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Obesity-related SNPs and weight gain following first-line antiretroviral therapy

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Key points

We studied the association of obesity-related SNPs with weight and BMI increase at 96 weeks after ART initiation in treatment-naïve patients. A significant association was found between *ZC3H4* rs3810291 GG and *BCDIN3D/FAIM2* rs7138803 GG genotypes with weight and BMI increase.

Abstract

Background

We studied the association of obesity-related SNPs (OR-SNPs) with weight gain after antiretroviral therapy (ART) in people with HIV (PWH).

Methods

Participants were ART-naïve PWH from the Spanish HIV Research Cohort who started ART from 2014 onwards and had blood/DNA deposited in the cohort Biobank. The primary outcome was change in weight at 96 weeks after starting ART. We genotyped 14 OR-SNPs from a meta-analysis of GWAS body mass index (BMI) loci. Changes over time in weight and BMI were studied using adjusted linear mixed models (A-LMM).

Results

A total of 1,021 PWH were included. The mean weight gain over 96 weeks was 2.90 (95% CI: 2.54 - 3.26) Kg. Factors associated with higher weight gain were female sex, birth in Sub-Saharan Africa, prior AIDS, CD4+ < 200 cells/uL, HIV-RNA > 100,000 copies/mL, negative HCV serology, and use of tenofovir alafenamide. A significant association was found between *ZC3H4* rs3810291 GG genotype and *BCDIN3D/FAIM2* rs7138803 GG genotypes polymorphisms and weight and BMI increase. The estimated adjusted mean (SE) of weight gains were 4.26 (0.56) Kg in *ZC3H4* rs3810291 GG carriers and 2.66 (0.19) Kg in AA/AG carriers ($P=0.007$). Likewise, the estimated means (SE) weight gain at 96 weeks were 3.35 (0.29) Kg in *BCDIN3D/FAIM2* rs7138803 GG carriers and 2.51 (0.24) Kg in AG/AA carriers ($P=0.020$).

Conclusions

Genetic factors may play a role in weight gain after ART initiation. Further work is needed to replicate our findings and understand how the identified SNPs lead to higher weight gain in this context.

Introduction

Obesity is a pandemic that contributes decisively to the increase in morbidity and mortality from cardiovascular disease, diabetes, chronic kidney disease, non-alcoholic steatohepatitis, and cancer [1, 2]. Obesity is caused by an imbalance between income and energy expenditure influenced by environmental, biological, and genetic factors [3, 4].

Overweight and obesity are also growing problems in people living with HIV (PWH), among whom the initiation of combined antiretroviral therapy (ART) is often associated with weight gain [5-11]. The weight gain after the onset of ART can be partly explained by the attenuation of systemic inflammation and accelerated catabolism characteristic of active HIV infection [12] and, in some patients, by the resolution of opportunistic infections and gastrointestinal dysfunction that could adversely affect appetite and nutrient absorption. Other factors associated with higher weight gain after the onset of ART include black race, female gender, high viral load, and low CD4 + lymphocyte count [6-8, 11-14].

In recent randomized clinical trials in ART-naïve PWH, it has been found that weight gain is greater with regimens that include integrase inhibitors (INSTI) (such as dolutegravir [DTG], bictegravir [BIC], and to a lesser extent elvitegravir [EVG]) compared with non-nucleoside reverse transcriptase inhibitors (NNRTI) (such as efavirenz [EFV] and to a lesser extent rilpivirine [RPV]). Similarly, weight gain is greater with regimens that include tenofovir alafenamide (TAF) compared to those that include tenofovir disoproxil fumarate (TDF) or abacavir [15-17]. So far, the mechanisms that explain these differences are unknown. However, TDF has been associated with weight loss in HIV-negative individuals taking preexposure prophylaxis therapy, a finding that could be attributed to some gastrointestinal side effects associated with TDF [18]. The clinical implications of weight gain after ART initiation are not well understood, but it has been associated with an increased risk of diabetes mellitus and cardiovascular disease [9, 19].

Although lifestyle and diet are of paramount importance, genetic factors also play a decisive role and interact with environmental factors to produce obesity [20, 21]. Recent data from genome-wide association studies (GWAS) have shown several loci associated with body mass index (BMI), implicating genes and pathways related to synaptic function, glutamate

signaling, insulin secretion/action, energy metabolism, lipid biology, and adipogenesis [22-25]. These studies estimated that common genetic variations could account for as much as 21% of BMI variation.

To the best of our knowledge, the role of genetic factors in weight gain after ART initiation in treatment naïve PWH has not been analyzed so far. Our objective was to study the association of polymorphisms of genes potentially involved in obesity with weight gain after ART initiation.

Methods

Study Design

Ours was a retrospective observational study using data and samples from PWH included in the Cohort of the Spanish AIDS Research Network (CoRIS), a prospective cohort of PWH older than 13 years, naïve to ART at study entry, seen for the first time from January 1, 2004, in 46 participating centers from 13 of 17 Regions in Spain [26]. The CoRIS database collects demographic and clinical data, HIV transmission category, ART history, previous opportunistic diseases, specific non-AIDS diseases, and serological and immunovirological data. Since 2014, height and weight have been recorded at participant recruitment in the cohort. Weight has also been recorded at follow-up visits. Blood and other biological samples from PWH in CoRIS were collected at recruitment and then yearly and stored in the Spanish HIV BioBank [27]. The Strengthening the Reporting of Genetic Association Studies (STREGA) guidelines were used to ensure the reporting of the study (**Supplementary Table 1**) [28]. The Ethics Committee for Research with Medicines of Hospital General Universitario Gregorio Marañón approved the study (Internal Ref#: RIS EPICLIN 01-2020).

Participants

Eligible participants for this study were PWH who started ART as of January 1, 2014, who had weight and height information within the 24 weeks before the start of ART and at 96 ($\pm$ 24) weeks from the beginning of ART, and who had at least one blood or DNA sample deposited in the CoRIS Biobank.

Variables

Outcomes: The primary outcome variable was a change in weight at 96 weeks after starting ART. Secondary outcomes included a change in BMI and a 10% weight gain.

Exposure variables: We genotyped 14 obesity-related SNPs selected from a meta-analysis of GWS BMI loci [25], see **Supplementary Table 2**. The selection criterion was to have the lowest *P*-value in the GWAS and to be compatible in the multiplex DNA genotyping assay evaluated by "in silico" simulation analysis among the 14 selected SNPs. Several polymorphisms with low *P*-values could not be included in the multiplex genotyping assay because their "in silico" analysis indicated they were incompatible with other selected SNPs.

Adjustment variables: Gender at birth, age, baseline BMI, world region of birth, HIV transmission category, level of education, baseline CD4+ T-cell counts, baseline HIV-RNA load, prior AIDS-defining conditions, serological status against HCV and HBV, ART drug regimen (categorized by type of anchor drug and type of nucleoside reverse transcriptase inhibitor [NRTI] backbone), and underlying comorbidities (diabetes mellitus, arterial hypertension, chronic heart disease, and non-AIDS related cancer).

Laboratory methods: Genomic DNA was extracted from peripheral blood with the Qiagen kit (QIAamp DNA Blood Midi / Maxi; Qiagen, Hilden, Germany). DNA genotyping was performed in the Spanish National Genotyping Center (CeGen; <http://www.cegen.org/>) using the iPLEX® Gold technology and Agena Bioscience's MassARRAY platform (San Diego, CA, USA). All SNPs had a DNA genotyping success rate greater than 95%.

Statistical analysis

To describe the sociodemographic and clinical characteristics of the study population at ART initiation, we used frequency tables for categorical variables and median and interquartile ranges for continuous variables. To describe weight and BMI at baseline and 96 weeks and their differences, we used means and 95% percent confidence intervals. Multivariable linear regression analysis was used to identify baseline characteristics independently associated with weight gain over the 96 weeks study period.

The Hardy-Weinberg equilibrium (HWE) was evaluated by the Chi-square test, considering the equilibrium when $P > 0.05$. For genetic association analysis, adjusted linear or logistic regressions were carried out as appropriate to assess the influence of SNPs on the variables

of interest. Different inheritance models (dominant, recessive, and additive) were tested, selecting the model that best fitted the data.

Unadjusted and adjusted linear mixed models for longitudinal data were used to account for repeated measures of weight and BMI, with SNPs and time, and their interaction was taken as fixed effects and the patient as a random effect. The selection of potential variables for adjustment was based on statistical criteria and the underlying conceptual framework regardless of statistical significance.

Statistical analyses were performed using Stata software (version 15.0; Stata Corporation, College Station, Texas, USA). Least-square means and their standard error (SM) were reported for these models. The level of significance was established at 0.05 (2-tailed).

Results

As of November 30, 2019, data from 16,759 PWH were registered in the CoRIS database, and 1,021 met the study inclusion criteria.

Patients' characteristics

The baseline characteristics of the study participants are shown in **Table 1**. In brief, 88.15% were male, the median age was 35 years, 6.56% were obese, 71.30% were MSM, 59.84% had an upper secondary or university education, and 65.23% were native-born Spaniards. In relation to HIV, 5.88% had had prior AIDS-defining conditions, the median baseline CD4+ T-cell count was 426 cells/uL, 15.57% had < 200 CD4 cells/uL, the median HIV-RNA load was 45,986 copies/mL, and 33.20% had > 100,000 HIV-RNA copies/mL. The most frequent ART regimens were the combination of two nucleoside reverse transcriptase inhibitors (NRTIs) plus one INSTI in 62.98%, 2 NRTIs plus one NNRTI in 17.92%, and 2 NRTIs plus one boosted protease inhibitor (bPI) in 11.75%; the most frequent NRTI backbone was TDF plus emtricitabine (FTC) in 44.27%, followed by abacavir (ABC) plus lamivudine (3TC) in 33.69%, and TAF plus FTC in 15.67%. Hepatitis C virus (HCV) antibodies were present in 5.0%, and hepatitis B virus (HBV) surface antigen (HBsAg) was present in 2.15%. The most frequent comorbid condition was arterial hypertension (14.89%).

Weight and BMI changes

The mean weight of the study population at baseline and 96 weeks were 72.86 and 75.76 Kg, respectively, with a mean increase in weight over the study period of 2.90 Kg, $P < 0.001$ (**Figure 1A**). Likewise, the mean BMI values at baseline and 96 weeks were 24.24 and 25.21 Kg/m², respectively, with a mean increase in BMI over the study period of 0.98 Kg/m², $P < 0.001$ (**Figure 1B**).

Factors independently associated with higher weight gain over the 96 weeks study period by multivariable linear regression analysis were female sex, birth in Sub Saharan African countries, a prior AIDS-defining condition, a CD4⁺ T-cell count < 200 cells/uL, an HIV viral load $> 100,000$ copies/mL, absence of HCV antibodies, and use of TAF/FTC as the NRTI backbone (**Table 2**). Taking normal BMI at baseline as a reference, obesity was independently associated with less weight gain 96 weeks after starting ART.

Association of SNPs with weight and BMI changes

By unadjusted linear mixed model analysis, with weight and BMI increases over the 96 weeks as the outcome, a significant interaction was identified for two of the 14 obesity-related SNPs analyzed: *ZC3H4* (Zinc Finger CCCH-Type Containing 4) rs3810291 (recessive model) and *BCDIN3D* (*BCDIN3* Domain Containing RNA Methyltransferase)/*FAIM2* (Fas Apoptotic Inhibitory Molecule 2) rs7138803 (dominant model). No significant effect was found for the interaction of the remaining 12 obesity-related SNPs on the time-course of weight and BMI (**Supplementary Table 3**). Regarding genetic diversity based on the ethnic group or origin, Locke et al. [25] observed that *ZC3H4* rs3810291 and *BCDIN3D/FAIM2* rs7138803 polymorphisms had comparable effects across ancestries (European, African American, and Asian). Thus, we analyzed patients from different regions of birth together.

The results of linear mixed models adjusted by age, sex, BMI at baseline, country of birth, prior AIDS-defining conditions, CD4⁺ cell count, HIV-RNA viral load, type of ART regimen according to anchor drug, and NRTI backbone are shown in **Figure 2**. The estimated adjusted mean (standard error [SE]) of weight gains at 96 weeks were 4.26 (0.56) Kg in *ZC3H4* rs3810291 GG carriers and 2.66 (0.19) Kg in AA/AG carriers ($P = 0.007$; **Figure 2A**). Likewise, the estimated means (SE) weight gain at 96 weeks were 3.35 (0.29) Kg in *BCDIN3D/FAIM2* rs7138803 GG carriers and 2.51 (0.24) Kg in AG/AA carriers ($P = 0.020$; **Figure 2B**). Moreover, the estimated adjusted mean (SE) of BMI gains at 96 weeks were 1.41 (0.19) Kg/m² in *ZC3H4*

rs3810291 GG carriers and 0.91 (0.07) Kg/m² in AA/AG carriers ($P=0.010$; **Figure 2C**). Likewise, the estimated mean (SE) BMI gains at 96 weeks were 1.15 (0.10) Kg/m² in *BCDIN3D/FAIM2* rs7138803 GG carriers and 0.84 (0.08) Kg/m² in AG/AA carriers ($P=0.014$; **Figure 2D**).

A statistically significantly higher frequency of weight gain >10% was found among individuals with *ZC3H4* rs3810291 GG genotype in comparison with those with AA/AG genotypes: 37/163 (22.7%) vs. 133/856 (15.5%), respectively ($P=0.029$). However, no statistically significant difference in weight gain >10% was found between *BCDIN3D/FAIM2* rs7138803 GG and AG/AA carriers (90/494 (18.2%) vs. 80/524 (15.3%), respectively ($P=0.209$).

Discussion

We studied the association of DNA polymorphisms of genes potentially involved in obesity with weight gain following ART in 1,021 treatment naïve PWH recruited in CoRIS since 2014. At 96 weeks after ART initiation, the mean weight increase was 2.90 Kg. Out of 14 obesity-related SNPs analyzed, a significant association was found between *ZC3H4* rs3810291 GG and *BCDIN3D/FAIM2* rs7138803 GG genotypes with weight and BMI increase.

To detect any published study analyzing the contribution of genetics to weight gain after initiation of ART in PWH, we searched PubMed and EMBASE up to August 6, 2022, using the terms: (HIV OR AIDS) (ANTIRETROVIRAL THERAPY OR ANTIRETROVIRALS) (WEIGHT OR OBESITY OR BMI) (SNPs OR (Single Nucleotide Polymorphism)) with no language restrictions. Out of 78 manuscripts retrieved with this search, no one analyzed the role of genetic factors in weight gain after starting ART.

In a substudy of the ADVANCE trial, which compared DTG plus either TDF or TAF with EFV and FTC plus TDF in PWH who had not received ART in the prior six months, it was found that CYP2B6 metabolizer genotype was strongly associated with weight gain over 48 weeks in the EFV arm [29]. Researchers found that extensive metabolizers gained the most weight, whereas slow metabolizers lost weight. Of note, CYP2B6 extensive metabolizers in the EFV arm had similar weight gain to participants in the DTG arm. The authors concluded that the more significant weight gain documented on DTG-based ART compared with EFV-based ART

could be due to impaired weight gain in PWH with CYP2B6 intermediate or slow metabolizer genotypes, who presumably had increased EFV concentrations causing toxicity, rather than off-target effects of DTG affecting appetite or metabolism [29].

We found that individuals with *ZC3H4* rs3810291 GG genotype (16.0% of the population studied) had significantly higher adjusted weight and BMI gain than those with AA/AG genotype. We also found an association between *ZC3H4* rs3810291 GG genotype and weight gain >10%. *ZC3H4* is a protein of unknown function, and the *ZC3H4* rs3810291 polymorphism is located in the 3' untranslated region of the *ZC3H4* gene, probably affecting the posttranscriptional regulation [30, 31]. *ZC3H4* rs3810291 A allele has been associated with BMI in the general population [23]. Of note, in a recent study that examined the effect of 32 confirmed genetic loci for obesity on eating behaviors in US men and women, variants in/near *ZC3H4* were found to be positively associated with emotional eating and uncontrolled eating, suggesting that its link to obesity may, in part, be mediated by eating behaviors [32]. This is of interest as many documented genetic loci for obesity are expressed or known to operate in the brain, particularly in the hypothalamus, underscoring a probable neuronal element to the predisposition to obesity by regulating energy balance, appetite, and reward-seeking behavior [33, 34].

We also found an association between the *BCDIN3D/FAIM2* rs7138803 GG genotype (48.5 % of the population studied) and higher weight gain and BMI after ART initiation. *BCDIN3D/FAIM2* rs7138803 is in an intergenic region in chromosome 12, upstream of the *BCDIN3D* gene and downstream of the *FAIM2*, and large-scale GWAS have identified associations of rs7138803 with BMI and obesity [22, 23]. The mechanisms through which *BCDIN3D/FAIM2* rs7138803 lead to increased adiposity are poorly understood.

To further explore the relationship between *ZC3H4* rs3810291 and *BCDIN3D/FAIM2* rs7138803 variants and gene expression in human tissues, we used the GTEx (Genotype-Tissue Expression) Portal [35]. We found that rs3810291 polymorphism may contribute to the expression of *SUMO-Activating Enzyme Subunit 1 (SAE1)*, a regulatory gene in metabolic homeostasis and obesity [36]. Likewise, we found an association between rs7138803 A allele with higher expression of *C1QL4/CTRP11* (Complement C1q Like 4/C1q/Tumor Necrosis

Factor-related Protein 11) in the thyroid [35]. In animal models, *C1QL4/CTRP11* expression has been found to modulate food intake, energy expenditure, and insulin sensitivity [37].

We did not find an association between rs1558902 in the fat mass and obesity-associated (*FTO*) gene and weight gain after ART initiation. The *FTO* gene is the one that has a more potent genetic association with obesity. It is located on chromosome 16 and encodes a dioxygenase dependent on alpha-ketoglutarate. It is essential in managing energy homeostasis and regulating body weight by increasing energy intake and reducing satiety [38, 39]. In our population, 35.2% of individuals were homozygous for the *FTO* rs9939609 polymorphism risk allele (AA genotype), a figure concordant with the finding found approximately in 39% of European adults [38]. We also did not find an association between the polymorphism rs6567160, near melanocortin 4 receptor (*MC4R*) gene, and weight gain after ART initiation; this gene with a strong genetic association with obesity plays an essential role in the regulation of food intake, energy expenditure and catabolism [24, 25].

Our study is limited because data were not adjusted by multiple comparisons. However, we did not do a random search for a meaningful result. On the contrary, we carried out a clinical-orientated study with hypotheses grounded on theory and multiple previous reports that supported the selection of obesity-related SNPs. In this situation, adjusting the "P-value" after multiple tests may penalize relevant results [40, 41]. Besides, we do not think the association between SNPs and weight gain could be a false positive since the distribution of patients in each category of the outcome variables, according to genotypes, showed a prominent "effect size." Another limitation is that rs3810291 and rs7138803 are in high linkage disequilibrium with other different SNPs, and we cannot rule out that these SNPs could be the causal polymorphism. Finally, our work is also limited by the lack of information about diet and exercise.

Our study has several strengths, including the inclusion of participants recruited in a prospective cohort of ART-naïve PWH with high-quality data in whom the factors independently associated with weight gain over the study period were concordant with what has been found in recent clinical trials. Besides, the frequency of the *FTO* rs1558902 risk allele among our population is also concordant with what has been found among European adults, which bespeaks the quality of the genetic analysis.

In conclusion, our findings suggest that genetic factors play a role in weight gain after ART initiation; notwithstanding, further work is needed for replication studies to confirm our results and eventually to understand how the polymorphisms in/near ZC3H4 and BCDIN3D/FAIM2 could lead to higher weight gain in this clinical context. Conceivably, individual obesity-related SNPs could help to develop risk scores for predicting overweight and obesity and their related diseases and using them to make clinical decisions when selecting antiretroviral regimens.

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Conflict of interests

Juan Berenguer reports honoraria for advice or public speaking from GILEAD, Glaxo Smith Kline (GSK), JANSSEN, MSD, and ViiV Healthcare; and grants from GILEAD, MSD, and ViiV Healthcare. Inmaculada Jarrín reports honoraria for advice or public speaking from GILEAD, and ViiV Healthcare; and grants from MSD. Juan González-García reports honoraria for advice or public speaking from GILEAD, JANSSEN, MSD, and ViiV Healthcare. David Dalmau reports honoraria for public speaking from GILEAD, JANSSEN, and ViiV Healthcare. Julián Olalla reports honoraria for advice or public speaking from GILEAD, JANSSEN, MSD, and ViiV Healthcare; and grants from GILEAD, MSD, and ViiV Healthcare. Pere Domingo reports honoraria for advice, public speaking or grants from AbbVie, Boehringer-Ingelheim, Bristol-Myers Squibb, GILEAD, Glaxo Smith Kline (GSK), MSD, JANSSEN, Pfizer, Theratechnologies, and ViiV Healthcare. José M Bellón, Cristina Díez, María A Jiménez-Sousa, Cristina Roca, David Dalmau, Carmen Herrero, Francesc Villarroya, and Salvador Resino have nothing to disclose.

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Figure captions

Figure 1

Violin plots showing the baseline and 96 weeks distribution and mean values of weight (A) and BMI (B).

Figure 2

Estimated means (SE) of weight and BMI at baseline and 96 weeks according to genotypes in *ZC3H4* rs3810291 (A, C) and *BCDIN3D/FAIM2* rs7138803 (B, D). Adjusted linear mixed models for longitudinal data accounted for repeated measures with SNPs and time as fixed effects and the patient as a random effect. *P* values refer to the significance of the interaction (i.e., the impact of SNPs on the time-course of the variable). Variables for adjustment were age, sex, BMI at baseline, country of birth, prior AIDS-defining conditions, CD4+ cell count, HIV-RNA viral load, type of ART regimen according to anchor drug, and NRTI backbone.

The genotype frequencies for the *ZC3H4* rs3810291 SNP were 84.0% (856/1019) for AA/AG and 16.0% (163/1019) for GG genotype. Likewise, the genotype frequencies for the *BCDIN3D/FAIM2* rs7138803 SNP were 51.5% (524/1018) for AG/AA and 48.5% (494/1018) for GG genotype; one individual could not be genotyped for *BCDIN3D/FAIM2* rs7138803 SNP.

Table 1. Baseline characteristics of 1,021 study participants

Characteristic	Total (No.=1,021)
Demographics	
Male Sex - No. (%)	900 (88.15)
Age Median (IQR) - yr.	35.76 (29.59 - 43.23)
BMI at baseline	
< 18.5 (low weight)	41 (4.02)
18.5 - 24.9 (normal weight)	626 (61.31)
25 - 29.9 (overweight)	287 (28.11)
≥ 30 (obesity)	67 (6.56)
HIV transmission mechanism - No. (%)	
Men who have sex with men	728 (71.30)
Heterosexual sex	239 (23.41)
Injection drug use	25 (2.45)
Other/Unknown	29 (2.84)
Level of education - No. (%)	
None/Compulsory	219 (21.45)
Upper secondary/University	611 (59.84)
Other/Unknown	191 (18.71)
Region of birth - No. (%)	
Spain	666 (65.23)
Western Europe	24 (2.35)
Eastern Europe	22 (2.15)
Sub Saharan Africa	30 (2.94)
Northern Africa	14 (1.37)
Latin America	259 (25.37)
Other	5 (0.49)
Unknown	1 (0.10)
HIV data at baseline	
Prior AIDS defining conditions - No. (%)	60 (5.88)
CD4+ cell count Median (IQR), cells/uL	426 (274 - 591)
CD4+ cell count categories - No. (%)	
< 200 cells/uL	159 (15.57)
200 - 349 cells/uL	214 (20.96)
350 - 499 cells/uL	242 (23.70)
≥ 500 cells/uL	388 (38.00)
Unknown	18 (1.76)
HIV RNA Median (IQR), copies/mL	45,986 (14,900 - 160,216)
HIV RNA copies/mL - No. (%)	
< 10,000	193 (18.90)
10,000 - 100,000	467 (45.74)
> 100,000	339 (33.20)

Unknown	22 (2.15)
Antiretroviral therapy	
Regimen according to anchor drug - No. (%)	
2 NRTI + 1 INSTI	643 (62.98)
2 NRTI + 1 NNRTI	183 (17.92)
2 NRTI + 1 bPI	120 (11.75)
Other regimens	21 (2.06)
Unknown	54 (5.29)
Type of NRTI backbone - No. (%)	
TDF/FTC	452 (44.27)
ABC/3TC	344 (33.69)
TAF/FTC	160 (15.67)
Other	11 (1.08)
Unknown	54 (5.29)
Hepatitis C virus antibodies	
Negative	698 (68.36)
Positive	51 (5.00)
Unknown	272 (26.64)
Hepatitis B surface antigen	
Negative	754 (73.85)
Positive	22 (2.15)
Unknown	245 (24.00)
Comorbid conditions - No./with data (%)	
Arterial hypertension	152 (14.89)
Non-AIDS non-liver related cancer (active)	11 (1.08)
Cardiovascular disease (ischemic/heart failure)	6 (0.59)
Diabetes mellitus	6 (0.59)
Chronic renal failure	2 (0.20)

Abbreviations. IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; bPI, boosted protease inhibitor; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; ABC, abacavir; 3TC, lamivudine; TAF, tenofovir alafenamide.

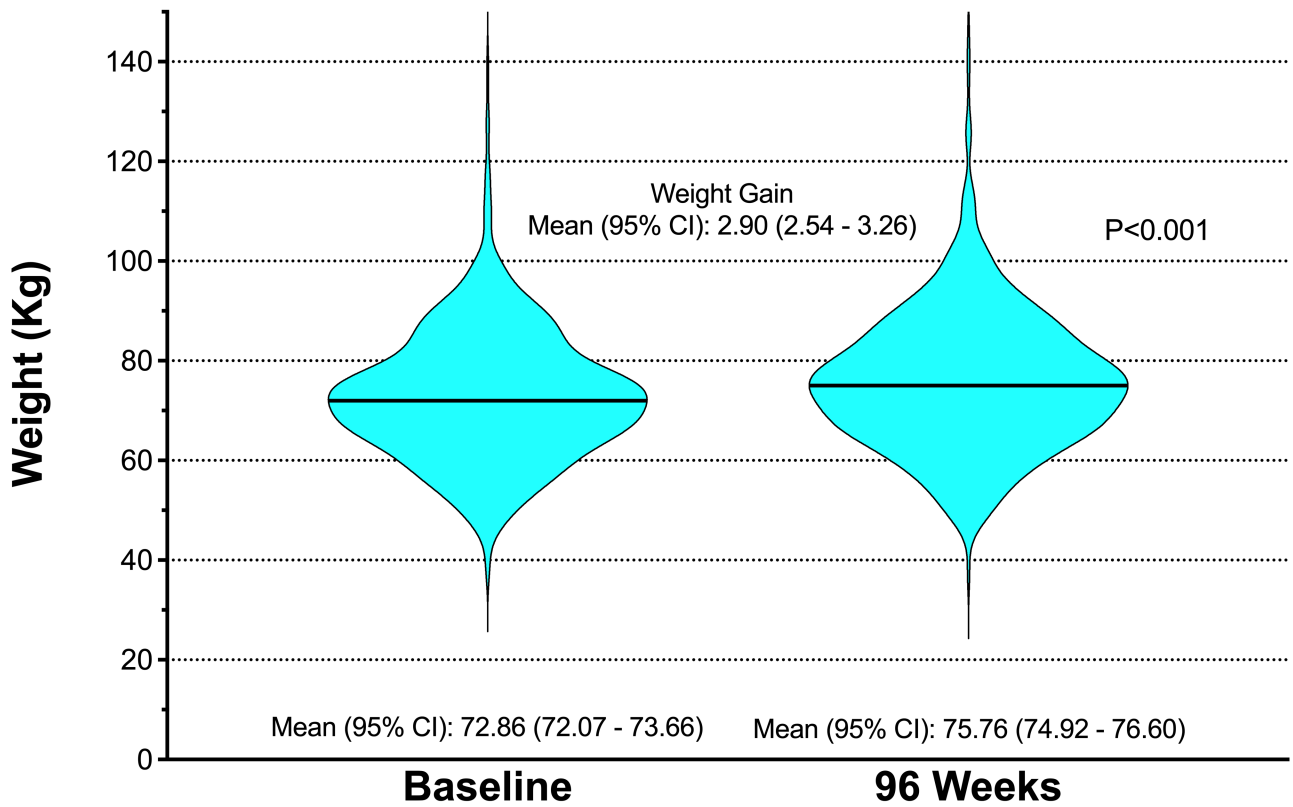
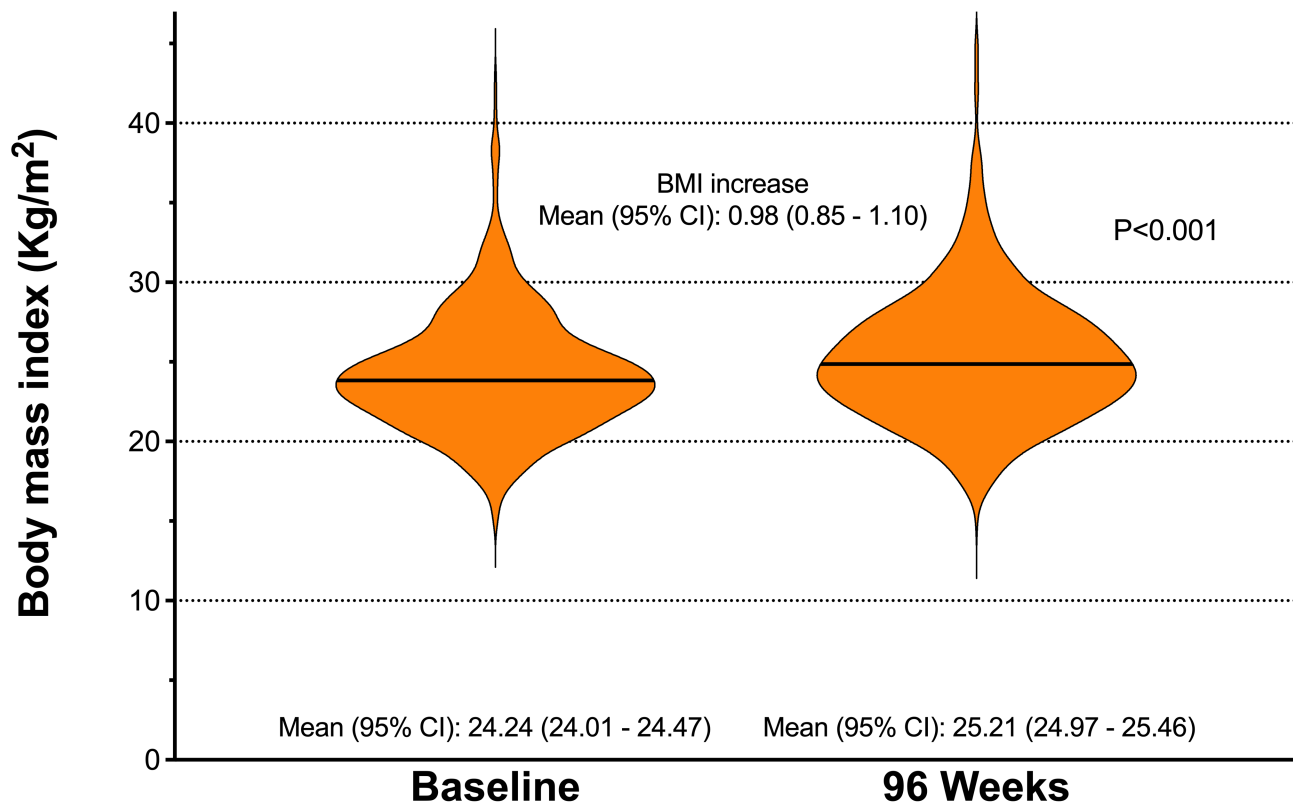
Table 2. Factors associated with weight gain at 96 weeks after the initiation of antiretroviral therapy by multivariable linear regression analysis

	Mean difference in weight gain (kg)	Lower bound 95% CI	Upper bound 95% CI	P value
Gender				
Male	Ref.			
Female	1.339	0.058	2.620	0.041
Age - years				
<30	Ref.			
30-39	-0.046	-0.886	0.795	0.915
40-49	-0.431	-1.413	0.550	0.388
50-59	0.353	-1.007	1.713	0.610
≥60	-0.164	-2.637	2.308	0.896
BMI at baseline				
< 18.5 (low weight)	0.660	-1.029	2.349	0.443
18.5 - 24.9 (normal weight)	Ref.			
25 - 29.9 (overweight)	0.104	-0.670	0.870	0.792
≥ 30 (obesity)	-1.692	-3.083	-0.302	0.017
HIV transmission mechanism				
Men who have sex with men	Ref.			
Injection drug use	1.577	-0.940	4.093	0.219
Heterosexual sex	-0.187	-1.214	0.840	0.721
Other/Unknown	-0.143	-2.178	1.892	0.890
Level of education				
None/Compulsory	Ref.			
Upper secondary/University	0.286	-0.575	1.147	0.515
Other/Unknown	0.248	-0.803	1.300	0.643
Region of birth				
Spain	Ref.			
Western Europe	0.222	-2.004	2.448	0.845
Eastern Europe	-0.495	-2.793	1.803	0.673
Sub Saharan Africa	2.684	0.609	4.758	0.011

Northern Africa	-1.007	-3.839	1.824	0.485
Latin America	0.231	-0.564	1.026	0.568
Other	0.649	-4.065	5.363	0.787
Unknown	4.250	-6.732	15.232	0.448
Prior AIDS defining conditions				
No	Ref.			
Yes	4.164	2.586	5.741	<0.001
CD4+ cells/uL				
< 200	Ref.			
200 - 349	-3.593	-4.804	-2.383	<0.001
350 - 499	-3.640	-4.876	-2.404	<0.001
≥ 500	-3.514	-4.703	-2.326	<0.001
Unknown	-0.247	-3.871	3.378	0.894
HIV RNA copies/mL				
< 10,000	Ref.			
10,000 - 100,000	0.286	-0.643	1.215	0.546
> 100,000	1.346	0.310	2.382	0.011
Unknown	-1.266	-4.495	1.964	0.442
Hepatitis C virus antibodies				
Negative	Ref.			
Positive	-2.060	-3.828	-0.291	0.023
Unknown	0.414	-0.470	1.298	0.358
Hepatitis B surface antigen				
Negative	Ref.			
Positive	-2.166	-4.460	0.128	0.064
Unknown	-0.736	-1.659	0.186	0.118
ART type of regimen				
2 NRTI + 1 NNRTI	Ref.			
2 NRTI + 1 bPI	-0.131	-1.394	1.131	0.838
2 NRTI + 1 INSTI	0.490	-0.523	1.503	0.343

Other regimens	0.241	-2.984	3.467	0.883
Unknown	0.345	-1.283	1.972	0.678
ART type of NRTI backbone				
TDF/FTC	Ref.			
TAF/FTC	1.076	0.018	2.135	0.046
ABC/3TC	-0.668	-1.538	0.202	0.132
Other	0.470	-3.869	4.810	0.832
Unknown	-			
Comorbid conditions				
Diabetes mellitus				
No	Ref.			
Yes	-2.218	-6.785	2.349	0.341
Arterial hypertension				
No	Ref.			
Yes	-0.485	-1.444	0.473	0.321
Cardiovascular disease				
No	Ref.			
Yes	-1.375	-5.991	3.240	0.559
NANL-related cancer				
No	Ref.			
Yes	-1.521	-4.708	1.667	0.349

Abbreviations. ART. antiretroviral therapy; NRTI. nucleoside reverse transcriptase inhibitor; NNRTI. non-nucleoside reverse transcriptase inhibitor; bPI. boosted protease inhibitor; INSTI. integrase strand transfer inhibitor; TDF. tenofovir disoproxil fumarate; FTC. emtricitabine; ABC. abacavir; 3TC. lamivudine; TAF. tenofovir alafenamide; NANL. non-AIDS non-liver.

A**B**

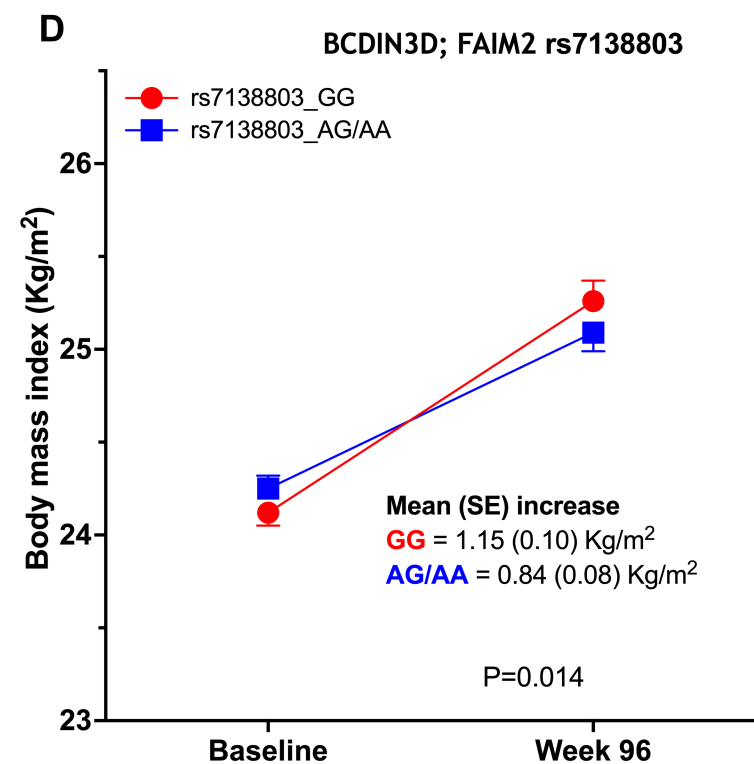
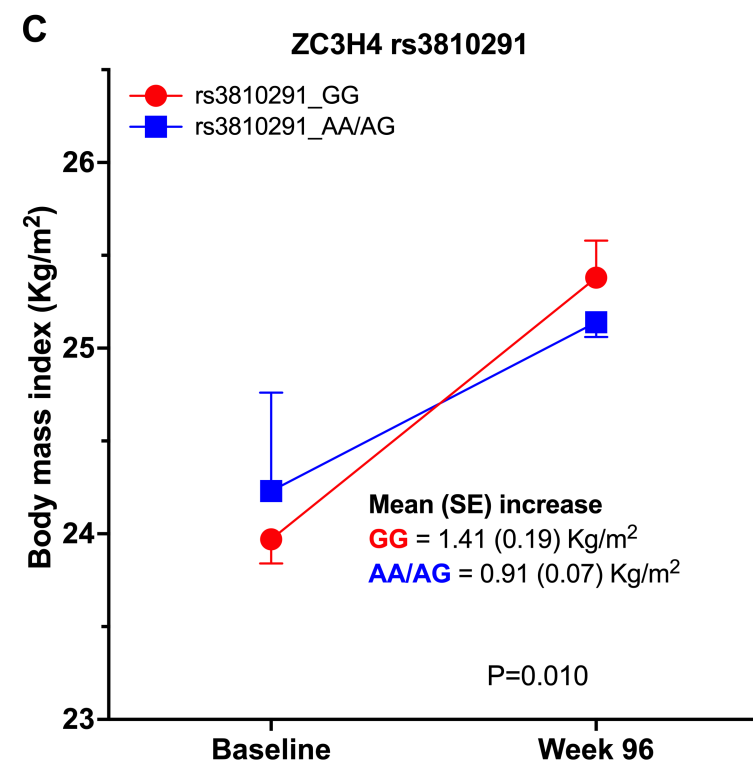
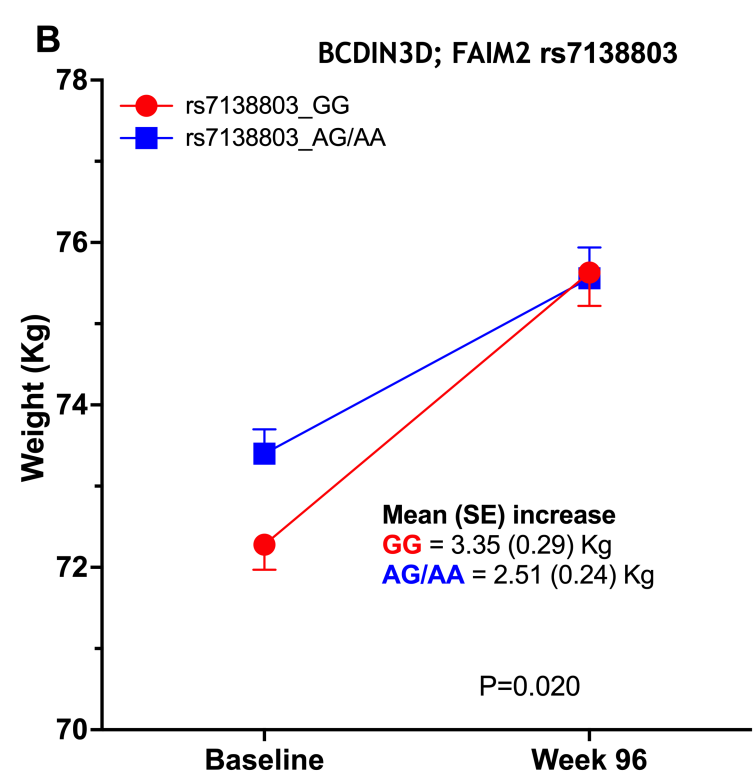
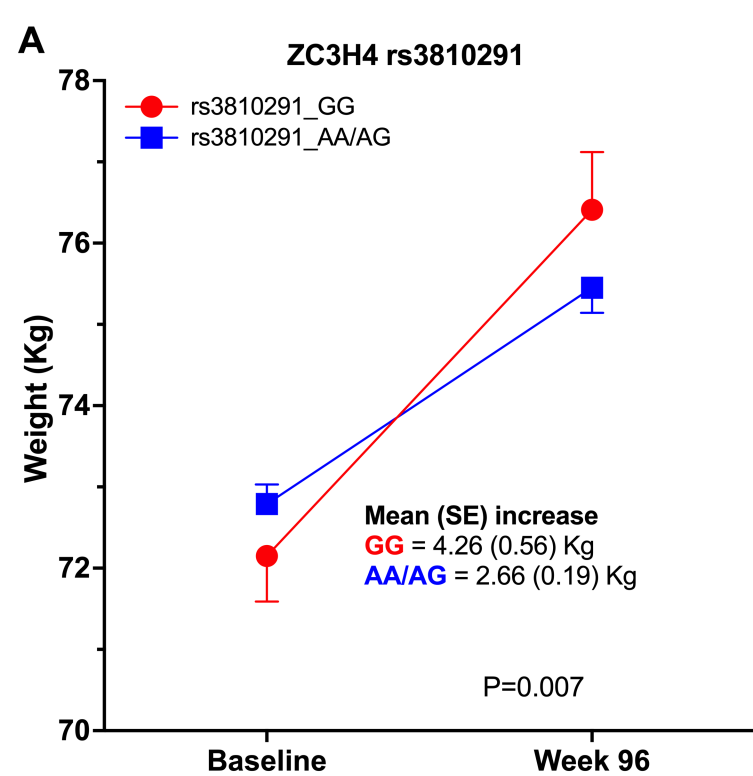


Table S1. STrengthening the REporting of Genetic Association Studies (STREGA) Reporting Recommendations, Extended from the STROBE Statement (Extension for Genetic Association Studies highlighted in yellow). Little J, et al. Ann Intern Med 2009; 150(3): 206-15.

	Item No.	Recommendation	Relevant text from manuscript
Title & abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	Obesity-related SNPs and weight gain following first-line antiretroviral therapy
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	We studied the association of obesity-related single nucleotide polymorphisms (SNPs) with weight gain after antiretroviral therapy (ART) in people with HIV (PWH).
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Overweight and obesity are also growing problems in people living with HIV (PWH), among whom the initiation of combined antiretroviral therapy (ART) is often associated with weight gain.
Objectives	3	State specific objectives, including any prespecified hypotheses	Our objective was to study the association of polymorphisms of genes potentially involved in obesity with weight gain after ART initiation.
		State if the study is the first report of a genetic association, a replication effort, or both.	To the best of our knowledge, the role of genetic factors in weight gain after ART initiation in treatment naïve PWH has not been analyzed so far.
Methods			
Study design	4	Present key elements of study design early in the paper	Ours was a retrospective observational study using data and samples from PWH included in The Cohort of the Spanish AIDS Research Network (CoRIS).
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	 a prospective cohort of PWH older than 13 years, naïve to ART at study entry, seen for the first time from January 1, 2004, in 46 participating centers from 13 of 17 Regions in Spain. The CoRIS database collects demographic and clinical data, HIV transmission category, ART history, previous opportunistic diseases, specific non-AIDS diseases, and serological and immunovirological data. Since 2014, height and weight have been recorded at participant recruitment in the cohort. Weight has also been recorded at follow-up visits. Blood and other biological samples from PWH in CoRIS were collected at recruitment and then yearly and stored in the Spanish HIV BioBank
Participants	6	(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Eligible participants for this study were PWH who started ART as of January 1, 2014, who had weight and height information within the 24 weeks before the start of ART and at 96 (± 24 weeks) from the beginning of ART, and who had at least one blood or DNA sample deposited in the CoRIS Biobank.
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed	NA
		Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.	Eligible participants for this study were PWH who have started ART as of January 1 st . 2014, who have weight and height information within the 24 weeks prior to the start of ART and at 96 (± 24 weeks) from the beginning of ART, and who have at least one blood or DNA sample deposited in the CoRIS Biobank.
Variables	7	(a) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.	Outcomes: The primary outcome variable was a change in weight at 96 weeks after starting ART. Secondary outcomes included a change in BMI and a 10% weight gain.
		(b) Clearly define genetic exposures (genetic variants) using a widely used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).	We genotyped 14 obesity-related SNPs selected from a meta-analysis of GWs BMI loci ²⁵ , see Supplementary Table 2. The selection criterion was to have the lowest <i>P</i> -value in the GWAS and to be compatible in the DNA genotyping assay evaluated by "in silico" simulation analysis among the 14 selected SNPs.
Data sources/measurement	8*	(a) For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group.	See Item n° 5 above

		(b) Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele-calling algorithm used, and its version), error rates, and call rates. State the laboratory/center where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.	Genomic DNA was extracted from peripheral blood with the Qiagen kit (QIAamp DNA Blood Midi / Maxi; Qiagen, Hilden, Germany). DNA genotyping was performed in the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) using the iPLEX® Gold technology and Agena Bioscience's MassARRAY platform (San Diego, CA, USA). All SNPs had a DNA genotyping success rate greater than 95%.
Bias	9	(a) Describe any efforts to address potential sources of bias.	Adjustment variables: Gender at birth, age, baseline BMI, world region of birth, HIV transmission category, level of education, baseline CD4+ T-cell counts, baseline HIV-RNA load, prior AIDS-defining conditions, serological status against HCV and HBV, ART drug regimen (categorized by type of anchor drug and type of nucleoside reverse transcriptase inhibitor [NRTI] backbone), and underlying comorbidities (diabetes mellitus, arterial hypertension, chronic heart disease, and non-AIDS related cancer).
		(b) For quantitative outcome variables, specify if any investigation of potential bias resulting from pharmacotherapy was undertaken. If relevant, describe the nature and magnitude of the potential bias, and explain what approach was used to deal with this.	NA
Study size	10	Explain how the study size was arrived at	As of November 30, 2019, data from 16,759 PWH were registered in the CoRIS database, and 1,021 met the study inclusion criteria.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	To describe the sociodemographic and clinical characteristics of the study population at ART initiation, we used frequency tables for categorical variables and median and interquartile ranges for continuous variables. To describe weight and BMI at baseline and 96 weeks and their differences, we used means and 95% percent confidence intervals.
		If applicable, describe how effects of treatment were dealt with.	NA
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Multivariable linear regression analysis was used to identify baseline characteristics independently associated with weight gain over the 96 weeks study period. Unadjusted and adjusted linear mixed models for longitudinal data were used to account for repeated measures of weight and BMI, with SNPs and time, and their interaction was taken as fixed effects and the patient as a random effect. The selection of potential variables for adjustment was based on statistical criteria and the underlying conceptual framework regardless of statistical significance.
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	NA
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
		(f) State whether Hardy-Weinberg equilibrium was considered and, if so, how.	The Hardy-Weinberg equilibrium (HWE) was evaluated by the Chi-square test, considering the equilibrium when $P > 0.05$.
		(g) Describe any methods used for inferring genotypes or haplotypes.	For genetic association analysis, adjusted linear or logistic regressions were carried out as appropriate to assess the influence of SNPs on the variables of interest. Different inheritance models (dominant, recessive, and additive) were tested, selecting the model that best fitted the data.
		(h) Describe any methods used to assess or address population stratification.	Regarding genetic diversity based on the ethnic group or origin, Locke et al. ²⁵ observed that <i>ZC3H4</i> rs3810291 and <i>BCDIN3D/FAIM2</i> rs7138803 polymorphisms had comparable effects across ancestries (European, African American, and Asian). Thus, we analyzed patients from different regions of birth together.
		(i) Describe any methods used to address multiple comparisons or to control risk of false-positive findings.	See limitations in the discussion section
		(j) Describe any methods used to address and correct for relatedness among subjects.	NA
		State software version used, and options (or settings) chosen	Statistical analyses were performed using Stata software (version 15.0; Stata Corporation, College Station, Texas, USA).

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	As of November 30, 2019, data from 16,759 PWH were registered in the CoRIS database, and 1,021 met the study inclusion criteria.
		Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.	As of November 30, 2019, data from 16,759 PWH were registered in the CoRIS database, and 1,021 met the study inclusion criteria.
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	See Table 1 and related text in the results section.
		Consider giving information by genotype.	See Table S3 (Supplementary material) and related text in the results section.
		(b) Indicate number of participants with missing data for each variable of interest	See Table 1 and related text in the results section.
		(c) Cohort study—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Cohort study—Report numbers of outcome events or summary measures over time	See Figure 1 and related text in the results section.
		Report outcomes (phenotypes) for each genotype category over time.	See Figure 2 and related text in the results section.
		Report numbers in each genotype category.	Table S3 (Supplementary material) and related text in the results section.
		Report outcomes (phenotypes) for each genotype category.	See Figure 2 and related text in the results section.
		(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
Main results	16	(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
		(d) Report results of any adjustments for multiple comparisons.	See limitations in the discussion section
		(a) Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
		Other analyses	17
(c) If detailed results are available elsewhere, state how they can be accessed.	NA		
Summarize key results with reference to study objectives	See the first paragraph of the discussion		
Discussion			
Key results	18	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	See paragraphs 2 to 5 of the discussion
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	Antepenultimate paragraph of the discussion
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	Penultimate paragraph of the discussion
Generalizability	21	Discuss the generalizability (external validity) of the study results	Last paragraph of the discussion
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	See acknowledgements

Table S2. Obesity-related SNPs genotyped selected from a meta-analysis of GWS BMI loci in European people*

SNP	Chromosome:Position (GRCh38)	Notable gene(s)	Alleles	P value
rs1558902	16:53,769,662	<i>FTO</i>	A/T	7.51E-153
rs6567160	18:60,161,902	<i>MC4R</i>	C/T	3.93E-53
rs13021737	2:632,348	<i>TMEM18</i>	G/A	1.11E-50
rs10938397	4:45,180,510	<i>GNPDA2; GABRG1</i>	G/A	3.21E-38
rs543874	1:177,920,345	<i>SEC16B</i>	G/A	2.62E-35
rs2207139	6:50,877,777	<i>TFAP2B</i>	G/A	4.13E-29
rs11030104	11:27,662,970	<i>BDAF</i>	A/G	5.56E-28
rs3101336	1:72,285,502	<i>NEGR1</i>	C/T	2.66E-26
rs7138803	12:49,853,685	<i>BCDIN3D; FAIM2 (D)</i>	A/G	8.15E-24
rs3888190	16:28,878,165	<i>ATXN2L; SBK1; SULT1A2; TUFM</i>	A/C	3.14E-23
rs12446632	16:19,924,067	<i>GPRC5B; IQCK</i>	G/A	1.48E-18
rs2112347	5:75,719,417	<i>POC5; HMGCR; COL4A3BP</i>	T/G	6.19E-17
rs3810291	19:47,065,746	<i>ZC3H4</i>	A/G	4.81E-15
rs12429545	13:53,528,071	<i>OLFM4</i>	A/G	1.09E-12

*Locke AE, et al. Nature **2015**; 518(7538): 197-206. Chromosome and position data were updated according to the GRCh38 (Genome Reference Consortium Human Build 38) assembly.

Table S3. Distribution of polymorphisms among the study population for the 14 obesity-related SNPs and the association with weight and BMI changes by unadjusted linear mixed model analysis.

Gene	SNP	Alleles (%)		HW (<i>P</i>)	Weight (<i>P</i> -value)			BMI (<i>P</i> -value)		
					Dominant	Recessive	Additive	Dominant	Recessive	Additive
<i>FTO</i>	rs1558902	T (64.8)	A (35.2)	0.063	0.086	0.629	0.221	0.063	0.545	0.174
<i>MC4R</i>	rs6567160	T (83.2)	C (16.8)	0.946	0.946	0.851	0.977	0.928	0.947	0.992
<i>TMEM18</i>	rs13021737	G (84.4)	A (15.7)	0.718	0.875	0.163	0.322	0.943	0.140	0.297
<i>GNPDA2; GABRG1</i>	rs10938397	A (59.4)	G (40.6)	0.253	0.951	0.430	0.694	0.946	0.360	0.639
<i>SEC16B</i>	rs543874	A (81.8)	G (18.2)	0.130	0.262	0.378	0.171	0.270	0.396	0.185
<i>TFAP2B</i>	rs2207139	A (82.0)	G (18.0)	0.005	0.679	0.392	0.506	0.745	0.333	0.475
<i>BDNF</i>	rs11030104	A (80.6)	G (19.4)	0.001	0.264	0.316	0.125	0.224	0.378	0.128
<i>NEGR1</i>	rs3101336	C (64.4)	T (35.6)	0.789	0.398	0.452	0.616	0.357	0.383	0.544
<i>BCDIN3D; FAIM2</i>	rs7138803	G (68.4)	A (31.6)	0.011	0.018	0.125	0.046	0.016	0.146	0.046
<i>ATXN2L; SBK1; SULT1A2; TUFM</i>	rs3888190	C (61.9)	A (38.1)	0.123	0.197	0.242	0.140	0.144	0.227	0.101
<i>GPRC5B; IQCK</i>	rs12446632	G (86.4)	A (13.6)	0.826	0.507	0.475	0.671	0.603	0.556	0.770
<i>POC5; HMGCR; COL4A3BP</i>	rs2112347	T (63.3)	G (36.7)	0.187	0.537	0.310	0.366	0.608	0.261	0.351
<i>ZC3H4</i>	rs3810291	A (61.5)	G (38.5)	0.106	0.103	0.008	0.026	0.109	0.009	0.029
<i>OLFM4</i>	rs12429545	G (84.7)	A (15.3)	<0.001	0.284	0.464	0.519	0.302	0.457	0.536

Abbreviations: **SNP**, single nucleotide polymorphism; **HW (*P*)**; *P*-value Hardy-Weinberg equilibrium. ***FTO***, Fat Mass and Obesity Associated; ***MC4R***, Melanocortin 4 Receptor; ***TMEM18***, Transmembrane Protein 18; ***GNPDA2***, Glucosamine-6-Phosphate Deaminase 2; ***GABRG1***, Gamma-Aminobutyric Acid Type A Receptor Subunit Gamma1, ***SEC16B***, SEC16 Homolog B Endoplasmic Reticulum Export Factor; ***TFAP2B***, Transcription Factor AP-2 Beta; ***BDNF***, Brain Derived Neurotrophic Factor; ***NEGR1***, Neuronal Growth Regulator 1; ***BCDIN3D***, BCDIN3 Domain Containing RNA Methyltransferase; ***FAIM2***, Fas Apoptotic Inhibitory Molecule 2; ***ATXN2L***, Ataxin-2-like protein; ***SBK1***, SH3 Domain Binding Kinase 1; ***SULT1A2***, Sulfotransferase Family 1A Member 2; ***TUFM***, Elongation Factor Tu, Mitochondrial; ***GPRC5B***, G Protein-Coupled Receptor Class C Group 5 Member B; ***IQCK***, Motif Containing K; ***POC5***, POC5 centriolar protein; ***HMGCR***, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; ***COL4A3BP***, Collagen type IV alpha-3-binding protein; ***ZC3H4***, Zinc Finger CCCH-Type Containing 4; ***OLFM4***, Olfactomedin 4.