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MAJOR ARTICLE

Contribution of Genetic Background and Clinical Risk Factors to Low-Trauma Fractures in Human Immunodeficiency Virus (HIV)-Positive Persons: The Swiss HIV Cohort Study

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Background. The impact of human genetic background on low-trauma fracture (LTF) risk has not been evaluated in the context of human immunodeficiency virus (HIV) and clinical LTF risk factors.

Methods. In the general population, 6 common single-nucleotide polymorphisms (SNPs) associate with LTF through genome-wide association study. Using genome-wide SNP arrays and imputation, we genotyped these SNPs in HIV-positive, white Swiss HIV Cohort Study participants. We included 103 individuals with a first, physician-validated LTF and 206 controls matched on gender, whose duration of observation and whose antiretroviral therapy start dates were similar using incidence density sampling. Analyses of nongenetic LTF risk factors were based on 158 cases and 788 controls.

Results. A genetic risk score built from the 6 LTF-associated SNPs did not associate with LTF risk, in both models including and not including parental hip fracture history. The contribution of clinical LTF risk factors was limited in our dataset.

Conclusions. Genetic LTF markers with a modest effect size in the general population do not improve fracture prediction in persons with HIV, in whom clinical LTF risk factors are prevalent in both cases and controls.

Keywords. antiretroviral therapy; genetics; HIV infection; low-trauma fracture; osteoporosis.

Bone health is a major long-term concern in human immunodeficiency virus (HIV)-positive persons. Large observational studies have documented increased rates of osteoporosis and fractures in HIV-positive individuals compared with the general population. The pathogenesis of bone disease during HIV infection is incompletely defined; a high prevalence of smoking and substance use, low body weight, hepatitis C coinfection, vitamin D deficiency, as well as the effects of HIV itself, HIV-associated inflammation, and certain antiretroviral therapy (ART) agents have been implicated [1–6].

Bone mineral density and low-trauma fractures (LTFs) have a strong hereditary component [7, 8]. Genome-wide association studies (GWAS) have identified 6 common genetic variants that reproducibly contribute to either osteoporosis or fracture risk in the general population [9]. The primary aim of the present study was to quantitate the contribution of these gene variants to the risk of LTF in HIV-positive participants enrolled in the Swiss HIV Cohort Study (SHCS), in the context of clinical, HIV-related, and antiretroviral factors contributing to LTF risk. A secondary aim was the assessment of 61 single-nucleotide polymorphisms (SNPs) associated with bone mineral density in the reference paper [9]. We included only LTF that were validated in writing by the treating HIV physician. To our knowledge, this represents the first genetics-fracture study undertaken in HIV-positive persons.

METHODS

Study Population, Inclusion, and Exclusion Criteria

Eligible participants included HIV-positive persons enrolled in the SHCS (www.shcs.ch) [10], with ≥2 years follow-up after January 1, 1998. The study was approved by the local ethics committees. Participants provided written informed consent for genetic testing. Low-trauma fracture cases included all SHCS participants who developed a LTF during the study period, which was from SHCS enrollment until December 31, 2014. For participants with >1 LTF event, only the first event was included. Eligible participants who remained fracture-free during

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the study period were potential controls. Because most previous fracture GWAS in the general population were conducted in populations of European descent [9], the present report is restricted to participants of European descent.

Low-Trauma Fractures

The treating HIV physician confirmed in writing the presence, date, body site, and low trauma nature of all fractures. Low-trauma fractures were defined as resulting from a fall at standing height or less, at walking speed or less, and at age ≥18. For asymptomatic vertebral fractures detected by radiologic imaging, diagnosis relied on the attending radiologist's report and the imaging date was used. Fractures of the skull, cervical spine, fingers, and toes were excluded because these are typically considered traumatic in nature. Fractures occurring in patients with a history of malignancy were excluded to avoid including pathological fractures. The annualized LTF rate in the SHCS was calculated as the number of LTFs divided by the number of person-years of follow-up.

Case-Control Matching: Nongenetic Analyses

Controls were matched to cases by gender, and using incidence density sampling [11], on ART start and stop dates, which are recorded in the SHCS database. Patients were eligible as controls if their ART start date was within 1 year of the case's and if they were still receiving ART at the time of the case's fracture. All eligible controls were identified for each case, 5 of whom were selected at random (if <5 controls were available, all were selected). Any control matched to a case was removed from the list of eligible controls. Cases with no matching controls (n = 9) were dropped, and participants with unconfirmed LTF were excluded from the set of eligible controls. The expected effect of incidence density sampling is to correct for differences in ART compounds in use at different times during the study period, a correction that would not be achieved if matching were based only on ART duration. To ensure similar duration of observation, follow-up ended on the date of the fracture of the corresponding case for both cases and controls.

Case-Control Matching: Genetic Analyses

For the analyses that include genotype data (GWAS and logistic regression including genetic risk scores), the patient set was produced with the same procedure as the nongenetic analyses (see above), with the following differences: (1) only patients with available deoxyribonucleic acid or genome-wide genotyping data were included; (2) the "difference in ART start date" criterion was relaxed from 1 year to 2 years; and (3) up to 2 controls (instead of 5) were allowed per case (this has the effect of lowering the rate at which eligible controls are exhausted, thus lowering the probability of a case being dropped for lack of matching controls).

Genotyping: Quality Control

Deoxyribonucleic acid obtained from peripheral blood mononuclear cells was genotyped using the Human OmniExpress 24 array (n = 100 cases) or in the context of previous GWAS (n = 3 cases and 206 controls) [12–14]. Missing genotype imputation was performed with Mach [15, 16] and minimac [17, 18] using 1000 Genomes Phase 3 population data as reference haplotypes, resulting in a total of >6 million SNPs available for analysis. Imputed markers were filtered on minor allele frequency (excluded if <0.01) and imputation quality using Mach's reported r-squared measure (excluded if <0.3). Participants were filtered based on cryptic relatedness (dropped if identity by state >0.12). We used a modified Eigenstrat approach to control for the possibility of spurious associations resulting from residual population stratification [19]. This method derives the principal components of the correlations among common (minor-allele frequency, >5%) gene variants, which reflect population ancestry, and corrects for those correlations in the subsequent association tests by integrating the coordinates of the significant principal component axes as covariates (Eigenstrat covariates) in the models.

Nongenetic Low-Trauma Fractures Risk Factors

We tested LTF associations of nongenetic factors by conditional logistic regression, both univariable or multivariable. Analyses were done using R [20] and PLINK [21]. The variables included in the model were defined a priori, based on their contribution to LTF in large observational studies [1–3, 5, 6]. All variables entered the model as the information available closest to the fracture date of the corresponding case. Human immunodeficiency virus-related variables included CD4 count, CD4 nadir, cumulative exposure to tenofovir disoproxil fumarate, protease inhibitor use with/without ritonavir, and hepatitis C virus (HCV) coinfection, defined as detectable HCV ribonucleic acid at least once during SHCS follow-up. Other variables were height, weight, alcohol consumption (≥3 standard units daily consumption vs less [22]), smoking (current/ever smoking vs never [23]), injection drug use, corticosteroid use (ever used oral prednisone 5 mg equivalent for ≥3 months [24]), and parental hip fracture history [25]. The variables found to be associated with LTF risk in the multivariable model were retained, together with the genetic risk score quartile, to form the genetic model.

Genetic Association Analyses

We built an a priori defined genetic risk score using 6 SNPs (or a proxy with r2 > 0.8) with known fracture association at GWAS-wide significant $P < 5 \times 10^{-8}$ in the reference paper [9] (Supplementary Table 1). Based on these SNPs we applied the following: (1) an additive genetic score (number of risk alleles; heterozygous = 1, homozygous = 2; ie, scores ranged from 0 to 12, with higher scores indicating increased LTF risk); and (2) an additive weighted genetic score that takes into account the effect size reported in the reference paper [9]. For a SNP with, eg, a fracture odds ratio (OR) of 1.15, reference allele = 0, heterozygous = 0.15, and homozygous risk allele = 0.3. The

numbers obtained for each SNP were added to create an individual weighted genetic risk score, and study participants were divided into 4 genetic score quartiles.

Exploratory Genetic Association Analyses

The study was designed for replication of the 6 SNPs identified in GWAS in the general population [9]. However, because GWAS arrays were used for genotyping of all study participants, good practice calls for reporting genome-wide results. Thus, first, all genotyped or imputed SNPs were separately tested for association with LTF by logistic regression. We used Bonferroni adjustment, taking into account the number of tested SNPs, to correct for multiple testing. Second, we considered separately the 61 SNPs mapping to genes associated with bone mineral density in the reference paper [9]; 3 SNPs were not interrogated because they were neither present in the array nor reliably imputed. The distribution of association *P* values was compared between these groups and all other SNPs genotyped on the array using the 2-sample Kolmogorov-Smirnov test.

RESULTS

Participants: Fractures

Between January 1, 1998 and December 31, 2014, 158 confirmed LTFs were recorded in SHCS participants during 116 994 person-years of follow-up, for a LTF rate of 1.35 per 1000 person-years. After incidence density sampling, the study population for the analysis of nongenetic LTF risk factors included 158 cases and 788 clinical controls (cumulative follow-up, 16 278 person-years). Low-trauma fracture sites included spine (n = 70), femoral neck (n = 28), foot (without toes; n = 25), ankle (n = 17), humerus (n = 11), wrist (n = 10), ribs (n = 20), and other sites (n = 15). The characteristics of participants are shown in Table 1. The median age at first LTF was 52 years, and 34% of cases were women.

Associations Between Nongenetic Factors and Low-Trauma Fractures

In univariable analysis, LTF risk was associated with age, height, and corticosteroids exposure ≥3 months, and trends were noted for smoking and parental history of hip fracture (Table 2). In multivariable analysis, LTF risk was associated with height, corticosteroids, and smoking. Weight, CD4 nadir, HCV coinfection, alcohol, injection drug use, cumulative exposure to tenofovir disoproxil fumarate, and protease inhibitors (both with and without ritonavir) were not associated with LTF risk.

No Association Between Genetic Risk Score and Low-Trauma Fractures

The study population for genetic analyses included 103 cases and 206 controls. In the multivariable model that included genetic background, the additive genetic score was not associated with LTF. Odds ratios were 0.11 (95% confidence interval [CI], 0.01–1.57), 0.20 (95% CI, 0.01–3.25), and 0.42 (95% CI, 0.03–5.22), respectively, for the second, third, and fourth genetic score quartiles (most unfavorable genetic background), compared with the first quartile (most favorable genetic background). In this full model with genetics, age (OR, 1.00; 95% CI, 0.91–1.09), height (OR, 1.00; 95% CI, 0.89–1.13), smoking (OR, 1.06; 95% CI, 0.19–6.00), or corticosteroid exposure (OR, 0.67; 95% CI, 0.07–6.66) did not contribute to LTF risk either (all $P > .05$). Results were similar when a weighted genetic score was applied (data not shown). Removing parental hip fracture history did not change the estimates for the genetic score (Table 3).

Table 1. Characteristics of the Cases and Controls[a]

	Cases (n = 158)	Controls (n = 788)
Male gender[b], n (%)	104 (66)	520 (66)
Age (years), median (IQR)	52 (46–60)	49 (45–54)
Presumed mode of HIV transmission, n (%)		
Heterosexual	55 (34.8)	180 (22.8)
MSM	48 (30.4)	302 (38.5)
IDU	46 (29.1)	288 (36.6)
other/unknown	9 (5.6)	18 (2.3)
Smoking, n (%)		
Never	58 (37)	184 (23)
Past	26 (16)	157 (20)
Current	74 (47)	447 (57)
pack-years, median (IQR)	9 (0–20)	13 (0–20)
Alcohol ≥3 units per day, n (%)	77 (51)	392 (49)
Injection drug use, n (%)	46 (29)	288 (37)
Weight (kg), median (IQR)	67.5 (59.3–75.3)	68.2 (60.3–77)
Height (cm), median (IQR)	171 (164.2–175)	173 (167–179)
Hepatitis C coinfection, n (%)	42 (26.6)	231 (29.3)
Parent hip fracture, n (%)	35 (22.2)	129 (16.4)
Corticosteroid exposure ≥3 mo, n (%)	17 (11)	25 (3)
Duration of tenofovir disoproxil fumarate treatment (years), median (range)	2.1 (0–9.3)	2.7 (0–9.5)
Duration of protease inhibitor treatment (years), median (range)		
with ritonavir	0.9 (0–14.8)	0.8 (0–15.1)
without ritonavir	4.1 (0–14.9)	3.2 (0–15.7)
$CD4^+$ T-cell count (cells/μL)		
median (range)	442 (27–1943)	512 (5–1888)
nadir (range)	119 (0–740)	141 (0–1075)

Abbreviations: HIV, human immunodeficiency virus; IDU, injection drug use; IQR, interquartile range; MSM, men who have sex with men.

[a] Data are no. (%) of participants, unless otherwise indicated. For cases and controls, values refer to those closest to the fracture date of the corresponding case.

[b] Controls were matched to cases on gender.

Vertebral Versus Nonvertebral Fractures

We also ran the same models considering vertebral or nonvertebral fractures separately (Supplementary Tables 2 and 3). We did not observe any association between genetic score and vertebral or nonvertebral fractures.

Exploratory Genome-Wide Analyses

As expected, we did not observe any association between human genetic variants and LFT in a genome-wide SNP analysis (Supplementary Figure 1). The 61 SNPs mapping to genes associated with bone mineral density in the reference paper [9] were also not associated with LTF risk (data not shown).

Table 2. Contribution of Nongenetic Factors to Risk of Low-trauma Fractures in Univariable and Multivariable Analyses, Odds Ratio (95% Confidence Interval)

Variable	Univariable Analysis	*P* Value	Multivariable Analysis Without Genetic Background	*P* Value
Age, per additional year	1.04 (1.02–1.06)	<.01	1.02 (1.00–1.04)	.06
Current/past smoking	0.98 (0.97–1.00)	.07	0.58 (0.38–0.90)	.02
≥3 units alcohol/day	1.01 (0.99–1.02)	.38	1.11 (0.77–1.62)	.57
Weight, per additional kg	0.99 (0.98–1.04)	.15	1.00 (0.98–1.01)	.81
Height, per additional cm	0.94 (0.91–0.96)	<.01	0.95 (0.92–0.98)	<.01
Hepatitis C coinfection	0.86 (0.58–1.29)	.49	1.16 (0.68–1.96)	.59
Injection drug use	0.68 (0.46–1.01)	.06	0.86 (0.49–1.52)	.60
CD4 nadir	1.00 (1.00–1.00)	.95	1.00 (1.00–1.00)	.26
Cumulative tenofovir exposure, per additional year	0.97 (0.90–1.04)	.35	0.97 (0.90–1.05)	.44
Protease inhibitor (with ritonavir) exposure, per additional year	1.01 (0.95–1.07)	.76	1.04 (0.96–1.12)	.35
Protease inhibitor (without ritonavir) exposure, per additional year	1.04 (0.98–1.09)	.20	1.05 (0.99–1.12)	.13
Parental history of hip fracture	1.46 (0.96–2.23)	.08	1.35 (0.84–2.14)	.21
Corticosteroid exposure ≥3 mo	4.15 (2.08–8.24)	<.01	3.12 (1.48–6.58)	<.01

[a] Current or past smoking vs never smoking.

[b] ≥3 units alcohol/day vs less.

DISCUSSSION

Our study, to our knowledge, represents a first attempt to assess the contribution of human genetic variation to LTF in HIV-positive persons. A genetic risk score, which was defined a priori and captures the joint effect of 6 common SNPs with established LTF association in GWAS in the general population [9], had a negligible impact on LTF risk when considered in the context of established demographic, clinical, and HIV-related risk factors. Possible explanations for this include the relatively young age of our HIV-positive patients and the smaller proportion of women (approximately one third), compared with the reference paper (approximately two thirds) [9].

Our negative genetic results seem robust; first, we restricted the analysis to SNPs with established LTF association [9]; second, all SNPs were analyzed jointly, thereby avoiding false-positive results typically derived from candidate gene studies [26]; third, we performed rigorous quality control of the genotyping data, excluding population outliers and correcting for residual population stratification. Fourth, the genetic score did not capture the established effect of family history; this observation cannot be explained by overfitting, because removing family history from the model did not significantly change the genetic results.

Even though the SHCS population is aging and includes >9000 patients under active follow-up, a limited number of participants have experienced LTF. Therefore, our study was limited by sample size, in contrast to our previous genetic studies of dyslipidemia and diabetes mellitus in HIV in which similar numbers of cases allowed us to capture the effect of common SNPs [27, 28]. Thus, a larger study might have found genetic associations. Because demonstration of the modest effect size of the SNPs assessed in the genetic-LTF reference study in the general population required meta-analysis including >130 000 persons [9], longer follow-up and larger studies in aging HIV-positive populations are important. Our exploratory, genome-wide analyses did not provide any novel insight into the genetics of LTF. This was expected, because the study was designed to assess SNPs with validated LTF association and was not powered for genome-wide discovery of novel variants. Additional limitations include the lack of information on bone mineral density, diet, vitamin D serum levels, physical activity, menopause status, vitamin D supplements, and non-HIV medications other than corticosteroids in our dataset. Finally, no lateral spine x-rays were obtained, which have revealed a significant prevalence of vertebral fractures in HIV-positive persons in Italy [29].

Table 3. Odds Ratio for Low-Trauma Fracture According to Genetic Risk Score Quartile, Multivariable Models With and Without Parental Hip Fracture History[a]

Genetic Risk Score Quartile	Model With Parental History of Hip Fracture	*P* Value	Model Without Parental History of Hip Fracture	*P* Value
Genetic score quartile 2 vs quartile 1	0.12 (0.01–1.63)	.11	0.12 (0.01–1.57)	.11
Genetic score quartile 3 vs quartile 1	0.21 (0.12–3.49)	.28	0.20 (0.01–3.25)	.26
Genetic score quartile 4 vs quartile 1	0.43 (0.03–5.61)	.36	0.42 (0.03–5.22)	.50

[a] Data in parentheses are 95% confidence intervals.

The prevalence of several clinical LTF risk factors noted in previous studies in HIV [3, 6, 30] was similar in cases and controls in our dataset and did not contribute to increased LTF risk, including smoking, injection drug use, and HCV coinfection. Indeed, the prevalence of current smoking, past smoking, and the number of pack-years was each greater in controls than in cases, resulting in lower LTF ORs in smokers than in nonsmokers. This most likely is a chance finding given the robust association of smoking with LTF in large-scale meta-analysis in the general population [23]. The duration of exposure to tenofovir and protease inhibitors did not contribute to LTF risk. This was also in contrast to some large-scale analyses in HIV-positive populations [5] and likely, at least in part, was because we selected only controls who started ART contemporarily with corresponding cases, and because follow-up was censored at the fracture date in both controls and corresponding cases, ensuring similar time under observation (including similar duration of ART exposure).

Strengths of this study are the inclusion of all LTFs that occurred in a large, well established, observational HIV study during a 17-year period. All fractures were confirmed, and their low trauma nature ascertained in writing by the treating physician using a standardized definition, thereby overcoming constraints of registry-based studies [3, 5], and perhaps resulting in lower LTF rates in the SHCS (1.35 LTF per 1000 person-years) than in, eg, the US Veterans Aging Cohort Study (2.6 per 1000 person-years) [6] and in the Women's Interagency HIV Study (5.6 per 1000 person-years) [31].

CONCLUSIONS

In summary, our findings, based on the available, limited number of LTFs that have been recorded in the SHCS, suggest that genetic testing is presently not a useful predictor or monitoring tool for bone health in HIV.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious* Diseases online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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