Contribution of genetic background and clinical D:A:D risk score to chronic kidney disease in Swiss HIV-positive persons with normal baseline estimated glomerular filtration rate

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Summary: A polygenic risk score, summarizing information from >86000 single nucleotide polymorphisms, predicts chronic kidney disease risk in Swiss HIV-positive persons. The genetic effect size is similar to the clinical D:A:D score and similar to exposure to potentially nephrotoxic antiretrovirals.

Abstract

Background: In HIV, the relative contribution of genetic background, clinical risk factors, and antiretrovirals to chronic kidney disease (CKD) is unknown.

Methods: We applied a case-control design and performed genome-wide genotyping in white Swiss HIV Cohort participants with normal baseline estimated glomerular filtration rate (eGFR >90 mL/min/1.73 m^2). Uni- and multivariable CKD odds ratios (OR) were calculated based on the D:A:D score that summarizes clinical CKD risk factors and a polygenic risk score that summarizes genetic information from 86613 single nucleotide polymorphisms..

Results: We included 743 cases (79% male; median age, 42 years; baseline eGFR 106 mL/min/1.73 m^2) with confirmed eGFR drop to <60 mL/min/1.73 m^2 (n=144) or $\geq$25% eGFR drop to <90 mL/min/1.73 m^2 (n=599), and 322 controls (eGFR drop <15%; 81% male; median age, 39 years, baseline eGFR 107 mL/min/1.73 m^2). Polygenic risk score and D:A:D score contributed to CKD. In multivariable analysis, CKD ORs were 2.13 (95% confidence interval, 1.55-2.97) in participants in the 4th (most unfavorable) vs. 1st (most favorable) genetic score quartile; 1.94 (1.37-2.65) in the 4th vs. 1st D:A:D score quartile; and 2.98 (2.02-4.66), 1.70 (1.29-2.29), and 1.83 (1.45-2.40), per 5-years exposure to atazanavir/ritonavir, lopinavir/ritonavir, and tenofovir disoproxil fumarate, respectively. Participants in the 1st genetic score quartile had no increased CKD risk, even if they were in the 4th D:A:D score quartile.

Conclusions: Genetic score increased CKD risk similar to clinical D:A:D score and potentially nephrotoxic antiretrovirals. Irrespective of D:A:D score, individuals with the most favorable genetic background may be protected against CKD.

Key words: HIV infection, chronic kidney disease, genetics, clinical risk factors, antiretroviral therapy

Introduction

Chronic kidney disease (CKD) is a major long-term concern in HIV-positive persons.[1-4] The D:A:D study, the largest consortium of observational HIV studies with rigorous endpoint ascertainment and validation, has documented clinical and HIV-related risk factors for CKD, which can be summarized in a 9-item risk score.[5] HIV-positive persons with low, medium and high risk D:A:D score had a 1:393, 1:47, and 1:6 chance of developing CKD over 5 years.[5] In addition, the D:A:D study described atazanavir/ritonavir (ATV/r), lopinavir/ritonavir (LPV/r), and tenofovir disoproxil fumarate (TDF) as being associated with an increased CKD incidence rate in HIV-positive persons with normal kidney function at baseline.[6]

CKD has a strong hereditary component.[7-9] Genetic studies of CKD in HIV have focused on HIV-associated nephropathy (HIVAN) which develops predominantly in persons of African ancestry with untreated HIV infection, and there is a strong association with *APOL1* gene variants.[10,11] Candidate gene studies have suggested an association of e.g. *ABCC2* polymorphisms and TDF-associated kidney dysfunction in HIV, but were limited by the assessment of single or few gene variants only, by their cross-sectional design, and small study populations.[12,13]

Genome-wide association studies (GWAS) have now identified >50 common genetic variants that reproducibly contribute to CKD in the general population.[7-9] The aim of the present study was therefore to quantitate the contribution of genome-wide genetic variation to CKD in HIV-positive participants. Analyzed in the context of clinical risk factors (summarized in the D:A:D score) and potentially nephrotoxic antiretroviral drugs, we hypothesized that genetic background may partially explain CKD risk in HIV. Our study represents the most comprehensive genetics–CKD evaluation undertaken to date in HIV-positive persons.

Methods

Study population. Eligible participants included HIV-positive persons enrolled in the Swiss HIV Cohort Study (www.shcs.ch), with ≥3 months follow-up after 1.1.2004. The study was approved by the respective local ethics committees. Participants provided written informed consent for genetic testing. Baseline was defined as first estimated glomerular filtration rate (eGFR) measured after 1.1.2004. CKD cases included participants with normal baseline eGFR (>90 mL/min/1.73 m^2; using the CKD-EPI formula) who developed a CKD event during follow-up, as defined in the D:A:D study[6] and in the renal subproject of the START trial, i.e. eGFR drop to <60 mL/min/1.73 m^2, confirmed over a ≥90 day period. Because only 1% of D:A:D study participants with normal baseline eGFR later experienced an eGFR drop to <60 mL/min/1.73 m^2 [6]), we also included participants who developed mild CKD, [14] defined as >25% eGFR drop to <90 mL/min/1.73 m^2, confirmed over a ≥90 day period. To better separate the phenotypes of cases and controls, and thereby to increase power to detect genetic effects, [15,16] only participants with ≤15% eGFR drop at last SHCS follow-up were eligible as controls. Only controls with GWAS genotyping data already available were included. Because previous CKD GWAS in the general population were conducted in populations of predominantly European descent,[7-9] the study was restricted to participants of European descent.

Case-control matching. We performed 1:1 matching. The last available eGFR measurement of controls had to be after the CKD event date of the corresponding case. Matching was done using incidence density sampling, [11] i.e. controls were required to have the first available eGFR measurement +/- 1 year of the corresponding case. In other words, controls were matched on similar follow-up *duration*, and their observation *period* was at similar calendar times, in an effort to correct for differences in potentially nephrotoxic ART compounds in use at different times and other differences during the study period (**Supplementary Methods**). Since we had more cases than controls, only a subset of cases was successfully matched, and we therefore repeated the matching process 2000 times with random re-sampling from cases and controls.[17] This bootstrap resampling method yielded effect estimates (CKD odds ratio) for both D:A:D score and genetic

score with appropriately narrow confidence intervals (**Supplementary Figure 1**).

Genotyping. DNA samples obtained from peripheral blood mononuclear cells were genotyped with the Infinium CoreExome-24 BeadChip (Illumina, San Diego, CA), or in the context of previous GWAS in the SHCS (**Supplementary Methods**),

Non-genetic CKD risk factors. Only variables included in the D:A:D score[5] were used, i.e. mode of HIV transmission, hepatitis C co-infection, age, baseline eGFR, gender, CD4 nadir, hypertension, prior cardiovascular disease, and diabetes mellitus. Each antiretroviral agent is recorded with start and stop dates in the SHCS database. We adjusted only for those ART exposures that contributed to CKD in patients with normal baseline eGFR in the D:A:D study,[6] i.e. cumulative exposure to ATV/r, LPV/r, and TDF. Hypertension was defined as blood pressure $\geq$140/90 mmHg or use of antihypertensive medication. Diabetes mellitus was diagnosed with confirmed plasma glucose >7.0 mmol/L (fasting) or >11.1 (non-fasting), or use of antidiabetic medication.

Genome-wide Polygenic Risk Score. The effect estimate for each SNP included in the polygenic risk score ("genetic score") was obtained from the summary statistics in a recent genetic meta-analysis reference paper of eGFR.[7] The genetic score was calculated with PRSice (v1.1.3b),[18] using p-value thresholding to identify the best model, because including common variants of smaller effect sizes in addition to only the genome-wide significant variants has been shown to increase the predictive power of genetic risk scores. [19-21] The final genetic score model included 86'813 independent SNPs after clumping (**Supplementary Methods**).[7]

Statistical analyses. Univariable and multivariable conditional logistic regression analyses were used to estimate associations of the different quartiles of the genetic and D:A:D scores with CKD events for each of the 2000 case-control sets. The indicators of quartiles and not the scores themselves were included in the models. In multivariable analyses, we included the cumulative exposure to ATV/r, LPV/r, and TDF per 5 years use until the event date among cases, or, for controls, up to the CKD event date of the corresponding case. To assess any potential effect

modification of the D:A:D score by the genetic score, we added a model with an interaction term between genetic and D:A:D scores. The average odds ratio was then calculated as the antilog of the mean of the 2000 log-transformed odds ratios, and the 95% confidence interval was based on the 2.5 and 97.5 percentiles. We used Stata/SE 15.1 (StataCorp, College Station, TX, USA).

Sensitivity analyses. To capture the genetic effect in subgroups of participants who develop different degrees of kidney impairment, we performed sensitivity analyses, defining CKD as either (i) eGFR drop to <60 mL/min/1.73 m^2; (ii) eGFR drop >25% to <70 mL/min/1.73 m^2 ; (iii) or as eGFR drop to <60 mL/min/1.73 m^2 OR of $\geq$40%. In further sensitivity analyses, we excluded participants treated with; (i) dolutegravir, (ii) any integrase inhibitor, (iii) cobicistat, and (iv) rilpivirine, because these ART agents can increase serum creatinine (eGFR) without changing the actual GFR [22,23]. To quantify the potential bias introduced by the imbalance of matching frequencies we added a sensitivity analysis in which cases and controls were weighted with the inverse probability of being sampled, i.e. participants who were sampled less often were attributed more weight.

Exploratory genome-wide association analysis and analysis of previously published candidate SNP. In an exploratory GWAS, we separately tested all genotyped or imputed SNPs on the genetic arrays for association with CKD (**Supplementary Methods**). We also attempted to replicate previously published associations between candidate SNPs (**Supplementary Table 1**) and CKD by extraction of the p-values from the exploratory GWAS. A SNP was considered replicated if found nominally significant (P<0.05).

Results

Participants, CKD events. We included 743 cases with confirmed eGFR drop to <60 mL/min/1.73 m^2 (n=144) or eGFR drop >25% to <90 mL/min/1.73 m^2 (n=599). We included 335 controls with eGFR drop of <15% during the observation period, of whom 322 were successfully matched to a case. All cases were matched 377-2000 (out of 2000) times, with a median (IQR) of 660 (565-916) times. Only 6 cases were matched <500 times. All analyses are therefore based on 1065 participants whose baseline characteristics are shown in **Table 1.** There were 20% women and the median age at CKD event date was 42 years. Cases and controls had similar baseline eGFR (106 mL/min/1.73 m^2); cases were slightly older, less likely to be injection drug users or to be hepatitis C co-infected, had lower CD4 nadir, were more likely to have diabetes, and exposure to ATV/r, LPV/r, and TDF was longer.

CKD risks according to clinical D:A:D score, genetic score, and ART, univariable analyses. CKD odds ratio was associated with D:A:D score, genetic score, and cumulative ATV/r, LPV/r, and TDF exposure in univariable analyses (**Figure 1A**). Compared to the first (most favorable) D:A:D score quartile, participants in the 2nd, 3rd, and 4th (most unfavorable) quartiles had CKD odds ratios (OR) of 1.51 (95% confidence interval, 1.11-2.03), 1.77 (1.36-2.35), and 2.32 (1.70-3.06), respectively. Compared to the 1st (most favorable) genetic score quartile, participants in the 2nd, 3rd, and 4th (most unfavorable) quartiles had CKD OR of 1.12 (0.86-1.46), 1.46 (1.16-1.84), and 1.88 (1.47-2.45), respectively. Cumulative 5-year exposure to ATV/r, LPV/r, and TDF was associated with CKD OR of 2.93 (2.05-4.45), 1.64 (1.32-2.06), and 1.96 (1.59-2.52), respectively.

CKD risks according to clinical D:A:D Score, genetic score, and ART, multivariable analyses. CKD odds ratio remained associated with D:A:D score, genetic score, and cumulative ATV/r, LPV/r, and TDF exposure in multivariable analyses (**Figure 1A**). Compared to the first D:A:D score quartile, participants in the 2nd, 3rd, and 4th quartiles had CKD odds ratios (OR) of 1.43 (1.00-2.00), 1.53 (1.11-2.10), and 1.94 (1.37-2.65), respectively. Compared to the 1st genetic score quartile, participants in the 2nd, 3rd, and 4th quartiles had CKD OR of 1.25 (0.89-1.77), 1.70 (1.26-

2.27), and 2.13 (1.55-2.97), respectively. Cumulative 5-year exposure to ATV/r, LPV/r, and TDF was associated with CKD OR of 2.98 (2.02-4.66), 1.70 (1.29-2.29), and 1.83 (1.45-2.40), respectively.

Interaction of clinical and genetic risk score, adjusted for ART exposure. To evaluate whether genetic background modifies the clinical CKD odds ratio captured in the D:A:D score, we introduced an D:A:D score - genetic score interaction term to the multivariable model. The low CKD risk in the most favorable 1st D:A:D score quartile was not significantly modified by the participant's genetic score quartile (**Figure 2, Supplementary Table 2**). Participants in the 2nd D:A:D score quartile only had a significantly increased CKD odds ratio when they were in the most unfavorable (4th) genetic score quartile, when compared to the most favorable profile (D:A:D quartile 1, genetic score quartile 1). For participants in the highest (4th) CKD risk D:A:D score quartile there was no evidence for an increased CKD odds ratio when they had the most favorable genetic score (1st quartile).

Sensitivity analyses – additional CKD case definitions. When restricting the analyses to CKD cases with eGFR drop to <60 mL/min/1.73 m^2 (n=144), this case population was older, had lower baseline eGFR, and time to CKD event was longer compared to the entire case population (**Table 1**). CKD odds ratio remained associated with D:A:D score, genetic score, and cumulative ATV/r, LPV/r, and TDF exposure in uni- and multivariable analyses (**Figure 1B**). In multivariable analysis, compared to the first D:A:D score quartile, participants in the 2nd, 3rd, and 4th quartiles had CKD OR of 1.22 (.78-1.97), 2.71 (1.93-3.94), and 11.97 (7.61-22.17), respectively. Compared to the 1st genetic score quartile, participants in the 2nd, 3rd, and 4th quartiles had CKD OR of 2.74 (1-90-4.18), 2.33 (1.67-3.46), and 2.79 (1.81-4.43), respectively (see also **Supplementary Table 3**).

Results were similar when applying the intermediate CKD case definitions (i.e. >25% eGFR drop to <70 mL/min/1.73 m^2 [n=449]; or as eGFR drop to <60 mL/min/1.73 m^2 OR of $\geq$40%; n=204) (**Supplementary Table 3).**

Sensitivity analyses – exclusion of certain ART agents. When participants treated with

dolutegravir (n=146) were excluded, genetic score remained significantly associated with CKD but the effect size was slightly attenuated (**Supplementary Table 4)**. For example, in the 4th vs. 1st genetic score quartile, CKD OR was 1.80 (1.34-2.47) and 1.96 (1.33-2.95) in univariable and multivariable models, respectively. When all participants treated with any integrase inhibitor (n=244) were excluded, genetic score remained significantly associated with CKD but the effect size was attenuated (**Supplementary Table 5**). For example, in the 4th vs. 1st genetic score quartile, CKD OR was 1.58 (1.15-2.20) and 1.68 (1.10-2.61) in univariable and multivariable models, respectively. When participants treated with rilpivirine and cobicistat were excluded, results remained essentially unchanged (**Supplementary Tables 6-7**).

Sensitivity analysis – weighting of cases and controls with the inverse probability of being sampled. Results remained very similar when patients who were sampled less often get more weight (**Supplementary Table 8, Supplementary Figures 3 and 4**).

Exploratory GWAS, candidate SNP replication analysis. In exploratory GWAS, no SNPs were found to be genome-wide significant (P<5e-8, **Supplementary Figure 2**). Of 59 previously published candidate SNPs, 2 SNPs replicated as nominally significant, with P-values of 0.03 and 0.05 in the GWAS (**Supplementary Table 1)**.

Discussion

Our findings suggest that in white HIV-positive individuals an unfavorable genetic background increases the incidence of CKD approximately 2-fold. This genetic effect size was similar to the well validated D:A:D score [5,6], and similar to the CKD effect of 5 years treatment with LPV/r or TDF, but smaller than the CKD effect of 5 years ATV/r treatment. The genetic score appears robust, because in multivariable analyses and in sensitivity analyses, it remained independently associated with CKD after adjusting for D:A:D score and for potentially nephrotoxic ART. To our knowledge, this is the first application of a genome-wide polygenic risk score and its integration with clinical risk factors and ART exposure to better explain individual CKD risk in HIV-positive persons.

Our results further suggest that the individual CKD risk captured in the D:A:D score can additionally be stratified by knowledge of genetic background, based on our identification of a clinically relevant interaction between genetic score and D:A:D score. Most importantly, even individuals in the highest clinical risk category (4^{th} D:A:D score quartile) were protected against CKD if they had the most favorable genetic background (1^{st} genetic score quartile). Therefore, a favorable genetic background might explain why certain HIV-positive persons with high clinical CKD risk may not develop CKD, even in the presence of multiple clinical risk factors. Conversely, the most unfavorable genetic background was associated with CKD even with a relatively low D:A:D score (2^{nd} quartile), but was not associated with CKD with the lowest risk D:A:D quartile, highlighting the interaction of genetic and clinical CKD risk factors.

The polygenic risk score may predict more severe CKD better than milder degrees of CKD. The effect size of unfavorable genetic background increased from an approximately 2-fold to an almost 3-fold increased CKD odds ratio, when restricting the analyses to those with eGFR drop to <60 mL/min/1.73m^2. In these participants, D:A:D score was the strongest predictor of CKD, with the effect size increasing from approx. 2-fold increased CKD odds ratio, as in the entire case population, to an approx. 12-fold increase. This was not unexpected, because the variable with by

far the largest effect size in the D:A:D score is age, [5] and those with eGFR drop to <60 mL/min/1.73m^2 were older (median age 45 vs. 42 years in the entire case population). In addition, the D:A:D score was developed in a population with eGFR drop to <60 mL/min/1.73m^2), [5] and not in the much larger segment of individuals with eGFR 60-89 mL/min/1.73m^2.

We exploited clinical, laboratory, and HIV-related data from >1000 HIV-positive participants prospectively followed at regular intervals in the well-established Swiss HIV Cohort Study. This allowed the consideration of all relevant CKD-related risk factors and co-morbidities,[5] and of potentially nephrotoxic ART.[6] The polygenic CKD risk score we used summarizes the genome-wide risk captured by >86'000 SNPs.[7-9] We applied rigorous quality control of the genotyping data, excluded population outliers and corrected for residual population stratification. As in our previous genetic studies of dyslipidemia,[24] diabetes mellitus,[25] coronary artery disease events,[26] and osteoporotic fractures,[27] we based SNP selection on large previous GWAS meta-analyses in the general population.[7-9] As expected, we were unable to confirm most previous candidate-gene kidney association studies in HIV.[12,13]

CKD definitions rely on ultimately arbitrary degrees of eGFR drop, therefore we used a CKD case definition (normal baseline eGFR with subsequent drop to <60 mL/min/1.73 m^2) extensively validated in the D:A:D study[6] and in the renal substudy of the START trial.[28] Because this degree of CKD is uncommon (1% of D:A:D participants [6]), we also included participants who developed less severely decreased kidney function. The polygenic risk score was robust, i.e. it predicted CKD independent of the definition used. As expected, applying a rigorous control definition (longitudinal eGFR drop of <15%) limited the number of controls available, but this allowed us to achieve clear phenotypic separation of cases and controls and to thereby better capture the genetic effects. The issue of fewer controls than cases was successfully addressed by applying a well validated procedure, bootstrap resampling from cases and controls,[17] which yielded effect estimates for D:A:D score and genetic score with appropriately narrow confidence intervals.

Our results apply to individuals of European descent. Because of the relatively small number of women and persons >65 years of age included in our study, the results should be cautiously extrapolated to these populations. Additional studies are needed to confirm preliminary findings from trans-ethnic GWAS meta-analyses which suggest that genetic results may potentially be generalized from persons of European descent to persons of African descent.[8]

In conclusion, genetic background may provide CKD risk information complementary to that afforded by traditional CKD risk factors and antiretroviral regimen. Knowledge of an adverse genetic CKD predisposition might further emphasize the rationale to avoid potentially nephrotoxic antiretroviral and other drugs, and to optimize management of other factors contributing to CKD risk, including hypertension and diabetes. The clinical value of genetic testing will rely on demonstration of improved CKD risk stratification in prospective studies. This was beyond the scope of our study. Finally, CKD odds ratios of the genetic score were attenuated when patients treated with integrase inhibitors were excluded, highlighting the interest in future studies that quantitate the genetic effect in patients using different modern ART combinations.

Notes

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Table 1: Characteristics of cases and controls.

		Entire Case Population (n=743)	**Cases with eGFR drop to <60 mL/min/1.73 m^2 (n=144)**	**Controls** (n=322)
Male gender, n (%)		587 (79)	109 (76)	261 (81)
Age (years), median (interquartile range)		42 (36-47)	45 (41-54)	39 (34-44)
Baseline eGFR (mL/min/1.73 m^2), median (interquartile range)		106 (99-113)	100 (95-107)	107 (98-115)
Median (IQR) time from baseline to CKD date (years), median (IQR)		7.74 (4.98 - 10.81)	9.72 (7.37-12.18)	n.a.
Presumed mode of HIV transmission, n (%)	heterosexual	201 (27)	39 (27)	87 (27)
	MSM	380 (51)	64 (44)	126 (39)
	IDU	137 (18)	34 (24)	101 (31)
	other	25 (3)	7 (5)	8 (2)
Current* smoking, n (%)		410 (55)	78 (54)	241 (76)
Hepatitis C co-infection, n (%)		198 (27)	42 (29)	141 (44)
Duration of atazanavir-ritonavir treatment (years), median (IQR)	All participants** Ever exposed***	0 (0-0.97) 2.49 (0.78-4.94)	0 (0-2.77) 3.61 (1.42-6.81)	0 (0-0.0) 1.63 (0.18-3.48)
Duration of lopinavir-ritonavir treatment (years), median (IQR)	All participants** Ever exposed***	0 (0-1.17) 2.11 (0.70-4.92)	0 (0-1.55) 2.76 (1.11-5.2)	0 (0-0.10) 1.65 (0.62-4.00)
Duration of tenofovir disoproxil fumarate treatment (years), median (IQR)	All participants** Ever exposed***	4.52 (1.76-7.03) 5.11 (2.67-7.38)	6.68 (2.76-9.18) 7.19 (3.85-9.60)	1.75 (0-5.21) 4.19 (1.47-6.05)
CD4+ T-cell count nadir (IQR), (cells/μL)		209 (64-370)	152 (43-295)	280 (150-405)
Hypertension		89 (12)	22 (15)	40 (12)
Prior cardiovascular disease		12 (1.6)	3 (2.1)	5 (1.6)
Diabetes mellitus		23 (3.1)	6 (4)	5 (1.6)

Notes. Data are no. (%) of participants, unless otherwise indicated. *at baseline +/- 1 year. **all CKD cases and controls, irrespective of whether ever treated with the respective ART drug or not. ***only those CKD cases and controls who were ever treated with the respective ART drug. CI, confidence interval; eGFR, estimated glomerular filtration rate; IDU, injection drug use; MSM, men who have sex with men; n.a., not applicable

Figure 1: CKD odds ratio according to quartiles of genetic score, quartiles of D:A:D score, and per 5-year antiretroviral exposures

Note. Uni- and multivariable conditional logistic regression of associations with CKD. Results are pooled estimates from 2000 re-sampled 1:1 case-control pairs involving 743 cases and 322 controls. Multivariable models are adjusted for all variables displayed, i.e. for genetic score, D:A:D score, and drug exposures, respectively.

Figure 2: CKD odds ratio according to quartiles of genetic score, quartiles of D:A:D score, adjusted for antiretroviral exposures

Note that the adjusted odds ratios and 95% confidence intervals displayed here in Fig. 2 are tabulated in **Supplementary Table 2**.

Note. Results from two conditional logistic regression analyses of associations with CKD. Results are pooled estimates from 2000 re-sampled 1:1 case-control pairs involving 743 cases and 322 controls. The leftmost four bars show estimates for quartiles of the D:A:D risk score adjusted for drug exposure to ATV/r, LPV/r, and TDF, without consideration of genetic score. Participants are then stratified into 16 groups by genetic score quartile (quartile 1, 2, 3, and 4) and by D:A:D score quartile (quartile 1, 2, 3, and 4), and these odds ratios are also adjusted for ATV/r, LPV/r, and TDF exposure. The first of these 16 groups, i.e., participants who are in D:A:D score quartile 1 and in genetic score quartile 1, is the reference (odds ratio = 1, without confidence interval).

Figure 1

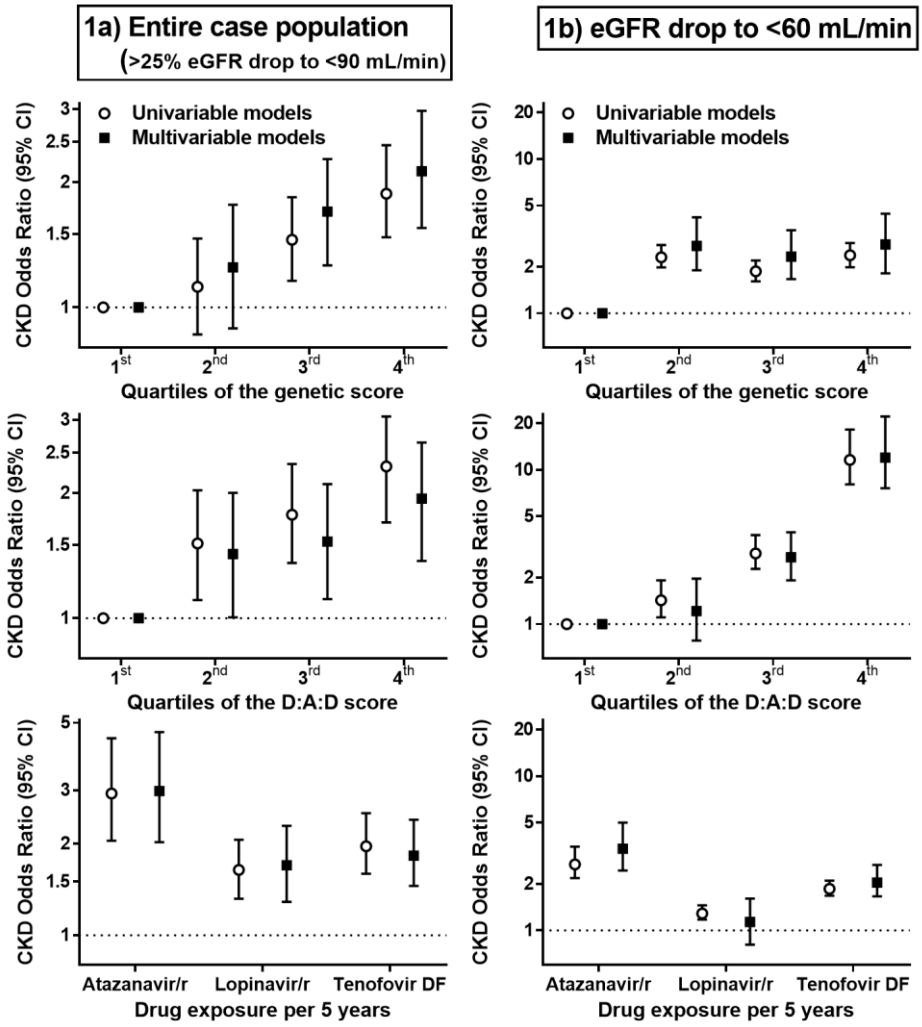

Figure 2