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Neurocognitive outcomes in indonesians living with HIV are influenced by polymorphisms in the gene encoding purinergic P2X receptor 7

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ABSTRACT

The advent of effective antiretroviral therapy (ART) has decreased the prevalence and severity of HIV-associated neurocognitive disorders (HAND), but milder forms of HAND remain despite optimal treatment. Neuronal injury and loss due to inflammation may mediate HAND. *P2X7R* encodes purinergic P2X receptor 7 which influences neuroinflammatory pathways and carries polymorphisms associated with sensory neuropathy in HIV patients. We assessed associations between *P2X7R* polymorphisms and neurocognitive outcomes in Indonesian patients (n = 59) as they commenced ART and after 3, 6 and 12 months. Z-scores were calculated over 5 domains using local controls and evaluated as continuous variables. Optimal linear regression models identified polymorphisms influencing attention, memory, executive function, motor speed and total cognitive function at each time point. rs504677 was associated with lower executive and motor speed Z-scores at 0, 3, 6, and 12 months, and with memory at 0 and 12 months. Memory was positively influenced by carriage of the rs208296 minor allele at 0, 3 and 6 months and by carriage of the rs208307 minor allele at 0 and 12 months. Higher attention Z-scores associated with carriage of minor alleles of rs1653598 after 0 and 12 months. These also positively influenced executive function and motor speed after 0–6 months. This study identifies polymorphisms in *P2X7R* which influence domain-specific neurocognitive outcomes in HIV+ Indonesians prior to and shortly after commencing ART. This implicates purinergic P2X receptor 7 in the pathogenesis of HAND.

1. Introduction

Effective antiretroviral therapy (ART) has decreased the prevalence and severity of HIV-associated neurocognitive disorders (HAND), but milder forms of HAND remain a serious complication of HIV infection (Estiasari et al., 2015; Heaton et al., 2010a; Cysique et al., 2014). Neurocognitive impairment affects up to 50% of ART naive Indonesian patients with <200 CD4 T-cells/μl, and improvements in neurocognitive function after 6 months of ART are influenced by age, education and CD4 T-cells/μl (Estiasari et al., 2015, 2020). In vivo and in vitro studies of the neuropathology of HAND identify neuroinflammation as a crucial mediator of neurocognitive impairment [reviewed in (Ru and Tang, 2017)]. HIV-infected monocytes and CD4 T-cells cross the blood-brain barrier, resulting in infection and activation of microglia and astrocytes. These cells release proinflammatory cytokines and chemokines including tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), interleukin-1β (IL-1β), chemokine (C–C motif) ligand 2 (CCL2), and viral proteins including transactivator of transcription (Tat) and glycoprotein 120 (gp120), resulting in release of adenosine triphosphate (ATP), intracellular influx of calcium ions (Ca^{2+}), and oxidative stress. This leads to further inflammation and neuronal and synaptic dysfunction characteristic of neurocognitive impairment and HAND (Ru and Tang, 2017).

Purinergic P2X receptor 7 (P2X7R) is an ATP-gated non-specific cation channel involved in neuroinflammatory pathways [reviewed in (Alves et al., 2020)]. P2X7R is highly expressed in the brain and is activated by high levels of ATP released from damaged cells, triggering

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an influx of Ca^{2+} and efflux of potassium ions (K^+) (Alves et al., 2020). P2X7R-dependent intracellular depletion of K^+ drives the assembly of the nucleotide-binding domain (NOD)-like receptor protein 3 (NLRP3) inflammasome, promoting the accumulation of caspase-1 which cleaves pro-IL-1β and releases mature IL-1β (Giuliani et al., 2017). P2X7R activation induces NADPH oxidase-dependent production of IL-6 from astrocytes (Munoz et al., 2020) and the release of TACE (TNFα converting enzyme) which subsequently increases release of TNFα from microglia (Barberà-Cremades et al., 2017). Moreover, in a rodent model of cognitive dysfunction induced by gp120, P2X7R expression was significantly higher in the hippocampus compared to control groups (Liu et al., 2017). In rat primary cultured microglia, application of gp120 stimulated P2X7R expression, increased concentrations of TNFα and IL-1β, and resulted in microglial apoptosis (Chen et al., 2017). In human astrocyte and neuron cultures, treatment with Tat resulted in calcium-dependent upregulation of P2X7R, release of CCL2 from astrocytes, and direct and indirect neuronal death (Tewari et al., 2015).

The gene encoding P2X7R (*P2X7R*) is highly polymorphic and located in a region of high linkage disequilibrium (LD) on chromosome 12. We have associated single nucleotide polymorphisms (SNP) and haplotypes within *P2X7R* with HIV-associated sensory neuropathy (a common neurological complication of HIV infection affecting peripheral nerves) in Indonesians and Africans (Gaff et al., 2019a, 2020; Goullee et al., 2016; Safri et al., 2020). Furthermore, SNP in *P2X7R* have been associated with neuroinflammatory and neuropsychological conditions including bipolar disorder, multiple sclerosis, and Alzheimer's disease (Sanz et al., 2014; Oyanguren-Desez et al., 2011; McQuillin et al., 2009). In the present study, we assessed associations between SNP in *P2X7R* and neurocognitive outcomes across five neurocognitive domains in HIV+ Indonesians as they commenced ART and after 3, 6 and 12 months.

2. Materials and methods

2.1. Participants

The JakCCANDO study conducted in Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia (Wulandari et al., 2017) recruited 82 adults with horizontally-acquired HIV infection (Estiasari et al., 2015, 2020), with <200 CD4 T-cells/μl, a Karnofsky performance score 70–100, living in Jakarta, and providing written and informed consent to participate in the study. Participants with a history of head injury, stroke, recurrent seizures, severe depression (Hamilton Depression Scale) (Estiasari et al., 2015), neurological deficits which may interfere with neurocognitive evaluation, pregnancy, breastfeeding and current use of illicit drugs were excluded. To establish a normative value, we recruited 82 local healthy controls similar in proportion of males (48% vs 68%), age (30 vs 31 years) and education (85% completed 9 years of education vs 78%) using the same criteria, plus no declared history of HIV risk behaviour. Subjects were assessed for pulmonary tuberculosis (TB), plasma HIV RNA was quantitated using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Tests (version 2.0) and CD4 T-cell counts were determined using standard flow cytometric techniques. CD4 T-cells/μl and plasma HIV-RNA were assessed before ART and after 3, 6 and 12 months. The study was approved by the Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo National General Hospital, and Curtin University ethics committees.

2.2. Neurological assessments

All participants underwent baseline neurological assessment for five cognitive domains as per published methods (Estiasari et al., 2015, 2020). Follow up assessments were completed for HIV+ participants after receiving ART for 3, 6 and 12 months. Tests included Forward Digit Span to evaluate attention, Animal Naming Test to assess fluency, Rey Auditory Verbal Learning Test (immediate recall, delayed recall and learning over trials) to assess memory, Trail Making Test A and B to asses executive function, and Grooved Peg Board to assess motor speed. Z-scores for each domain were calculated by subtracting the neurocognitive test result from the mean normative value (obtained from the healthy controls) and dividing by the standard deviation of the normative value. The total cognitive Z-scores are an average of the Z-scores represented by each cognitive domain. Of the 82 participants, 21 participants did not complete follow-up clinical and neurocognitive assessments due to withdrawal from ART (n = 4), pregnancy (n = 2), death (n = 4), relocation (n = 4), and loss of contact (n = 7).

2.3. Genotyping

DNA samples were available for 59 of the 61 participants with complete neurological assessments. DNA samples were adjusted to 50 ng/μl and diluted 1:1 with TaqMan® OpenArray™ Genotyping Master Mix. Samples were genotyped for 20 SNP across *P2X7R* (Supplementary Table 1) using custom TaqMan® OpenArray™ Real-Time PCR Plates with the QuantStudio 12 K Flex Real-Time PCR System (Life Technologies, NY) and genotypes were assigned manually using the TaqMan® Genotyper Software and assessed as a dominant model (homozygous major allele versus hetero- or homozygous carriage of the minor allele). rs2230911 and rs3751144 were invariably co-inherited, so rs2230911 was excluded from analyses. Two SNP which failed to genotype in >10% of samples (rs208288 and rs1653609), two SNP that were monoallelic (rs10160951 and rs2230912), and three SNP that were carried by <10% of this group (rs1169737, rs17525767 and rs2857585) were excluded from analyses.

2.4. Statistical analyses

The effect of markers of HIV disease on neurocognitive Z-scores in HIV+ participants were assessed for each domain at 0 and 12 months on ART using Wilcoxon matched-pairs signed rank tests in GraphPad Prism version 8.2.1 for Windows (GraphPad Software, La Jolla, CA, USA). Multiple linear regression models were generated for Z-scores at all four time points, for each of the five cognitive domains and the total cognitive scores (Tables 2–5). Models included the SNP from *P2X7R* and demographic and clinical variables previously associated with neurocognitive outcomes; age, CD4 T-cells/μl and education (years) (Estiasari et al., 2020). Optimal models were determined using backward elimination of demographic and genetic predictors with $p > 0.1$ using the 'olsrr' v0.5.3 package (Hebbali, 2020) for the R programming language (Team, 2020). Models with a $p < 0.05$, an adjusted R-squared ≥ 0.1 and including one or more SNP are considered significant and discussed further (Tables 2–5). Regression coefficients are represented by Beta.

3. Results

3.1. Markers of HIV disease and cognitive function improved over 12 months on ART

Demographic, clinical and neurocognitive outcomes in this subset of 59 participants reflects the parent cohort, and demonstrate improvement of markers of HIV disease and neurocognitive function as described previously (Estiasari et al., 2015, 2020). The median (range) CD4 T-cell/μl and $\log_{10}$ of plasma HIV RNA/ml was 67 (2–199) and 5.15 (2.64–6.68) at baseline, and improved to 288 (44–763) and 1.30 (1.30–6.32), respectively, at 12 months on ART (Table 1), so ~60% of participants had undetectable plasma HIV RNA after 12 months of ART (Estiasari et al., 2020). TB was identified in 27/59 (46%) participants at baseline but did not influence Z-scores at baseline or at 12 months of ART ($p > 0.05$ for all cognitive domains; Supplementary Table 2). Z-scores improved between baseline and 12 months of ART ($p < 0.05$ for all cognitive domains; Table 1), with the exception of memory ($P = 0.67$; Table 1).

Table 1
Markers of HIV disease and neurocognitive outcomes improved over 12 months on ART.

	Time on ART				*0m vs 12m*
Variable[a]	0 months	3 months	6 months	12 months	*P*[b]
Age (years)	31 (19–48)				
Male Gender[c]	43 (73%)				
Education (years)	12 (6–16)				
TB co-infection[c]	27 (46%)				
CD4 T-cells/μl	67 (2–199)	189 (7–601)	208 (6–516)	288 (44–763)	<0.0001
Log_{10} HIV RNA/ml	5.15 (2.64–6.68)	1.67 (1.30–5.23)	1.30 (1.30–5.23)	1.30 (1.30–6.32)	<0.0001
Z-Attention	−0.78 (−2.44 to 1.81)	−0.53 (−1.94 to 2.23)	−0.36 (−1.94 to 1.81)	−0.11 (−1.94 to 2.23)	0.0004
Z-Fluency	−0.57 (−3.67 to 1.57)	−0.33 (−2.48 to 3.71)	−0.10 (−2.48 to 1.22)	−0.10 (−2.48 to 3.48)	<0.0001
Z-Memory	−2.06 (−6.92 to 1.22)	−2.46 (−6.92 to 1.22)	−2.49 (−6.96 to 1.16)	−2.72 (−6.19 to 1.25)	0.67
Z-Executive	0.26 (−5.94- to 1.29)	0.63 (−15.03 to 1.52)	0.77 (−3.00 to 1.50)	0.93 (−2.88 to 1.57)	<0.0001
Z-Motor Speed	−0.14 (−7.90 to 1.01)	0.21 (−8.88 to 1.43)	0.58 (−1.40 to 2.96)	0.65 (−2.82 to 1.75)	<0.0001
Z-Total Cognitive Function	−0.84 (−4.82 to 0.67)	−0.45 (−7.24 to 1.33)	−0.35 (−1.99 to 1.13)	−0.25 (−2.05 to 1.17)	<0.0001

[a] Median (range).
[b] Wilcoxon matched-pairs signed rank test between 0 and 12 months.
[c] n (%).

Table 2
SNP in *P2X7R* influence neurocognitive Z-scores prior to commencing ART.

Variable	Beta	95% CI[a]		*P*
		2.5%	97.5%	
Attention: Adjusted $R^2 = 0.112$ Model P = 0.030				
rs11065464	0.44	−0.002	0.89	0.05
rs504677	−1.03	−1.88	−1.18	0.02
rs1653598	1.13	0.26	2.00	0.01
Fluency: Adjusted $R^2 = 0.140$ Model P = 0.002				
Education	0.53	0.20	0.86	0.002
Memory: Adjusted $R^2 = 0.231$ Model P = 0.005				
rs208296	1.03	0.02	2.03	0.05
rs208307	2.62	1.01	4.24	0.002
rs503720	−2.20	−4.78	0.37	0.09
rs504677	−3.25	−5.42	−1.09	0.004
rs1653598	2.51	−0.07	5.08	0.06
Executive Function: Adjusted $R^2 = 0.310$ Model P = 0.0003				
Education	0.53	0.20	0.86	0.003
rs208307	0.78	−0.07	1.63	0.07
rs504677	−2.14	−3.22	−1.05	0.000
rs1653598	1.49	0.52	2.46	0.003
Motor Speed: Adjusted $R^2 = 0.260$ Model P = 0.0004				
Education	0.62	0.20	1.04	0.005
rs504677	−2.19	−3.39	−0.98	0.001
rs1653598	2.27	1.03	3.51	0.001
Total Cognitive: Adjusted $R^2 = 0.321$ Model P = 0.0002				
Education	0.39	0.12	0.67	0.006
rs208307	1.03	0.33	1.73	0.005
rs504677	−1.86	−2.76	−0.96	0.000
rs1653598	1.02	0.22	1.82	0.01

[a] CI; Confidence Interval.

Table 3
SNP in *P2X7R* influence neurocognitive Z-scores after 3 months on ART.

Variable	Beta	95% CI[a]		*P*
		2.5%	97.5%	
Attention: Adjusted $R^2 = 0.176$ Model P = 0.006				
Age	−0.06	−0.10	−0.02	0.007
rs208307	−0.58	−1.12	−0.04	0.04
rs3751144	−0.54	−1.08	−0.01	0.05
Fluency: Adjusted $R^2 = 0.136$ Model P = 0.002				
Education	0.20	0.07	0.32	0.002
Memory: Adjusted $R^2 = 0.193$ Model P = 0.005				
Age	−0.10	−0.18	−0.03	0.008
rs1718125	1.07	1.04	2.04	0.03
rs208296	0.87	−0.05	1.80	0.06
rs12301635	−1.33	−2.49	−0.18	0.03
Executive Function: Adjusted $R^2 = 0.433$ Model P = 0.0000				
Age	0.07	−0.01	0.16	0.08
Education	0.31	0.12	0.50	0.002
rs208307	2.75	1.04	4.45	0.002
rs504677	−6.37	−8.59	−4.16	0.000
rs1653598	3.89	1.95	5.82	0.000
Motor Speed: Adjusted $R^2 = 0.334$ Model P = 0.0001				
Education	0.15	0.04	0.26	0.07
rs208307	1.05	0.09	2.01	0.03
rs504677	−2.84	−4.06	−1.61	0.000
rs1653598	1.81	0.71	2.91	0.002
Total Cognitive: Adjusted $R^2 = 0.324$ Model P = 0.0002				
Education	0.16	0.02	0.30	0.03
rs208307	1.54	0.26	2.83	0.02
rs504677	−3.85	−5.49	−2.21	0.000
rs1653598	2.69	1.22	4.16	0.001

[a] CI; Confidence Interval.

3.2. Linear regression models identified SNP from P2X7R as predictors of neurocognitive outcomes in all domains, except fluency

Linear regression models for the Z-scores of the five neurocognitive domains and total cognitive function at 0, 3, 6 and 12 months of ART were determined using backward elimination of covariables with $p > 0.1$ (Tables 2–5). Optimal models achieving analyses criteria of an adjusted $R^2 > 0.1$, model $p < 0.05$ and inclusion of ≥1 SNP were identified for all domains except fluency and are presented separately for each time point (Tables 2–5). Outcomes for each domain are described below.

Attention: Three SNP (rs504677, rs11065464 and rs1653598) remained in the optimal model before ART (Table 2), with rs1653598 remaining at 12 months (P = 0.006). Carriage of the minor allele of rs1653598 associated with positive attention outcomes at baseline and at 12 months (Tables 2 and 5). Attention also associated with rs10849849 and rs1718125 at 12 months (P = 0.001 and 0.005, respectively; Table 5).

Fluency: Models from all time points achieved an adjusted $R^2 > 0.1$ with $P < 0.001$, but none retained any *P2X7R* SNP. Fluency was consistently influenced by level of education (Tables 2–5).

Memory: The optimal model for memory outcomes before ART had an adjusted R^2 of 0.231 (P = 0.005) and included five SNP, of which rs208296, rs208307 and rs504677 were significantly associated with memory Z-scores ($P < 0.05$; Table 2). rs208296 remained in the optimal memory model at 3 months (P = 0.06; Table 3), and associated with

Table 4
SNP in *P2X7R* influence neurocognitive Z-scores after 6 months on ART.

Variable	Beta	95% CI[a]		*P*
		2.5%	97.5%	
Attention: Adjusted $R^2 = 0.026$ Model P = 0.117				
Education	0.06	0.00	0.14	0.12
Fluency: Adjusted $R^2 = 0.237$ Model P = 0.0002				
Age	−0.06	−0.11	−0.01	0.03
Education	0.22	0.10	0.34	0.001
Memory: Adjusted $R^2 = 0.202$ Model P = 0.002				
Age	−0.14	−0.21	−0.06	0.000
rs208296	0.94	0.00	1.88	0.05
rs1653598	−0.85	−1.78	0.09	0.07
Executive Function: Adjusted $R^2 = 0.221$ Model P = 0.0013				
Education	0.10	0.05	0.16	0.001
rs504677	−0.70	−1.27	−0.13	0.02
rs1653598	0.67	0.09	1.26	0.03
Motor Speed: Adjusted $R^2 = 0.135$ Model P = 0.016				
Age	−0.03	−0.06	−0.002	0.04
rs504677	−0.74	−1.42	−0.06	0.03
rs1653598	0.64	−0.06	1.33	0.07
Total Cognitive: Adjusted $R^2 = 0.320$ Model P = 0.0001				
Age	−0.05	−0.08	−0.02	0.001
Education	0.10	0.04	0.17	0.004
rs208296	0.33	−0.04	0.71	0.08
rs504677	−0.33	−0.70	0.04	0.08

[a] CI; Confidence Interval.

Table 5
SNP in *P2X7R* influence neurocognitive Z-scores after 12 months on ART.

Variable	Beta	95% CI[a]		*P*
		2.5%	97.5%	
Attention: Adjusted $R^2 = 0.182$ Model P = 0.009				
rs10849849	1.52	0.70	2.34	0.001
rs1718125	−1.08	−1.82	−0.34	0.005
rs11065464	0.46	−0.03	0.94	0.06
rs1653598	0.91	0.28	1.55	0.006
Fluency: Adjusted $R^2 = 0.137$ Model P = 0.002				
Education	0.67	0.52	1.10	0.002
Memory: Adjusted $R^2 = 0.058$ Model P = 0.087				
rs208307	1.71	0.13	3.28	0.03
rs504677	−1.61	−3.18	−0.03	0.05
Executive Function: Adjusted $R^2 = 0.277$ Model P = 0.007				
rs10849849	0.48	0.03	0.93	0.04
rs208307	0.75	0.12	1.39	0.02
rs504677	−1.03	−1.69	−0.36	0.003
rs3751144	−0.64	−1.11	−0.16	0.009
Motor Speed: Adjusted $R^2 = 0.129$ Model P = 0.010				
CD4 T-cells/μL	−0.001	−0.003	0.00	0.03
rs504677	−0.44	−0.86	−0.02	0.04
Total Cognitive: Adjusted $R^2 = 0.321$ Model P = 0.0002				
Age	−0.03	−0.06	−0.004	0.02
rs504677	−0.32	−0.67	0.04	0.08

[a] CI; Confidence Interval.

memory at 6 months (P = 0.05; Table 4). The rs208296 minor allele had a positive effect on memory outcomes at 0, 3 and 6 months (Tables 2–4). At 12 months, rs208307 and rs504677 were significantly associated with memory, but the model did not meet our analyses criteria (Table 5).

Executive function: Robust models were identified for executive function at 0, 3, 6 and 12 months after ART (Adjusted $R^2 = 0.22$ to 0.43, P = 0.007 to 0.0000; Tables 2–5). Three SNP (rs504677, rs208307 and rs1653598) consistently influenced executive outcomes after adjusting for education at 0, 3 and 6 months on ART and for age at 3 months on ART. Carriage of the minor allele of rs504677 associated with lower Z-scores at all four time points. Optimal models at baseline and 3 months retained rs208307 and rs1653598, at 6 months included rs1653598 and at 12 months included rs208307 (Tables 2–5).

Motor speed: The optimal model at baseline, 3 and 6 months included rs504677 and rs1653598 after adjusting for education (0 and 3 months; Tables 2 and 3) or age (6 months; Table 4). At 12 months, only rs504677 remained in the optimal model after adjustment for CD4 T-cell counts. The effects of age and CD4 T-cells/μl on motor speed Z-scores were marginal and rs504677 was consistently linked with lower Z-scores, as noted with other domains.

Total cognitive function: Optimal models at each time point reflected models for each domain at the corresponding time point. All included *P2X7R* SNP after adjusting for education (0, 3 and 6 months; Tables 2–4) and/or age (6 and 12 months; Tables 4 and 5). Accordingly, carriage of the minor allele of rs504677 is independently associated with lower Z-scores at baseline (P = 0.0006; Table 2) and at 3 months after ART ($p < 0.001$; Table 3), and remained in the optimal models at 6 and 12 months but did not reach significance (P = 0.08 and 0.08; Tables 4 and 5).

4. Discussion

This study provides unique insights into neurocognitive function across five domains in HIV+ Indonesians prior to commencing ART and at 3, 6 and 12 months of ART. Furthermore, we assessed associations between *P2X7R* SNP and the neurocognitive outcomes at these time points. This creates a large number of potential associations, and the longitudinal design complicates corrections for multiple comparisons. We address this by confining the discussion to associations evident in multiple domains or more than one timepoint. We report improvements of neurocognitive outcomes and associations with *P2X7R* SNP that vary by domain and time on ART.

By following patients responding to ART, we also shed light on the process of recovery of neurocognitive capacity. Patients began ART with <200 CD4 T-cells/μl, so they were experiencing a range of inflammatory symptoms affecting their general health and neurocognitive performance. With the exception of the memory domain, all Z-scores improved over time to approximate the healthy controls after 6–12 months (Estiasari et al., 2020), but rates differed by domain. It is therefore plausible that anti-inflammatory mechanisms were activated differentially or as a cascade. Rubin et al. demonstrated that levels of microglial activation varied between brain regions of virally-suppressed HIV+ individuals, and higher levels of microglial activation are inversely associated with neurocognitive outcomes in a domain-specific manner (Rubin et al., 2018). Additionally, immune responses can be affected by suboptimal adherence or changes to ART (García de Olalla et al., 2002). Short periods of non-adherence to ART have been associated with memory deficits (Obermeit et al., 2015). This may complicate longitudinal analyses. It is pertinent here that only ~60% of participants were virally suppressed after 12 months and several had changed their treatment regimens.

Memory was the only domain that did not improve over 12 months of ART (Table 1). Memory deficits have been described in individuals with viral suppression through effective ART (Rubin et al., 2018; Heaton et al., 2010b), but may also reflect the severity of HIV disease before treatment (Tozzi et al., 2007; Ellis et al., 2011). Participants in our study commenced ART with only 67 (2–199) CD4 T-cell/μl - which may enhance persistent neurological defects. Furthermore, the JakCCANDO cohort has a high burden of cytomegalovirus (CMV). CMV was associated with more severe HIV disease in a Thai population (Durier et al., 2013) and with neurocognitive impairment in older adults without HIV (Luz Correa et al., 2014). We associated the baseline burden of CMV with

memory and total cognitive outcomes after 6 months on ART in this population (Estiasari et al., 2020).

No alleles of *P2X7R* associated with fluency scores and optimal models consistently associated fluency with education (Tables 2–5). The cohort included individuals with only primary education and several who had completed university. The socioeconomic sequelae of this range in a large Asian city (Jakarta) provides further scope to identify an effect as "education". Older age has been associated with poorer fluency outcomes (Elgamal et al., 2011) but the cohort is relatively young and uniform in age [31 (19–48) years].

When we commenced this study, we anticipated associations with coding SNP which impact the function of P2X7R and therefore influence inflammation. We included six exonic SNP, but only two met our inclusion criteria for bivariate and multivariable analyses (Supplementary Table 1). One of the SNP excluded was rs2230912, a missense variant associated with neuropsychological disorders (McQuillin et al., 2009; Erhardt et al., 2007), as the minor allele was not detected in this population. This highlights differences in patterns of LD between ethnicities and may suggest P2X7R pathways differ between disease phenotypes. Our results instead identified five intronic SNP which influence neurocognitive outcomes in a time- and domain-specific manner in HIV+ Indonesians as they recover on ART. These will be discussed individually.

4.1. rs504677

rs504677 associated with lower Z-scores across all domains (except fluency) and at all four timepoints in executive function, motor speed and total cognitive function (Tables 2–5). rs504677 has a RegulomeDB score of 2b (https://regulome.stanford.edu), suggesting this SNP affects transcription factor binding and therefore may influence neurocognitive outcomes via the regulation of expression and splicing of *P2X7R* (Boyle et al., 2012). This variant has been linked to altered expression and splice variants of both *P2X7R* and the neighbouring gene encoding purinergic P2X receptor 4 (*P2X4R*) in the Gene Tissue Expression (GTEx) Portal (https://gtexportal.org/; accessed Nov 2020). Furthermore, rs504677 is in LD (r^2 and D' >0.90) with more than 25 intronic *P2X7R* SNP within a ±5000 base pair region in the 1000 Genomes East Asian (EAS) populations, and so may also mark a causal variant outside our genotyping panel (https://ldlink.nci.nih.gov/).

4.2. rs1653598

rs1653598 occurs in all optimal models prior to commencing ART (excluding fluency), with executive and motor speed domains at 3 and 6 months, memory at 6 months, and attention at 12 months (Tables 2–5). Carriage of the minor allele of rs1653598 is consistently linked to higher Z-scores. As with rs504677, this allele is linked with altered expression and splicing of *P2X7R* and *P2X4R* (https://gtexportal.org/), and is in LD with over 25 *P2X7R* SNP in the EAS population in a window ±5000 base pairs. One SNP in LD ($r^2 = 1.0$, D' = 1.0) is a gain-of-function variant, rs1718119 (https://ldlink.nci.nih.gov/). rs1718119 is associated with higher pain intensity scores in females with diabetic peripheral neuropathy (Ursu et al., 2014) and with inflammatory conditions including systemic lupus erythematosus, chronic obstructive pulmonary disease, and localised aggressive periodontitis (Chen et al., 2013; Dai et al., 2018; Harris et al., 2020). Homozygous carriage of the minor allele of rs1718119 is associated with an ATP-induced increase of IL-1β from monocytes and with increased levels of IL-6 in whole blood (Harris et al., 2020; Stokes et al., 2010). This fits with a role in HAND and warrants investigation.

4.3. rs11065464

The rs11065464 minor allele was explicitly linked with modest improvements in attention Z-scores at baseline and after treatment with ART for 12 months (Tables 2 and 5). This allele is linked in the GTEx Portal with altered expression of *P2X4R* and with altered splicing of calcium/calmodulin-dependent kinase kinase 2 (*CAMKK2*) and *P2X7R* (https://gtexportal.org/) but evidence of a pathological role for this SNP is lacking.

4.4. rs208296

Carriage of the minor allele of rs208296 is consistently associated with higher memory Z-scores in the optimal models at 0, 3 and 6 months (Tables 2–4). rs208296 has a regulomeDB score of 1f (https://regulome.stanford.edu) indicating a highly regulatory role, and is associated with altered expression of *P2X4R* and splicing of *CAMKK2* (https://gtexportal.org/). Carriage of the minor allele correlates with increased cold pain tolerance in a Finnish population (Kambur et al., 2018) suggesting a neurological role for this SNP. However, no studies assess its role in inflammation or memory/cognition.

4.5. rs208307

rs208307 associated with higher Z-scores in memory and executive domain optimal models at multiple timepoints. This allele was linked with HIV-SN in Africans treated with stavudine but not in Africans and Indonesians treated without stavudine (Gaff et al., 2019b, 2020; Goullee et al., 2016). rs208307 is located at an acceptor splice site in intron 6 and is associated with altered expression and splicing of *P2X7R* in the GTEx Portal (https://gtexportal.org/). Furthermore, carriage of the rs208307 minor allele associated with higher levels of P2X7R mRNA lacking exons 7 and 8, which is predicted to impair P2X7R function (Skarratt et al., 2020). It is plausible that associations between memory and executive Z-scores and rs208307 are mediated by higher levels of exon 7 and 8 skipping in P2X7R mRNA and warrants investigations. We were able to replicate our finding in Australian HIV patients (Gott et al., 2017). These were males (n = 49) aged 57 (45–73) years, of European descent and tested after >2 years on ART. We associated carriage of the minor allele with higher scores for verbal learning [49 (18–66) vs 41 (10–61), Mann Whitney P = 0.04] and verbal memory [50 (23–61) vs 40 (10–63), P = 0.009] (unpublished data).

Overall, this study provides novel insights in time- and domain-specific neurocognitive changes in HIV+ Indonesians over the first 12 months on ART. We identify five intronic polymorphisms which associate with neurocognitive outcomes in specific domains. These may influence the levels or isoforms of P2X7R expressed, or may be in LD with causal SNP. P2X7R is abundantly expressed in microglia and overexpression of P2X7R drives microglial activation (Monif et al., 2016; Chen et al., 2016). Microglial activation differs between regions of the brain in HIV+ individuals and correlates with domain-specific cognitive impairments, so it is plausible that our SNP may impact HIV-associated neurocognitive outcomes. Further genetic investigations in larger cohorts of defined ethnicity are warranted.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

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