SLC22A2 variants and dolutegravir levels correlate with psychiatric symptoms in persons with HIV

SLC22A2 Genetic Variants and Dolutegravir Trough Concentrations Correlate with Specific Psychiatric Symptoms in HIV-positive Patients on Dolutegravir

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Abstract (250 words)

Background Neuropsychiatric symptoms (NPs) have been reported with dolutegravir use. We hypothesized that increasing dolutegravir trough-concentrations (C_{trough}) and/or polymorphism in the *SLC22A2* gene, encoding for the organic cation transporter-2 (*OCT2*), that is involved in monoamine clearance in the CNS and inhibited by dolutegravir, might be implicated in the onset of NPs.

Materials and methods HIV-positive, consecutively enrolled patients treated with a dolutegravir-containing regimen underwent determination of allelic discrimination for *SLC22A2* 808 C>A polymorphism and dolutegravir C_{trough}. The Symptom Checklist-90-R (investigating 10 psychiatric dimensions and reporting a general severity index, "GSI"), a self-reported questionnaire and the Mini-International Neuropsychiatric Interview were offered to investigate current NPs. The effects of DtgC-t and *SLC22A2* gene variant on NPs were explored by multivariable analyses.

Results A cohort of 203 patients was analyzed: 71.4% were male, with median age of 51 years and 11 years of ART exposure. Median time on dolutegravir was 18 months. Dolutegravir was associated with different antiretroviral combinations (mainly lamivudine, 38.9%, and abacavir/lamivudine, 35.5%).

SLC22A2 CA genotype was independently associated with an abnormal GSI (aOR: 2.72; p=0.051), anxiety (aOR 2.97; p=0.028), hostility (aOR 3.76; p=0.012) and with moderate-to-severe headache (aOR: 5.55; p=0.037). Dolutegravir Ctrough predicted hostility (fourth versus first quartile, aOR: 6.70; p=0.007) and

psychoticism (fourth versus first quartile aOR 19.01; p=0.008). Other NPS were not associated to *SLC22A2* polymorphism nor DtgC-t.

Conclusions A variant of the OCT2-encoding gene, in addition to or in synergy with higher DtgC-t, could are associated with a set of NPs observed during dolutegravir therapy.

Main text (4500 words)

Introduction

Modern combination antiretroviral treatment (cART) is characterized by effective, safe and compact regimens that are associated with long-term benefits in people living with HIV; nonetheless during the first 12 months up to 20% of patients switch their initial therapy due to tolerability reasons.[1] International guidelines recommend integrase strand transfer inhibitors (INSTIs) as first-line options in combination with two nucleos(t)ide reverse transcriptase inhibitors (NRTIs).[2,3] Despite excellent safety, INSTIs have been variably associated to the onset of neurological/neuropsychological symptoms (NPs) including anxiety, sleep disturbances and headache.[4]

Dolutegravir, the latest approved INSTI, has been specifically studied in this setting. In phase III clinical trials no excess in NPs was observed with the exception of the SINGLE study: despite several relevant benefits insomnia was more commonly reported in the dolutegravir (10%) than in the efavirenz arm (7%).[5] After the drug was licensed, several cohort studies suggested a higher rate of discontinuation due to NPs in patients receiving dolutegravir as compared to elvitegravir or raltegravir; the highest rate was 13.7% in a Dutch study and adverse events included sleep disturbances (5.6%), fatigue and headache (4.3%), gastrointestinal complaints (3.8%) and NPs (2.5% including depression, anxiety, agitation, emotional instability and 1 case of

psychosis).[6-8] However other studies, including large cohorts from Europe and the US, did not confirm such observations and discontinuation rates were significantly lower.[9-11]

Beyond the controversies on the exact incidence of NPs in dolutegravir-receiving patients, several risk factors have been reported including female gender, older age and abacavir co-administration. A higher dolutegravir exposure has been associated with NPs in one study, but this was not confirmed in another cohort study.[12,13] In a recent case report, a reduced dolutegravir dose (50 mg every other day) and exposure in a female patient with low body mass index led to prompt resolution of her symptoms (dizziness, fatigue, insomnia and restlessness).[14] Notably, age and female gender have been associated with higher dolutegravir plasma concentrations; a recent study comparing dolutegravir 24-hour exposure in younger and older patients reported higher maximal concentrations and shorter sleep duration in those older than 60 years.[15]

Given the variability in NPs' incidence and severity, we aimed at investigating whether genetic predisposition may partially explain this phenomenon. In particular, we focussed on the role of a single nucleotide polymorphism (SNP) in the *SLC22A2* gene that encodes for Organic Cation Transporter 2 (OCT2), since dolutegravir has been shown to inhibit OCT2 function at clinically observed concentrations. Besides being expressed in renal tubular cells, OCT2 is broadly spread in the central nervous system, where it has been localized in neuronal cell bodies and presynaptic membranes, and at the choroid plexus where it is involved in the transport of a variety of intrinsic compounds (such as monoamine, dopamine, serotonin, histamine, creatinine and choline). [16,17] In mice, OCT2 was found to be expressed in the limbic system and to be involved in anxiety and depression-related behaviours. In addition, genetic deletion of *SLC22A2* produced a significant reduction in brain tissue concentrations of norepinephrine and serotonin and was shown to affect long-term response to treatment with antidepressants.[18]

Materials and methods

Study population

A cohort of HIV-positive patients from 2 clinical centers, treated with a dolutegravir-containing regimen since at least 5 days, were consecutively enrolled during the scheduled follow-up visit. Individuals were proposed to undergo blood sampling for the determination of allelic discrimination for *SLC22A2* 808 C>A SNP and for the evaluation of dolutegravir trough-concentrations (Ctrough). On the same day, a battery of testswere administered to investigate current neuropsychiatric symptoms. These included the self-report Symptom Checklist (SCL)-90-R exploring 10 psychiatric dimensions (somatization, obsessive-compulsive disorder, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism, sleep disorders),[19] as well as an ad-hoc self-reported questionnaire (investigating different symptoms occurring during the previous 4 weeks, including headache) and the Mini International Neuropsychiatric Interview Plus subscale[20].

The study was conducted in compliance with the Declaration of Helsinki and local review board regulations; all patients gave written informed consent before blood sampling and questionnaires' administration. The study was approved by the local Ethics Committee (protocol number: 7768/16).

Neuropsychiatric scores

Each of the 10 sub-scales from SCL-90-R comprises 6-13 items, and the scores of each dimension are calculated as the mean of the scores (from 0 to 4 on the basis of increasing intensity during the last week) of all the items included. Moreover, a Global Severity Index (GSI) is computed as the average score of all the 90 items. Both global and single dimensions scores >1 were considered abnormal.

From the self-reported symptoms questionnaire, only the information about the presence of headache during the previous 4 weeks was used for the present study. Self-reported headache was classified in two categories: absent/mild or moderate/severe.

Finally, the Mini International Neuropsychiatric Interview Plus subscale for suicide risk was administered by a specialized neuropsychologist to explore the presence of current suicidal ideation or recent suicide attempts.

Pharmacokinetics and PG methods and LODs

Plasma samples were collected before the administration of the next dose of cART (Ctrough). Dolutegravir concentrations were measured in plasma at every visit using a validated high-performance liquid chromatography (HPLC)/mass (MS) spectrometry method, with a limit of detection of 16 ng/mL.[21] For samples collected at times different from 24 hours since last drug intake, Ctrough was calculated by the following formula: Calculated Ctrough = C/[2^[(24-h)/19] where "C" is the measured concentration and "h" is time after drug intake; 19 hours was used as dolutegravir half-life.

Whole blood was stored at -20°C for pharmacogenetic analysis. Genomic DNA was extracted using QIAamp whole blood mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. *SLC22A2* 808 (rs316019) C>A polymorphism genotyping was conducted by real time PCR-based allelic discrimination with the use of standard methods (LightCycler 96, Roche, Monza, Italy). Primers, probes and PCR conditions are available on request.

Statistical analysis

The primary objective of the study was to investigate the potential association of dolutegravir Ctrough and *SLC22A2* gene variant on NPs.

A descriptive analysis was performed to explore demographic and clinical characteristics of the study population. At first, the prevalence of NPs in persons showing different *SLC22A2* 808 genotypes and increasing dolutegravir Ctrough were compared using parametric or non-parametric tests, as appropriate. The effects of dolutegravir Ctrough and *SLC22A2* SNP on every single neuropsychiatric dimension were thereby explored by univariate logistic regression. For dependent variables that resulted associated to *SLC22A2* polymorphism and/or to dolutegravir plasma levels, multivariate models were built up by adjusting for every other clinical variable that resulted associated to the outcome (at p values <0.100) at univariate analysis and for potential confounders. The choice of confounders was based on availability of data present in the current literature and by exploring variables that significantly differed among patients with higher dolutegravir Ctrough and different *SLC22A2* 808 C>A genotype.

Neuropsychiatric dimensions not related to *SLC22A2* variant nor to dolutegravir plasma exposure were also analyzed through multivariate logistic regression at a secondary analysis, in order to evaluate potential clinical risk factors for psychiatric impairment.

Results

Characteristics of study population

A cohort of 203 patients was analyzed. Of these, 71.4% were male, and the median age was 51 years (interquartile range, IQR, 43-57). The most common risk factors for HIV acquisition were heterosexual (44.8%) or homosexual (45.3%) contacts. Median time since HIV diagnosis was 13 years (IQR 5-21), with 11 years (IQR 4-18) of median exposure to antiretroviral therapy (ART) and a median time on dolutegravir of 18 months (IQR 10-25). At the time of blood sampling for OCT2-encoding gene typing and dolutegravir plasma level determination, dolutegravir was administered with lamivudine in 79 cases (38.9%), with abacavir/lamivudine in 72 (35.5%), tenofovir disoproxil fumarate/emtricitabine in 25 (12.3%), other potential OCT2 inhibitors, rilpivirine and cobicistat, in 9 (4.4%) cases, and other antiretroviral drugs in 18 (8.9%). Most patients (98.0%) had a plasma HIV-RNA <50 copies/mL, while median CD4 cell count was 650 cells/μL. A current psychiatric disease, as defined by the assumption of specific drug therapy, was present in 6 (3.0%) patients. Characteristics of the study population are summarized in table 1.

SLC22A2 808 CA genotype carriers were 31 (15.3%): compared to CC genotype carriers, they presented more often an active psychiatric disease (APD) at dolutegravir start (9.7% and 1.8% of persons with psychiatric disease had CA and CC genotype, respectively; p=0.048), but no statistically significant differences were found according to sex and other clinical characteristics.

Median dolutegravir Ctrough was 1492 ng/mL (IQR 981-2234). Females with heterosexual intercourses as risk factor for HIV transmission had higher dolutegravir Ctrough (median concentration: 1918 ng/mL; IQR 1193-2918; p=0.001), as compared with heterosexual males (1509 ng/mL; 1071-2269), MSM (1319 ng/mL; IQR 877-1968), male IDUs (825 ng/mL; IQR 469-1505) and female IDUs (1344 ng/mL; IQR 383-2898). Interestingly, younger patients ($\leq$ 51 years-old) showed a trend for higher dolutegravir plasma levels (1513

ng/mL versus 1373 ng/mL), even if this was not statistically significant (p=0.889). No difference in dolutegravir Ctrough emerged among different antiretroviral combinations (p=0.837), but lower concentrations were seen with abacavir/lamivudine (1380 ng/mL, IQR 955-2424) and rilpivirine or cobicistat-boosted darunavir (954 ng/mL, IQR 631-2600) compared to tenofovir disoproxil fumarate/emtricitabine (1521 ng/mL, IQR 976-2176) and lamivudine only (1492 ng/mL, IQR 1071-2221).

Neuropsychiatric symptoms, dolutegravir concentration and SLC22A2 genotyping results

According to the Symptom Checklist-90-R the prevalence of a pathological global severity index score was present in 35 (17.2%) individuals. Concerning every single dimension, a somatization disorder was present in 61 (30.0%) persons, obsessive-compulsive disorder in 56 (27.6%), a pathological interpersonal sensitivity in 26 (12.8%), depression in 51 (25.1%), anxiety and hostility in 38 (18.7%) patients each, phobic anxiety in 10 (4.9%), paranoid ideation in 38 (18.7%), psychoticism in 22 (10.8%) and sleep disorders in 96 (47.3%). Using the self-reported symptoms questionnaire, a moderate-to-severe headache in the preceding 4 weeks was reported by 14/201 (7.0%). Importantly, 15/199 (7.5%) reported suicidal ideation in the previous 2 weeks, whereas 2/199 patients (1.0%) also attempted to commit suicide in the previous month. Prevalence of NPs are summarized in figures 1 and 2.

Prevalence of *SLC22A2* CA genotype compared with CC was higher in patients showing an abnormal GSI (32.3% versus 14.6%, p=0.035), in those with anxiety (35.5% versus 15.8%; p=0.022), hostility (32.3% versus 16.4%; p=0.047), and moderate-to-severe headache (16.7% versus 5.3%; p=0.041). Higher mean dolutegravir Ctrough were observed in patients with hostility (2019 ng/mL versus 1344 ng/mL; p<0.001) and psychoticism (2138 ng/mL versus 1383ng/mL; p=0.003). No association of *SLC22A2* genotypes and dolutegravir Ctrough emerged with the other neuropsychiatric dimensions. In particular, both patients who attempted to commit suicide during the previous month had a *SLC22A2* CC genotype; one of them had lower dolutegravir plasma exposure (954 ng/mL) compared with median value, while the other one had higher concentration (1912 ng/mL). Patients with suicidal ideation in the last 2 weeks were more often CA genotype carriers (CA, 13.3% versus CC, 6.5%), although the difference did not reach statistical relevance (p=0.252). Finally, no relation with dolutegravir Ctrough emerged in patients with suicide ideation, who

conversely presented lower median concentrations (1383 ng/mL, IQR 793-1812) compared with patients free from suicide thoughts (1491 ng/mL, IQR 987-2250).

Role of SLC22A2 gene polymorphism and dolutegravir trough-concentrations on neuropsychiatric symptoms

At a univariate analysis, *SLC22A2* CA genotype (versus CC genotype) was found to be associated to GSI, anxiety, hostility, obsessive-compulsive disorder, depression and to moderate-to-severe headache ($p<0.100$ in every case). Increasing dolutegravir levels were associated to increased risk of hostility and psychoticism. Due to the increased prevalence of APD in CA genotype carriers, and considering the probability of a causal role of APD in increasing the risk of reporting NPs, this variable was treated as a potential confounder in multivariate models that included SLC22A2 CA genotype as the exposure variable. Age and gender are recognized risk factors for dolutegravir discontinuation due to neuropsychological symptoms;[6] moreover, both older age and female sex are associated to increased dolutegravir Ctrough:[13,14] these variables were therefore considered as confounders in multivariate models including dolutegravir as an exposure variable. Finally, considering the role of certain concomitant antiretroviral drugs in inhibiting OCT2 and their different properties of modulating dolutegravir exposure, and considering the possibility of a direct independent neurotoxic effect, antiretroviral drugs associated to dolutegravir were also considered as potential confounders variables in multivariate models that included OCT2-encoding gene variant and/or dolutegravir Ctrough.

At multivariate analysis, CA genotype confirmed its independent association with an abnormal GSI (versus CC, adjusted Odds Ratio, aOR: 2.72; $p=0.051$), anxiety (aOR 2.97; $p=0.028$) and hostility (aOR 3.76; $p=0.012$), as well as with an increased risk of self-reported moderate-to-severe headache during the last 4 weeks (aOR: 5.55; $p=0.037$); conversely, *SLC22A2* 808 polymorphism was not independently associated with depression nor with obsessive-compulsive disorder, after adjusting for confounders and other competing variables. Dolutegravir concentration showed an independent, dose dependent association with both

hostility (third versus first quartile, aOR: 5.26; p=0.021; fourth versus first quartile, aOR: 6.70; p=0.007) and psychoticism (third versus first quartile, aOR: 9.77; p=0.047; fourth versus first quartile aOR 19.01; p=0.008).

As expected, APD was an independent risk factor for abnormal GSI (aOR: 13.77; p=0.023), anxiety (aOR: 11.61; p=0.034), obsessive-compulsive disorder (aOR: 10.03; p=0.045), depression (aOR: 9.84; p=0.046) and for moderate-to-severe headache (aOR: 7.76; p=0.047), but not for hostility nor psychoticism. Concomitant antiretroviral drugs were also associated with the increased risk of neuropsychiatric disorders, particularly for obsessive-compulsive disorder, psychoticism and hostility. Tenofovir disoproxil fumarate/emtricitabine was associated with both obsessive-compulsive disorder (versus lamivudine-based dual therapy, aOR 2.86; p=0.044) and pathological hostility (versus lamivudine-based dual therapy, aOR: 3.28; p=0.060). Compared with lamivudine plus dolutegravir, psychoticism was more frequent with both abacavir/lamivudine (aOR: 5.40; p=0.018), tenofovir disoproxil fumarate/emtricitabine (aOR: 9.46; p=0.006) and rilpivirine or cobicistat-boosted darunavir (aOR: 13.33; p=0.024). Gender was not associated to any NPs, whereas age was only associated with hostility: differently from what expected, older age was a protective factor (per 10 years older, aOR: 0.63; p=0.034).

Interestingly, a trend for a protective association between previous cumulative exposure to efavirenz and abnormal GSI (per 1 year more, aOR: 0.81; p=0.072) and anxiety (per 1 year more, aOR: 0.81; p=0.056) was observed.

Complete results of the regression analysis are reported in tables 2 to 6.

Role of clinical variables on other neuropsychiatric symptoms

Somatization disorder, phobic anxiety, a pathological interpersonal sensitivity, paranoid ideation, sleep disorders and the presence of suicidal ideation in the last 2 weeks were not associated to *SLC22A2* SNP nor to dolutegravir Ctrough.

Among other clinically relevant variables, an association was found between the use of different concomitant antiretroviral combinations and both a pathological interpersonal sensitivity and paranoid ideation: compared to lamivudine-based two drug regimen, tenofovir disoproxil fumarate/emtricitabine

was associated to an increased risk of reporting a pathological interpersonal sensitivity (aOR: 8.21; p=0.006) after adjusting for potential confounders, whereas both tenofovir disoproxil fumarate/emtricitabine (aOR: 3.81; p=0.057) and rilpivirine or cobicistat-boosted darunavir (aOR: 5.78; p=0.045) were associated with an increased risk of paranoid ideation, after adjusting for APD (aOR: 14.11; p=0.013), age (per 10 years more, aOR: 0.64; p=0.049), use of lipid lowering drugs (aOR: 4.40; p=0.013), and other potential confounders.

An APD was also the only factor independently associated to phobic anxiety (aOR: 42.75; p=0.001) after adjusting for other confounders, as well as for suicidal ideation during the previous 2 weeks (aOR: 29.76; p<0.001).

Sleep disorders and somatization disturb were not associated to any of the explored variables.

Conclusions

Dolutegravir is a highly effective and generally well tolerated drug, that is recommended in different antiretroviral combinations for both naïve and treatment-experienced patients.[2,3] However, post-marketing studies highlighted a potential neuropsychiatric effect of dolutegravir, particularly in specific subgroups of patients, such as older patients and women.[6,7]

In this cohort of HIV-infected patients treated with different dolutegravir-containing antiretroviral regimens, an independent association of the *SLC22A2* 808 C>A polymorphism and of higher dolutegravir Ctrough with the increased risk of reporting neuropsychiatric symptoms emerged. CA genotype carriers were at higher risk of having an abnormal global severity index, and particularly they were more likely to report pathological scores of anxiety and hostility, as well as moderate-to-severe headache in the last 4 weeks prior to questionnaire's administration. Increasing dolutegravir plasma levels were also independently and dose-dependently associated to higher hostility risk, and with psychoticism (a continuum of disorders ranging from mild interpersonal estrangement to overt psychosis). At the same time, influence of dolutegravir on other psychiatric symptoms, especially sleep disturbances and suicide ideation, did not emerge.

OCT2 is a polyspecific, low-affinity carrier for a variety of physiological compounds and xenobiotics in mammals, including catecholamine, serotonine and choline neurotransmitters.[22] In mice experiments, OCT2, together with other subtypes of OCTs, is widely expressed throughout the forebrain, especially in anatomical structures of the limbic system, and takes part to the postsynaptic reuptake of the extraneuronal neurotransmitters (especially catecholamines) that escape from the high-affinity transporters.[23] The monoaminergic pathways control fundamental physiological functions within the central nervous system: among the others, serotonin has been implied in modulation of mood, aggression and sleep, while noradrenalin has been associated with arousal, mood and stress and dopamine with the control of motor function, motivation and reward, mood and cognition.[22] The role of OCT2 in modulating the response to stress in rodents has already been evidenced.[22] By decreasing the extracellular monoamine concentrations, OCT2 inhibits the corticosterone release driven by the hypothalamic-pituitary-adrenocortical system, and this in turn reduces stress and depression-like behaviors. Polymorphisms of the OCT2-encoding gene have already demonstrated influence substrates' transport activity[24,25] and several drugs have been shown to have an inhibitory effect on OCT2,[26] with a relevant influence in various clinical contexts. An interesting hypothesis has therefore been made concerning the potential role of different variants of the OCT2-encoding gene or of several OCT2-inhibiting drugs in enhancing the vulnerability to repeated adverse events, leading to chronic stress.[22] In line with this background, we found that different *SLC22A2* 808 genotypes, as well as increasing plasma levels of dolutegravir, a known OCT2 inhibitor, were associated to several neuropsychiatric symptoms in HIV-infected patients treated with dolutegravir. Particularly, the CA variant was associated to anxiety and this could be explained by a reduced OCT2 activity as compared to the CC variant. Indeed, as observed in depression paradigms in OCT2-deficient mice, that resulted insensitive to long-term venlafaxine treatment,[27] the minor transport activity of CA as compared to CC genotype carriers might trigger adaptative modifications of other neurotransmitters receptors over time, that in turn could lead to the expression of depressive symptoms such as anxiety.

An association with increased risk of hostility was found with both *SLCA22A* C>A SNP and increasing dolutegravir Ctrough. An increase in the activity of the limbic system, specifically of the amygdala (following a reduction of cortical control), has already been implied in the onset of aggressive behaviors and

disinhibition in humans?.[28] It is reasonable to hypothesize that an interference with neurotransmitters uptake could explain the higher frequency of this symptom observed in this group. CA genotype had a similar impact to that of higher dolutegravir levels on the risk of reporting hostility. Interestingly, this risk in CA carriers was greatly increased only for patients who fell within the lowest quartile of dolutegravir Ctrough (data not shown): it is possible that a stronger inhibition of OCT2 driven by dolutegravir overwhelms the effect of OCT2 gene variants on neurotransmitters re-uptake. Finally, a similar effect of dolutegravir was obtained analyzing psychoticism: increasing concentrations were associated with progressively increased risk of an abnormal score.

In a previous study by Yagura et al.,[12] increased median dolutegravir Ctrough were detected in Japanese, HIV-1 infected persons reporting central nervous systems symptoms (dizziness, headache, insomnia, restlessness and anxiety) compared with persons without those symptoms. Moreover, a recent European retrospective study[8] also showed higher median dolutegravir concentrations (1719 ng/mL) in patients who interrupted dolutegravir. In line with these reports, our findings are consistent with the hypothesis of a neuromodulating effect of dolutegravir, involving at least some of the analyzed NPs.

Contrary to previous reports showing an association of older age with NPs during dolutegravir treatment,[6] in this series older age was associated to a reduced risk of reporting some of the analyzed psychiatric symptoms, particularly hostility and paranoid ideation. However, in our population persons older than 60 years-old, the group at higher risk in the previous reports, were under-represented, and dolutegravir plasma exposure was slightly lower in persons over 51 years old as compared with younger people, which may partly explain this apparent discrepancy. In addition, the neuropsychiatric symptoms leading to dolutegravir discontinuation in the cited cohort (mainly insomnia and sleep disturbances) could have a different pathophysiological cause: indeed, sleep disturbances were not found to have any association with dolutegravir Ctrough nor with *SLC22A2* SNP.

Dolutegravir concentrations were slightly although not significantly higher in women than in men. Higher rates of dolutegravir discontinuations in women as evidenced in previous studies could have been at least partly explained by its increased concentrations.

An interesting association between concomitant antiretrovirals and risk of neuropsychiatric symptoms emerged in our work. Compared with lamivudine-based two drug regimen, a greater risk of psychoticism was seen with patients on rilpivirine or cobicistat boosted-darunavir: both drugs have a recognized inhibitory effect on OCT2, and this could have further increased the overall effect of dolutegravir; however, due to the lack of comparative data about the effect of different combinations with dolutegravir on the risk of neuropsychiatric symptoms, this hypothesis needs to be further explored. The combination of abacavir/lamivudine also increased the risk of psychoticism and was independent from dolutegravir concentrations: in a previous report[7] abacavir was associated to a higher risk of dolutegravir discontinuation, and a common metabolic pathway between abacavir and dolutegravir, that could in turn justify higher dolutegravir concentrations, was hypothesized as a causal mechanism. However, in our series patients on abacavir/lamivudine had lower dolutegravir concentrations as compared to those receiving other NRTIs combinations and, moreover, the effect of abacavir/lamivudine exposure was independent from dolutegravir Ctrough. Unexpectedly, the concomitant use of tenofovir disoproxil fumarate/emtricitabine was also associated with psychoticism, as well as with obsessive-compulsive disorder, hostility, interpersonal sensitivity and paranoid ideation. While emtricitabine, as well as lamivudine, are substrate, but not inhibitors of OCT2, data on the effect of tenofovir disoproxil fumarate on OCTs are conflicting;[29,30] however, due to the paucity of data concerning possible neuropsychiatric effects of tenofovir and emtricitabine,[31] a direct neurotoxicity of these compounds appears unlikely. Although an interference with OCT2 or other mediators of brain function could not be completely excluded, other unmeasured confounders could explain our findings, that therefore need further confirmation. Finally, a trend for a protective association emerged between previous cumulative exposure to efavirenz and psychiatric impairment (global score and anxiety): due to the plausible long-term effect of efavirenz exposure, a potential benefit of switching to a more tolerable regimen could not be excluded. However, caution is warranted when interpreting this association, because a selection bias is also plausible: in clinical practice, patients previously exposed to efavirenz could have lacked significant neuropsychiatric risk factors.

Our study suffers from some important limitations, that have to be acknowledged. Firstly, this is a cross-sectional analysis, that did not take into consideration a validated neuropsychiatric evaluation at the time of dolutegravir start and therefore could not assess whether psychiatric symptoms were already present before dolutegravir; also, the lack of a comparison arm to investigate potential differences in psychiatric symptoms onset makes it difficult to ascertain the unique role of dolutegravir in determining these alteration and firmly establish a cause-effect relationship. Moreover, patients recruited here were on a stable dolutegravir regimen, which represents a selection bias that excluded from our analysis individuals discontinuing dolutegravir due to side effects.

Our study also had important strengths that need to be underscored. To our knowledge, this is the first attempt to systematically investigate specific neuropsychiatric symptoms by a validated questionnaire, as well as with a psychiatric interview offered by a specialist neuropsychologist and a self-reported questionnaire, in HIV-1 infected individuals during dolutegravir therapy. Compared with a previous work[12] on this subject, we also extended its results to a larger, western European population. Moreover, a molecular pathophysiological mechanism underlying dolutegravir neuropsychiatric effect was postulated here for the first time, and our result give an insight that prompts further investigation: if confirmed by prospective studies, determination of variants of the OCT2-encoding gene and therapeutic drug monitoring of dolutegravir could become important tools to support treatment choices and monitoring.

Given the widespread use of dolutegravir in clinical practice, further studies aiming to determine the predictors and clinical relevance of the neuropsychiatric side effect of this drug are needed.

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