Association between serotonin transporter gene polymorphisms and increased suicidal risk among HIV positive patients in Uganda

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Abstract

Background: Persons living with HIV/AIDS (PLWHA) are at an increased risk of suicide. Increased suicidal risk is a predictor of future attempted and completed suicides and has been associated with poor quality of life and poor adherence with antiretroviral therapy. Clinical risk factors have low predictive value for suicide, hence the interest in potential neurobiological correlates and specific heritable markers of suicide vulnerability. The serotonin transporter gene has previously been implicated in the aetiology of increased suicidal risk in non-HIV infected study populations and its variations may provide a platform for identifying genetic risk for suicidality among PLWHA. The present cross-sectional study aimed at identifying two common genetic variants of the serotonin transporter gene and their association with increased suicidal risk among human immunodeficiency virus (HIV)-positive adults in Uganda.

Results: The prevalence of increased suicidal risk (defined as moderate to high risk suicidality on the suicidality module of the Mini Neuropsychiatric Interview (M.I.N.I) was 3.3% (95% CI, 2.0 – 5.3). The 5-HTTLPR was found to be associated with increased suicidal risk before Bonferroni correction (p-value = 0.0174). A protective effect on increased suicidal risk was found for the 5-HTTLPR/rs25531 S allele (p-value = 0.0046)- which directs reduced expression of the serotonin transporter gene (5-HTT).

Conclusion: The S allele at the 5-HTTLPR/rs25531 locus is associated with increased suicidal risk among Ugandan PLWHA. Further studies are needed to validate this finding in Ugandan and other sub-Saharan samples.

Keywords: Suicidal risk, Serotonin transporter (5-HTT) gene polymorphisms, HIV/AIDS, Uganda

Background

HIV/AIDS is associated with a considerable risk of suicide with studies reporting rates of between 7.8% to 43%, [1–5]. Increased suicidal risk is a predictor of future attempted and completed suicides, and has been associated with poor quality of life and poor adherence with antiretroviral therapy [6–8]. While considerable work has been undertaken to understand psychosocial and clinical risk factors for suicidal risk, these have low predictive value for suicide, hence the interest in potential neurobiological correlates and specific heritable markers of suicide vulnerability [1, 2, 9]. Evidence from genetic and epidemiological studies suggests that genes play an important role in the predisposition to suicidal behavior [10, 11]. There is also evidence that suicidal behavior may aggregate in families independently of the familial transmission of major depression [12], suggesting the existence of independent genetic risk factors for suicidal behavior [13]. A disturbance in serotonin (5-HT) transmission is the most frequently reported neurobiological abnormality associated with suicidal behavior [14, 15].

The serotonin transporter (5-HTT) is known to influence serotonergic transmission by regulating the duration of serotonin in the synaptic cleft. The gene encoding 5-HTT (5-HTT) is located on chromosome

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17 and is composed of 14 exons [16]. Several 5-HTT polymorphisms have been identified [17]. Two of the most commonly studied 5-HTT polymorphisms are an insertion/deletion polymorphism located 1.2 kb upstream of the transcription initiation site of the 5-HTT-linked polymorphic region (5-HTTLPR) and a variable number of tandem repeat polymorphism, located in the second intron of the gene [18] and abbreviated as STin2.VNTRs. The 5-HTTLPR polymorphism has been widely investigated for its role in increased suicidal risk and in other psychiatric disorders, mainly in western populations. [19, 20]. This polymorphism comprises two main alleles based on the presence or absence of a 44 base pair fragment. The first is a 14-repeat short (S) variant with less transcriptional activity and lower serotonin uptake from synaptic clefts and the second is the 16-repeat long (L) variant which has been found to possess more transcriptional activity [20]. In addition, Lesch et al. [9] found the S-allele to be significantly associated with reduced 5-HTT binding in the brain, and lower 5-HTT mRNA expression and 5-HT uptake in lymphoblasts, relative to the LL genotype [20]. A meta-analysis found a significant association between the 5-HTTLPR S allele and suicidal behavior [21]. The S allele was also found to increase the risk of developing a depressive episode, and suicidal ideation following exposure to stressful life events, early adverse environments, maltreatment and childhood physical and sexual abuse [19, 22–24].

More recently, a polymorphism has been reported in the 5-HTT gene on human chromosome 17, designated as rs25531 which is an A to G substitution [25]. The presence of A or G allele results in LA, SA, LG and SG alleles. LA and SA alleles are associated with increased transcriptional activity of the 5-HTT gene and are thus referred to as “overexpressing” alleles, while LG and SG are associated with reduced transcriptional activity of the 5-HTT, referred to as “low-expressing” alleles. A study by Roy et al. 2007 [26] found that the low-expressing alleles (LG and SG) increased the risk of suicidal behavior in male African-American substance dependent patients exposed to childhood trauma.

The STin2 VNTR is found in intron 2 and consists of multiple repeat copies of a 16–17 bp element [27]. Three major alleles have been described, containing 9 (STin 2.9), 10 (STin 2.10) and 12 (STin 2.12) repeats of the 16–17 bp repetitive element. These STin2 VNTRs have been found to support differential gene expression in vitro [20, 28, 29]. For example, Lovejoy et al. [28] demonstrated that the 9, 10 and 12 repeat elements within the VNTR domain yielded extremely high levels of enhancer activity relative to the cytomegalovirus supported control, with the 9-repeat allele exhibiting greater enhancer activity in an embryonic stem cell model. In addition, Ali et al. [30] reported on an interaction between the STin2 VNTR and 5-HTTLPR to regulate expression of the 5-HTT. They reported that the STin2.10 and STin2.12 variants, which alone did not support additional activity, in conjunction with the S-allele (S10 and S12) directed higher transcriptional activity of the 5-HTT. However, no study has examined this polymorphism and increased suicidal risk in the context of HIV/AIDS. In addition, the STin2 variant has not been widely studied in suicidal behavior. Only a few studies have shown a significant association between polymorphisms at this site and suicidal behavior [31].

There is a paucity of data from sub-Saharan Africa on genetic risk factors for increased suicidal risk. Additionally, globally, there is an absence of studies on genetic risk factors for suicidality among patients living with HIV/AIDS. To address these gaps we undertook a study to investigate the genetic risk factors associated with particular suicidal behavior phenotypes in the sub-Saharan African setting of Uganda among persons living with HIV/AIDS. We hypothesized that the overexpressing alleles of the serotonin transporter gene variants would confer increased suicidal risk to the bearer.

Methods
Study design
This cross-sectional, genetics sub-study was part of a bigger EDCTP funded project that primarily investigated risk factors for psychiatric disorders among adults with HIV/AIDS in Uganda [32]. All consenting eligible HIV-infected patients attending the study health facilities during the specified study period were enrolled until the required sample size (N = 600) was attained. To be eligible, study participants had to be registered at the participating HIV clinics, not on anti-retroviral therapy (ART), 18 years or older, fluent in English or Luganda (the local language into which the study instruments were translated), and physically and mentally well enough to complete the interview (not suffering from a severe physical and mental disorder to require immediate medical and psychiatric attention). Participants who had defaulted on their most recent clinic visit were excluded.

Participants
Study participants were recruited by the parent study from two HIV clinics run by The AIDS Support Organisation (TASO) at Entebbe hospital (semi-urban site) and Masaka hospital (rural site) between 6th May 2010 and 30th October 2012. Study participants completed a structured interview (undertaken by trained psychiatric nurses) that included, amongst
Genotyping
Genomic DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (Manchester, United Kingdom). Polymerase chain reactions (PCR) was carried out in 25 μl reaction volumes containing between 20 and 205 ng template DNA, 200 μM dNTP (Kapa Biosystems, Cape Town, South Africa), 5 μl of 10X Taq DNA polymerase buffer (Kapa Biosystems, Cape Town, South Africa), 0.625 units (U) Taq DNA polymerase (Kapa Biosystems, Cape Town, SA), and 0.5 μM of each primer, with bi-distilled water. All PCR-amplification reactions were performed in a GeneAmp PCR System 9700 (Perkin Elmer Biosystems, Foster City, CA, USA).

For the 5-HTTLPR/rs25531 polymorphism, an initial denaturation step was performed at 95 °C for 3 min. Thereafter, a denaturation step was performed at 95 °C for 15 s (s), followed by the primer annealing step, at 60 °C for 15 s, and an elongation step, at 72 °C for 15 s. A final elongation step, at 72 °C for 30 min, was then performed. The denaturation and extension steps were repeated for 35 cycles using 5′-HEX-CTGAGTATCAACGGCTGAGGAGC and 3′-TGGAGCTGAGGAGC.

Sample preparation and analysis on the ABI prism
The loci containing the 5-HTTLPR/rs25531, and STin2 VNTR polymorphisms were amplified individually. Following agarose electrophoresis to determine success of each PCR, the amplicons and the digested products were combined in a 1:1 ratio for allele-specific size discrimination analysis, which was performed on the ABI 3130 XL Genetic Analyzer (Applied Biosystems, Foster City, United States of America). The multiplexed samples were then sent to the Central Analytical Facilities (CAF) laboratory at Stellenbosch University, where analysis on the ABI prism was done.

Statistical analysis
Functions from R, a language and environment for statistical computing, and R packages genetics, haplo.stats and MASS, were used for all statistical analyses. R is freely available from URL http://www.R-project.org. Specifically, functions from genetics were used to derive genotype and allelic distributions and to test the Hardy-Weinberg Equilibrium (HWE), while allelic combinations and their frequencies were inferred with functions from haplo.stats.

Logistic regression was used to model the different definitions of increased suicidal risk susceptibility (dichotomous outcome: increased suicidal risk defined as either lifetime attempted suicide or past month attempted suicide) and ordinal logistic regression (function polr from R package MASS to model the 3 categories of increased suicidal risk (low, moderate and high risk), as defined by the suicidal risk assessment criteria of the suicidal risk module of the M.I.N.I. These models allowed us to control for potential confounders (study site, socio-economic status and major depressive disorder).
*P*-values for tests of association between the serotonin transporter gene polymorphisms and increased suicidal risk were generated. Firstly, these were obtained as nominal *p*-values without Bonferroni correction (α = 0.05). To perform a Bonferroni correction, the *p*-value threshold of 0.05 was divided by 9 (number of separate tests) to yield a corrected threshold *p*-value of 0.0056 (see Table 4).

**Ethical considerations**
Ethics approval was obtained from the Uganda Virus Research Institute Science and Ethical Committee (Ref # GC/127/11/08/04) and the Uganda National Council of Science and Technology (Ref# HS 1053). All study participants provided informed consent to participate in the study and for a blood specimen to be withdrawn from them for the genetics analyses. Study participants who were diagnosed with significant psychiatric problems were referred to mental health units at Entebbe and Masaka government hospitals and their care clinicians were notified and follow-up care was arranged.

**Results**
Socio-demographic and clinical variables are shown in Table 1.

**Prevalence of increased suicidal risk**
The prevalence of increased suicidal risk, according to the criteria for ‘moderate to high suicidal risk’ on the M.I.N.I., was 3.3% (95% CI, 2.0–5.3), with a lifetime rate of attempted suicide of 6.3% (95% CI, 4.5–8.8) and a past month rate of attempted suicide of 2.2% (95% CI, 1.2 to 4.0) (Table 2).

**Distribution of the alleles and genotypes for the 5-HTTLPR, 5-HTTLPR/rs25531 and STin2 VNTR**
The allele and genotype distributions of 5-HTTLPR, 5-HTTLPR/rs25531 and STin2 VNTR are presented in Table 3.

The genotype distribution at the STin2 VNTR and 5-HTTLPR deviated significantly from HWE (*p* = 0.0036 and *p* = 0.0054 respectively) (Table 4).

The estimated effect of the *L*<sub>A</sub> allele was to increase the odds of an individual being in a higher suicide risk category (from no to low, or low to moderate) by 53% (95% CI: 12% to 112%) before Bonferroni correction while the addition of an *S*<sub>A</sub> allele reduced the odds of an individual being in a higher suicide risk category by 43% (95% CI: 14 to 63%) (*p* = 0.0046) after Bonferroni correction.

There was also a significant association of the genotypes *L*<sub>A</sub>*L*<sub>A</sub>, *L*<sub>A</sub>*S*<sub>A</sub>, *L*<sub>A</sub>*L*<sub>G</sub> and *L*<sub>A</sub>*S*<sub>G</sub> (representing genotypes found to be associated with increased expression of 5-HTT) versus the genotypes *S*<sub>A</sub>*S*<sub>A</sub>, *S*<sub>A</sub>*L<sub>G</sub>, *L*<sub>L</sub>*G*<sub>G</sub> and *L*<sub>G</sub>*S*<sub>G</sub> (representing genotypes found to be associated with reduced expression of 5-HTT) with increased suicidal risk outcome (*p*-value 0.0145). However this association became non-significant after Bonferroni correction (Table 4). For the STin2 VNTR, the rare 9-repeat allele was absent in the sample.

**Discussion**
In this study, we investigated the genetic risk for increased suicidality among HIV positive persons in Uganda. To our knowledge, this is the first sub-Saharan African study to investigate the association of polymorphisms in 5-HTT and increased suicidal risk, and the first such study in the world to investigate this risk among persons living with HIV/AIDS. In this study, the

| **Table 1** Counts (%) of the characteristics of the study group |
|------------------|--|
| Characteristic   | Count (%) |
| Site             |          |
| Entebbe          | 214 (39) |
| Masaka           | 341 (61) |
| Sex              |          |
| Male             | 131 (24) |
| Female           | 424 (76) |
| Education Level  |          |
| Primary or less  | 406 (73) |
| Secondary and above | 147 (27) |
| Marital Status   |          |
| Married          | 274 (50) |
| Not married      | 279 (50) |
| Employment Status|          |
| Farmer           | 168 (31) |
| Not a farmer     | 382 (69) |
| Duration of awareness of HIV status |        |
| < 12 months      | 145 (26) |
| 12 months or more| 407 (74) |
| Religion         |          |
| Catholic         | 306 (55) |
| Protestant       | 107 (19) |
| Other            | 142 (26) |

Other: Muslims, Born-again Christians, Seventh Day Adventists etc. combined

**Table 2** The prevalence of increased suicidal risk as defined by three criteria of increased suicidal risk

| Increased suicidal risk | Prevalence | Per 100 (95% CI) |
|-------------------------|------------|-----------------|
| Attempted suicide life-time | 35 of 553 | 6.3 (4.5 to 8.8) |
| Attempted suicide past month | 12 of 537 | 2.2 (1.2 to 4.0) |
| No, Low, Moderate and High suicidal risk (according to MINI) | 18 of 547 | 3.3 (2.0 to 5.3) |

CI: Confidence Interval, MINI, Mini International Neuropsychiatric Interview
prevalence of moderate to high risk suicidality, according to the MINI, was 2.2%. Kinyanda and colleagues, 2012a [1], using a similar methodology in a study undertaken at government run HIV clinics at the semi-urban site of Entebbe, reported a prevalence of increased suicidal risk of 7.8% and lifetime attempted suicide of 3.9%. [1]. Rukundo and colleagues (2016), in a semi-urban HIV clinic in south-western Uganda and using a different method for assessing suicidal risk, reported a prevalence of suicidal ideation of 8.8% and lifetime attempted suicide of 3.1% [2]. These rates from Uganda are, however, much lower than those reported elsewhere: 26% for suicide ideation in the USA [3], 43% for suicidal ideation in China [4], 31% in United Kingdom [7], 21% in Australia [36] and 26.9% in Europe and America (from a systematic review) [5]. The reasons for these differences include diversity of assessment methods for suicide risk and differences in underlying suicide risk associated with particular HIV risk categories (in HIV endemic countries like Uganda the HIV risk category is the general population while in the west and in China the HIV risk categories are men who have sex with men and intravenous drug users). For a complete discussion, see Kinyanda and colleagues 2012a [1].

A recent review provided evidence for an association between 5-HTT and suicidal behavior [13]. 5-HTT has been a focus of investigation in affective disorders. A better understanding of these genetic variants and others that control 5-HTT function (including non-5-HTT sources of epistasis) will be important in predicting the effects of 5-HTT variation on a variety of emotional phenotypes associated with increased suicidal risk [37].

In the brain, impulses are passed from one nerve cell to another via a synapse. The pre-synaptic cell releases serotonin (5-HT) into the synaptic cleft, where it interacts with both post- and presynaptic receptors. At the presynaptic side, 5-HT activates 5-hydroxytryptamine (serotonin) receptor 1A (HTR1A), B (HTR1B), and D (HTR1D), which in turn relays the signal [38]. About 90% of 5-HT is sent back to the pre-synaptic cell. 5-HTT clears 5-HT from the synaptic clefts, regulating the strength and duration of serotonergic signaling. Ho et al., [39] observed a significant difference in 5-HTT availability in the thalamus (measured by positron emission tomography 5-HTT imaging technique) in major depression patients and healthy controls. Overexpression of 5-HTT leads to more serotonin transporter in the synaptic cleft [17, 20] and increases the efficiency of serotonin transportation back into the pre-synaptic cell resulting in serotonin not to linger at the synapse. This consequently leads to a shorter action of serotonin. Reduced serotonin activity is thus expected to be found in the neurons of individuals with the L_A allele. These individuals are probably at an increased risk of developing major depression, as

Table 3 Allele and genotype distribution for 5-HTTLPR, 5-HTTLPR and STin2.VNTRs polymorphisms of the serotonin transporter gene

| 5-HTTLPR | Count (Frequency) |
|----------|-------------------|
| L/L      | 358 (0.65)        |
| L/S      | 155 (0.28)        |
| S/S      | 38 (0.07)         |

5-HTT, serotonin transporter linked polymorphic region; L, long allele; S, short allele; L/L, long long; L/S, long short; S/S, short short genotypes; L_p and S_p are over expressing alleles while L_s and S_s are under expressing alleles of the 5-HTTLPR/rs25531 locus; STin2, Serotonin transporter intron 2; VNTRs, Variable number of tandem repeats; 10, 10 repeat VNTRs and 12, 12 repeat VNTRs for STin2 VNTRs

Table 4 Association between the 5-HTTLPR, rs25531 and STin2 VNTR and increased suicidal risk

| Polymorphism/allele | Suicide risk categories | No Bonferroni correction (α = 0.05) | With Bonferroni correction (α = 0.0056) |
|---------------------|-------------------------|-------------------------------------|------------------------------------------|
| STin2.VNTRs         | Genotype                | Allelic                             | Genotype | Allelic |
| rs25531             | 0.0732                  | 0.0732                              | 0.6125  | 0.6125  |
| 5-HTTLPR            | L_A                     | *0.0068                             | 0.0068  | 0.0068  |
|                     | S_A                     | *0.0046                             | 0.0046  | 0.0046  |
|                     | L_G                     | 0.3491                              | 0.3491  | 0.3491  |
|                     | S_G                     | 0.5686                              | 0.5686  | 0.5686  |

Func_Comb *0.0145 0.0145

*p values were corrected for study site, socio-economic status and major depressive disorder

*Significant effect, Func_Comb = Functional combination (over expressing (L_A, S_p) vs lower expressing (L_s, S_p) alleles). The STin2.VNTRs serotonin transporter intron 2 variable number of tandem repeats, rs25531, A to G single nucleotide polymorphism at position 25,531 of the serotonin transporter gene. 5-HTTLPR-rs25531, Serotonin transporter linked polymorphic region –rs25531 haplotype
dysfunction of serotonin neurotransmission has been associated with occurrence of major depressive disorder [40]. As depression has been found to be an independent predictor of moderate-to-high suicidal risk among ambulatory HIV patients in Uganda [1], we hypothesized that the overexpressing alleles would be the risk alleles for increased suicidal risk. A nominally significant association between the 5-HTTLPR and increased suicidal risk (p-value = 0.0174) was found with the S-allele being protective and the L-allele being a risk factor for suicidality. Previous studies of the 5-HTTLPR have found the S-allele to be a risk factor for increased suicidality [41], while others have found no significant association [42]. The discrepancy in findings may be related to sample size, sample ethnicity or other confounders such as sampling methods. Thus proper control for confounders is critical when replicating findings.

The 5-HTTLPR-rs25531 L_A allele was found to be associated with an increased risk of suicidality (p-value = 0.0068), with a 53% increase in the odds of an individual being in a higher suicide risk category (from no-to-low, or low-to-moderate risk) if they carry the L_A allele (95% CI: 12% to 112%), however this association became less significant after Bonferroni correction. The L_G and S_G alleles were not significantly associated with increased suicidal risk (p-values = 0.3491 and 0.5686 respectively), while individuals possessing the S_A allele were found to have a 43% (95% CI: 14% to 63%) reduction in odds of increased suicidal risk (p-value = 0.0046).

Our results are somewhat in line with those from previous studies. Henriette et al. [43] found a statistically significant protective effect of the 5-HTTLPR S-allele for suicide among individuals below 35 years of age with a contrasting statistically significant protective effect of the high expression genotype (L-allele) for individuals between 35 and 49 years in a Danish sample. We did not stratify for age, thus the significance of age suggested by Henriette was not tested for. In addition, Goldman et al., 2010 [44] observed low rates of depression, despite a high frequency of the S-allele, among an East Asian population. Also, among Caucasians of American descent, those with the S/S genotype had lower 5-HIAA levels than those with either L/L or L/S genotypes [45]. These findings seem to suggest that the S-allele is protective against depression in African-Americans and East-Asian populations, unlike in Caucasian populations, where it acts as a risk allele. The discrepancy amongst these different populations could be due to linkage disequilibrium between the S- allele and other 5-HTT polymorphisms, such as the functional single nucleotide polymorphisms (SNPs) at rs25532 and rs6355 [37], that may result in a reversal of the effect of the S/S genotype on serotonin turnover among Africans or African-Americans which may be absent in Caucasian populations.

The association of 5-HTTLPR with increased suicidal risk has been contradictory across various ethnicities. However, a recent review by Schild et al. [46] has reported a protective role of the S allele in Caucasian populations, whilst acting as a risk factor in non-Caucasian populations. In the present study, the S_A allele was protective against increased suicidal risk, a finding similar to that observed among Caucasians [46]. The discrepancy between findings of Goldman et al. [46] and those of Schild et al. [46] may be due to the fact that the non-Caucasians referred to by Goldman et al. [44] were of East Asian origin. Extrapolating previous findings seems difficult for samples of African ethnicity since there is paucity of data in the field. The contradiction in findings among Caucasians, Asians and African-Americans/Africans seems to suggest differences in 5-HT risk alleles and suicidality among different races, supporting the finding by Schild and colleagues [46] that ethnicity moderates the association between 5-HTTLPR and national suicide rates.

A nominally significant association between the STin2 VNTR and increased suicidal risk (p-value = 0.0285) was found in the present study. However, this association became non-significant after Bonferroni correction. The STin2.12 allele increased the odds of an individual falling into a higher suicidal risk category group, while the STin2.10 allele decreased these odds. A study by Bah and colleagues [47] found an association of the STin2.12 allele with a history of suicide attempts, which concurs with our findings. However, a previous study by Lopez de Lara and colleagues [48] found a significant association of suicide completion with having at least a copy of the STin2.10 allele. Another study by Lee and colleagues [31] found the STin2.10 allele to be associated with suicidal behavior. Findings by Lee et al. [31] and Lopez de Lara et al. [48] are in agreement, whilst contradicting those of the present study, which are in agreement with the findings of Bah et al. [47]. Mackenzie and Quinn, [49] reported the STin2.12 to possess greater transcription activity of the 5-HTT than the STin2.10. The STin2.12 could thus be compared to the overexpressing L_A allele whilst the STin2.10 could be compared to the less expressing S_A allele. As per our hypothesis that over expressing alleles of the 5-HTT confer increased suicidal risk to the bearer, the over expressing L_A allele of the 5-HTTLPR/rs25531 was found to be a risk allele while the lower expressing S_A allele was found to be protective against increased suicidal risk. Since the STin2.12 compares with L_A and STin2.10 compares with S_A in their capacity to direct transcription of the 5-HTT, this is interesting, as the finding at the polymorphic intron 2 region replicates that at the 5-HTTLPR.

Neither the 5-HTTLPR nor the STin2 VNTRs genotypes were in Hardy-Weinberg equilibrium (HWE).
There exist a number of reasons for deviation from HWE, including selection bias and genotypic errors. In the present study, it is likely that deviation from HWE could result from selection bias. Participants were not randomly selected from the general population, but were all HIV+ adults who were recruited from HIV care clinics. Another cause of the Hardy-Weinberg Disequilibrium (HWD) could have been genotyping errors as genotyped samples were not re-genotyped in order to eliminate the presence of genotypic errors. However, genotyping errors as a cause of HWD is contentious - McCarthy and colleagues [50] reported, in hypothetical data, that the presence of HWE was generally not altered by the introduction of genotyping errors [51], thus ruling out genotypic errors as the possible cause of the HWD. Finally, the violation of the assumptions for HWE could have been due to population stratification or selection bias as discussed above. Future studies should endeavour to control for these when sampling.

This is first study to investigate the interactions between 5-HTTLPR and 5-HTTLPR-rs25531 polymorphisms, STin2.VNTR polymorphisms and increased suicidal risk in PLWHA.

Limitations
Population stratification was not controlled for at analysis. We assumed that majority of our sample was of Bagandan ethnicity based on the language spoken (Luganda) and self report. There is a possibility that some participants were not Bagandan even though they could speak Luganda, indicating that we may have had an admixed sampled, resulting in violation of HWE. Future studies should focus on ethnicity using ancestry informative markers to delineate subjects genetically, rather than basing ethnic differentiation on language spoken and or self report.

The significant associations of the STin2 VNTRs observed became non-significant when we controlled for multiple testing using the Bonferroni correction. It is possible that the significance observed for the above named polymorphisms may have been due to chance. Replication studies in the same population are, therefore, needed to confirm these findings.

Conclusions
Both the LA allele of the polymorphism at the 5-HTTLPR/rs25531 locus and the 12-repeat of the polymorphism at STin2 region in the 5-HTT are risk alleles for increased suicidal risk among PLWHA in a Ugandan population.

Results of the present study require replication in a larger study, which is more equipped to address population stratification, and provides more power for association analyses.

There is need for further genotyping studies in Ugandan samples to confirm these findings.

The majority of previous studies have investigated the 5-HTT in Caucasian populations in Europe and America and more studies are needed in African populations.

Abbreviations
5-HIAA: 5-hydroxy indole acetic acid; 5-HT: Serotonin; 5-HTT: Serotonin transporter; 5-HTTLPR: Serotonin transporter linked polymorphic region; CSF: Cerebral spinal fluid; DNA: Deoxyribonucleic acid; EDCTP: European Developing Countries Clinical Trials Partnership; HIV/AIDS: Human Immunodeficiency Virus/ Acquired immunodeficiency syndrome; MINI: Mini International Neuropsychiatric Interview; PCR: Polymerase chain reaction; PLWHA: People living with HIV/AIDS; SNPs: Single nucleotide polymorphisms; STin2: Serotonin transporter intron 2; TASO: The Aids Supporting Organisation; VNTR: Variable number tandem repeat

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Availability of data and materials
All information gathered about study participants and their samples is confidential, with access limited to the research team. However, upon request, data from the MRC/UVRI Uganda Research Unit on AIDS is currently accessed under a data sharing policy via: http://www.mrcuganda.org/sites/default/files/publications/MRC_UVRI_Data_sharing_policy_December2015.pdf.

Consent for publication
No details, images or videos relating to any of the study participants are included in this manuscript.

Ethics approval and consent to participate
This study obtained ethics approval from both the Science and Ethics Committee of Uganda Virus Research Institute (Ref # GC/127/11/08/04), and the Health Research Ethics Committee of Stellenbosch University (Ref # N11/11/324). The parent EDCTP study obtained ethics approval from the Uganda National Council of Science and Technology (Ref # HS 1053). All study participants provided informed consent to participate in the study and for a blood specimen to be withdrawn from them for the genetics analyses.

Authors’ contributions
Concept: EK, AK, SS, SH; Data collection: EK, AK, NN, HB, JS. Data analysis: EK, AK, LvdM, SS; First draft: EK, AK, SS, SH, MJ, AN; Final revision: EK, AK, SS, SH, LvdM, MJ, AN, NN, HB, JS, PK; All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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