB/m)	9	3	0	12.5%
	75%	25%	0%	
***GCKR* (rs780094)**	**GG**	**GA**	**AA**	
Healthy Controls	53	77	22	39.8%
	35%	51%	15%	
HIV-Patients (all)	21	29	7	37.7%
	37%	51%	12%	
HIV-Patients without fatty liver disease(<238dB/m)	14	18	3	34.3%
	40%	51%	9%	
HIV-Patients with fatty liver disease (≥238 dB/m)	7	11	4	43.2%
	32%	50%	18%	
HIV-Patients with severe fatty liver disease (>260 dB/m)	3	9	0	37.5%
	25%	75%	0%	
***PPP1R3B* (rs4240624)**	**AA**	**AG**	**GG**	
Healthy Controls	127	25	0	8.2%
	84%	16%	0%	
HIV-Patients (all)	44	13	0	11.4%
	77%	23%	0%	
HIV-Patients without fatty liver disease(<238dB/m)	27	8	0	11.4%
	77%	23%	0%	
HIV-Patients with fatty liver disease (≥238 dB/m)	17	5	0	11.4%
	77%	23%	0%	
HIV-Patients with severe fatty liver disease (>260 dB/m)	8	4	0	16.7%
	67%	33%	0%	
***LYPLAL1* (rs12137855)**	**CC**	**CT**	**TT**	
Healthy Controls	109	39	4	15.5%
	72%	26%	3%	
HIV-Patients (all)	37	18	2	19.3%
	65%	32%	4%	
HIV-Patients without fatty liver disease(<238dB/m)	24	10	1	17.1%
	69%	29%	3%	
HIV-Patients with fatty liver disease (≥238 dB/m)	13	8	1	22.7%
	59%	36%	5%	
HIV-Patients with severe fatty liver disease (>260 dB/m)	6	6	0	25.0%
	50%	50%	50%	

We recruited 57 HIV-infected patients (median age: 48 years, range 30 – 72 years; 8 females) into this pilot study. Patients were on HAART for a median of 81 months (range 6 – 275 months) and had a median of 468 CD4+ cells/µl (range 128-1474 cells/µl). At the time of the study 31 and 26 patients were taking a NNRTI- and PI-based antiretroviral therapy, and 33 and 29 patients had prior exposure to PIs and D-drugs, respectively. 152 healthy volunteers (median age 39 years, range 21-67 years; 59 females) served as reference for the distribution of alleles in the background population. Informed consent was obtained from all patients prior to sample acquisition, and the study was approved by the local ethics committee of the University of Bonn, Germany. Genomic DNA was extracted from 200 µl EDTA-blood using the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Determination of the *PNPLA3* (rs738409), *CSPG3/NCAN* (rs2228603), *GCKR* (rs780094), *PPP1R3B* (rs4240624) and *LYPLAL1* (rs12137855) polymorphisms was performed by LightCycler real time PCR (Roche, Mannheim, Germany) using commercial LightSNiP (SimpleProbe) assays from TIB-MolBiol (Berlin, Germany) according to the manufacturer's recommendations. Using a "Fibroscan 502 Touch" device equipped with an M probe (echosens GmBH, Norderstedt) we assessed hepatic steatosis via the novel "controlled attenuation parameter", CAP technology (10). This non-invasive technique yields results in dB/m, which correspond to intrahepatic fat contents. Patients were classified as hepatic steatosis and severe hepatic steatosis, if CAP values were above 237 dB/m (corresponding to >10% fat) and 260 dB/m (corresponding to >33% intrahepatic fat), respectively. Genotype frequencies were determined and tested for consistency with the Hardy-Weinberg equilibrium using an exact test. Allele and genotype frequencies were compared between patient groups and controls by Pearson's goodness-of-fit chi^2 test and Armitage's trend test, respectively (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Via CAP technology, we identified 22 individuals to have hepatic steatosis and 12 patients to even have severe steatosis among our 57 patients. Table 1 summarizes the distribution of genotypes and the frequencies of minor alleles for the 5 genetic variants in healthy controls and the HIV patients stratified with respect to their hepatic steatosis. This table clearly illustrates that we could not find any significant differences between HIV-infected patients with a fatty liver and those HIV-infected patients without hepatic steatosis. Further more no significant difference between healthy controls and the patient cohort was detected, that would support an association between any of the 5 genetic variants and hepatic steatosis in our HIV patients. This finding suggests that unlike alcohol consumption or non-alcoholic steatohepatitis genetic variants in *PNPLA3* (rs738409), *CSPG3/NCAN* (rs2228603), *GCKR* (rs780094), *PPP1R3B* (rs4240624) and *LYPLAL1* (rs12137855) are not major risk factors for fatty liver disease associated with HIV infection and antiretroviral drugs. Of note, our observation may be also a hint to a fundamental difference in the pathogenesis between hepatic steatosis in HIV infection and that linked to nutritional abnormalities. Nevertheless genetic variants in other genes may still contribute to the risk of fatty liver disease in HIV infection and HAART.

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