The chitinases expression is related to Simian Immunodeficiency Virus Encephalitis (SIVE) and in HIV encephalitis (HIVE)

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ABSTRACT

Objectives: Human Immunodeficiency Virus (HIV) infection can induce neurocognitive complications classified as HIV-associated neurocognitive disorder (HAND). The most severe form of HAND is HIV-associated dementia (HAD). It is very likely that HAD is a secondary response to innate immunity activation induced by HIV infection. The chitinase family is associated with innate immunity cells; the expression of chitinase family members is greatly amplified during many infections, highlighting their potential biological roles in inflammatory diseases and in innate immunoactivation. In this study, we have investigated the relationship between chitinases and macrophage/microglial activation in the brain of HAD.

Methods: We analyzed microarray datasets obtained from NCBI (http://www.ncbi.nlm.nih.gov/) under accession number GDS4214 to examine the expression of chitinase family genes in hippocampal specimens of uninfected rhesus macaques (animal control) versus those with histopathologic evidence of Simian Immunodeficiency Virus Encephalitis (SIVE). All SIV-infected monkeys had neuropathological evidence of SIVE on post-mortem histopathological examination. A portion of hippocampus was preserved frozen at necropsy for later RNA extraction. The brain viral load (BVL) was determined on a sample of frontal lobe, containing both grey and white matter, and is given in $\log_{(10)}$ viral genome equivalents per microgram RNA. For these studies, we also used the open source tools Genome-scale Integrated Analysis of gene Networks in Tissues (GIANT) to identify the chitinase genes network.

Results: *CHIT1*, *CHI3L1* and *CHI3L2* mRNA levels were significantly increased in SIVE hippocampus as compared to non-infected control specimens (p=0.0009, p=2.66E-13 and p=2.53E-12, respectively). Furthermore, we found a negative correlation between CHIA vs BVL (r= -0.829 and p=0.006) and positive correlation between C1s and CHIA (r=0.837 and p=0.0050), CHIT1 *vs* CHI3L2 and SLC11A1 (r=0.901, p=0.001; r=0.798, p=0.01 respectively), CHI3L2 *vs* CHID1 (r=0.782, p=0.013) and CHID1 *vs* SLC11A1 (r=0.880, p=0.002).

Conclusions: These results suggest that chitinase gene expression is altered in SIVE and call for more studies examining whether this is a protective immunological reaction or a destructive tissue response to SIV infection.

KEY WORDS: Chitinases; HIV-1; SIVE; Hippocampus

ABBREVIATION USED: human immunodeficiency virus 1 (HIV-1); simian immunodeficiency virus (SIV); SIV encephalitis (SIVE); acquired immune deficiency syndrome (AIDS); Chitotriosidase (CHIT1); acidic mammalian chitinase (CHIA); chitinase 3-like-1 (CHI3L1); chitinase 3-like-2 (CHI3L2); chitinase domain–containing 1 (CHID1); spermine oxidase (SMOX); discoidin, CUB and LCCL domain containing 2 (DCBLD2); low density lipoprotein receptor class A domain containing 4 (LDLRAD4); ADAM metallopeptidase with thrombospondin type 1 motif, 2 (ADAMTS2); Ras-related associated with diabetes (RRAD); complement component 1, s subcomponent (C1S); peripheral myelin protein 22 (PMP22); metallothionein 1B (MT1B); homer homolog 3 (Drosophila) (HOMER3); protein tyrosine kinase 2 (PTK2); solute carrier family 11 (proton-coupled divalent metal ion transporter), member 1 (SLC11A1); tripartite motif containing 10 (TRIM10); purinergic receptor P2X, ligand-gated ion channel, 6 (P2RX6); apolipoprotein C-IV (APOC4); Rho guanine nucleotide exchange factor (GEF) 15 (ARHGEF15); Genome-scale Integrated Analysis of gene Networks in Tissues (GIANT); MultiExperiment Viewer (MeV); edge score (ES); multiple sclerosis (MS)

INTRODUCTION

Human Immunodeficiency Virus (HIV) infection can induce neurocognitive complications classified as HIV-associated neurocognitive disorder (HAND) [1]. According to standardized measures of cognitive dysfunction, it is possible to define three stages of HAND: *asymptomatic neurocognitive impairment* (ANI), *mild neurocognitive disorder* (MND) and *HIV-associated dementia* (HAD) [2]. The most severe form of HAND is HAD [3]. This condition, characterized by cognitive dysfunction, motor disorders and deficits in learning and memory, occurs in less than 5% of people who have access to antiretroviral therapy [4]. Highly active antiretroviral therapy (HAART), a major advance in the treatment of HIV infection, has improved the survival of HIV patients [5]. As HIV patients now live longer, the prevalence of HAND has increased [6]. HAD correlates better with activation of brain mononuclear phagocytes (macrophages and microglia) than with quantitative measures of brain infection [7]. It is very likely that HAD is a secondary response to innate immunity activation induced by HIV infection [7,8]. In fact, HIV-1 can infect macrophages and microglia directly via gp120 binding, which in turn may induce the release of pro-inflammatory factors and proteins that can be recognized as markers of microglial activation. Macrophages/microglial express CCR5 and CXCR4 [9] that are chemokine receptors on their surface in addition to CD4 [10] and viral gp120 binds via these receptors. The neurons and astrocytes have been reported to also possess CXCR4 and CCR5 receptors on their surface [11]. During the HIV-1 infection the macrophage/microglial enhances their production of lysosomal vesicles and here the virus is assembled and acquires antigens characteristic of this compartment [12].

The chitinase family is an ancient gene family, which has evolved to hydrolyze chitin and its derivative [13,14]. The chitinase family includes proteins both with and without glycohydrolase enzymatic activity against chitin, which are expressed in cells of the innate immune system. Chitotriosidase (CHIT1) and acidic mammalian chitinase (CHIA or AMCAse) are the only two true chitinases possessing chitinolytic activities [8]. The other members, chitinase-like-lectins (Chi-lectins) or chitinase-like proteins (C/CLPs), including chitinase 3-like-1 (CHI3L1, also called YKL40 or HC-gp39), chitinase 3-like-2 (CHI3L2, CHIL2, YKL-39), chitinase domain containing 1 (CHID1), are structurally homologous to CHIT1 and CHIA but lack the essential catalytic residues with the preservation of the substrate-binding cleft of the chitinases [15].

Chitinase expression is greatly amplified during many infections, highlighting their potential biological roles in inflammatory diseases and in innate immunoactivation [16,17]. CHIT1 and CHI3L1 have been reported to be upregulated in a variety of neurological degenerative disorders [18-20]. In cerebrospinal fluid (CSF) from AD patients, CHIT1 and CHI3L1 levels were higher compared to those found in neurologically normal control individuals [21-23]. Furthermore, high CHIT1 levels in CSF from ALS patients suggested a possible role in disease progression [18,24]. Most of these studies suggest that increase CSF chitinase activity reflects microglial activation as a response to or contributing factor in neurodegeneration. Recently, CHI3L1 was associated to the dendritic cell activation and differentiation [17]. High CSF CHI3L1 levels were recently described in macaques and humans with lentiviral encephalitis [25]. In addition, it has been shown that CHI3L1 expression may be induced in astrocytes in traumatic brain injury [26].

CHI3L2, another chitinase family member, has been reported to be overexpressed in AD brains [27]. Recently, increased CSF levels of CHI3L2 were measured in multiple sclerosis [28]. It is also one of the most expressed genes in glioblastomas but its function remains still obscure [29,30].

CHIA is one of the enzymes with true chitinase activity [31]. The relatively abundant expression of CHIA in the gastrointestinal tract and lung supports a possible role in the mucosal immune system and potentially also as a digestive enzyme [32]. The precise role of CHIA in immune-mediated diseases is not clear but many reports suggesting that chitinase activity exerts a beneficial effect by negatively regulating chitin-induced tissue infiltration of innate immune cells associated with allergy [33]. Some studies suggest that CHIA activity may be needed in the brain to protect from protozoan infections [34].

Very scarce information is available on CHID1. It can be used as a marker of alternative macrophage activation [35] and is expressed in some brain tumours [36]. Furthermore, CHID1 is expressed by specialized tissue macrophages (placenta, skin, gut and pancreas) and in cardiac and skeletal muscle by sinusoidal endothelial cells in liver, spleen, bone marrow and lymph nodes [37].

In this study, we test the hypothesis that chitinase expression may be regulated during macrophage and microglial activation in a macaque model of HAD.

METHODS

Selection of a microarray expression dataset and bioinformatics analysis

For this study, we analyzed microarray datasets obtained from NCBI (http://www.ncbi.nlm.nih.gov/) under accession number GDS4214 in order to examine mRNA levels of chitinase family genes in hippocampus specimens of uninfected rhesus macaques (animal control) versus those with histopathologic evidence of *Simian Immunodeficiency Virus Encephalitis* (SIVE). The MultiExperiment Viewer (MeV) software was used to identify different expression of chitinase genes and to generate expression heatmaps. In cases where multiple probes insisted on the same NCBI GeneID, we used those with the *highest variance*. Groups studied was constituted by 18 male rhesus macaques free of SIV, type D simian retrovirus and Herpes B virus. Nine monkeys were intravenously inoculated with a cell-free stock of a derivative of *SIVmac251* that had been subjected to in vivo passage [38] [39]. The SIV-infected monkeys were sacrificed after development of neurological signs of simian AIDS, after a range of 56–132 (mean, 92; median, 93) days post-inoculation. All SIV-infected monkeys had neuropathological evidence of SIVE on post-mortem histopathological examination. A portion of hippocampus was preserved frozen at necropsy for later RNA extraction. The *brain viral load* (BVL) was determined on a sample of frontal lobe, containing both grey and white matter, and is given in $\log_{(10)}$ viral genome equivalents per microgram RNA.

Complete experimental details can be retrieved in the publication by Gersten M et al [40].

For the chitinase gene pathway we used GIANT database (http://giant.princeton.edu/) setting the *tissue menu* in Hippocampus, the Network filter with *Minimum relationship confidence* 0.13 (mininum 0; maximum 1) and *Maximum number of genes* in 5 (minimum 1; maximum 50) to reduce the genes number to only with high average edge score (ES). No enriched biological processes were found. The relationship confidence score (Edge score) indicate how two genes are co-expressed in the same tissue.

GIANT leverages a tissue-specific gold standard to automatically up-weight datasets relevant to a tissue from a large data compendium of diverse tissues and cell-types. GIANT was created by the Laboratory for Bioinformatics and Functional Genomics in the Lewis-Sigler Institute for Integrative Genomics at Princeton University. GIANT tissue networks integrate 987 genome-scale datasets, encompassing ~38,000 conditions from ~14,000 publications and include both expression and interaction measurements. The resulting functional networks accurately capture tissue-specific functional interactions. These maps can answer biological questions that are specific to a single gene in a single tissue. This software can effectively reprioritize functional associations from a genome-wide association study (GWAS) and potentially identify additional disease-associated genes. The approach, named NetWAS, can be applied to any GWAS study, and does not require that the phenotype or disease have any known associated genes [41].

Statistical analysis

Data are expressed as intensity expression levels and presented as box and whiskers. For statistical analysis, Prism 7 software (GraphPad Software, La Jolla, CA, USA) and SPSS 20.0 for IBM was used. Parametric tests were used. One-way ANOVA and t-tests with Bonferroni correction were used to analyze the data and significance was determined at *$p<0.05$; **$p<0.005$;***$p<0.0005$. Correlations were determined using Pearson's correlation.

RESULTS

SIVE Hippocampus expresses significant high levels of Chitinases mRNA

The GDS4214 microarray analysis showed a significant regulation of three chitinases out of five in rhesus macaques hippocampus with histopathologic evidence of SIVE compared to the animal control. The only chitinase with chitinolytic activity, CHIT1, was expressed at significantly higher mRNA levels in SIVE hippocampus compared to control hippocampus ($p=0.0009$, Figure 1A). No significant regulation was observed for CHIA ($p=0.28$) (Figure 1B). CHI3L1 and CHI3L2 mRNA levels were significant upregulated in SIVE hippocampus ($p=2.66E-13$ and $p=2.53E-12$, respectively) compared with control (Figure 2A and B). No significant regulation was observed for CHID1 mRNA ($p=0.27$) when comparing the two groups (Figure 2C). These data confirm innate immunity activation in the hippocampus in SIVE.

Hippocampus chitinase network analysis reveals significant regulation in SIVE

To verify the chitinase activation in SIVE hippocampus, we performed a GIANT analysis (http://giant.princeton.edu/). The software identified the hippocampus network genes related to the chitinases. The analysis showed that CHIT1 was co-expressed in hippocampus with SLC11A1 (ES=0.332), TRIM10 (ES=0.269), P2RX6 (ES=0.267), APOC4 (ES=0.267) and ARHGEF15 (ES=0.264) (Figure 3A). We decided to perform an expression analysis of SLC11A1 mRNA in SIVE hippocampus and found a significant upregulation ($p=0.00059$) compared with control hippocampus (Figure 3A). The other gene network analysis showed that TRIM10, P2RX6, APOC4 mRNA were significantly regulated in SIVE hippocampus (Figure 4A) compared with control hippocampus. No significant regulation was observed in ARHGEF15 mRNA when comparing the two groups.

We identified the following genes to be co-regulated in hippocampus with CHI3L1: C1S (ES=0.230), PMP22 (ES=0.224), MT1B (ES=0.206), HOMER3 (ES=0.199) and PTK2 (ES=0.194). Only C1S, PMP22 and HOMER3 mRNAs were significantly regulated in SIVE hippocampus compared control hippocampus (Figure 4B). The expression of the highly co-expressed CS1mRNA showed a significant regulation in hippocampus of SIVE compared to control hippocampus (Figure 3B). The CHI3L2 network analysis showed the following genes to be co-regulated with CHI3L2: SMOX (ES=0.315), DCBLD2 (ES=0.312), LDLRAD4 (ES=0.247), ADAMTS2 (ES=0.239) and RRAD (ES=0.235). The gene with the highest

score, SMOX, was significantly regulated in SIVE hippocampus (p= 1.17E-05) compared with control hippocampus (Figure 3C). Among the other genes belonging to the network, SMOX, DCBLD2, LDLRAD4 and ADAMTS2 mRNAs were significantly regulated in SIVE hippocampus (Figure 4C). No significant regulation was observed for RRAD mRNA (p=0.78).

Chitinases family correlation with brain viral load

To examine if the chitinases were correlated with the BVL, we performed a Pearson's correlation of chitinases expression in SIV-infected monkeys who presented neuropathological evidence of SIVE. The BVL is expressed in $\log_{(10)}$ viral genome equivalents per microgram RNA [40] (Figure 5A). The analysis showed a strong positive correlation between the BVL and C1s (r=0.955, p=0.0007) (Figure 5B; Figure 6 and Table 1). Among the chitinases, only CHIA showed a negative correlation with BVL (r= -0.829 and p=0.006) (Figure 5C) (Figure 6) (Table 1). Positive correlation between C1s and CHIA (r=0.837 and p=0.0050) was observed too (Figure 5D) (Figure 6 and Table 1). Positive correlations between chitinases and their gene network were found for CHIT1 *vs*. CHI3L2 and SLC11A1 (r=0.901, p=0.001; r=0.798, p=0.01 respectively); CHI3L2 *vs.* CHID1 (r=0.782, p=0.013); CHID1 *vs.* SLC11A1 (r=0.880, p=0.002) (Figure 6 and Table 1).

DISCUSSION

In the present study, we have analyzed the transcriptomic profiles of chitinase gene family members in hippocampus specimens of macaques with SIVE. We showed that three chitinases (CHIT1, CHI3L1 and CHI3L2) among five were regulated during the virus encephalitis. Beside this, the most important chitinases related to the gene network in the hippocampus weere significantly regulated in SIVE. Additionally, we found that CHIA was negatively correlated with C1s mRNA levels and BVL.

To our knowledge, there is no information concerning the association between CHIT1 expression and SIVE. Our study is the first that assesses this relationship. The CHIT1 expression is linked to macrophages activation and with several lysosomal disorders, especially in Gaucher's disease [16,42]. Yasemin Gulcan et al. associated the serum CHIT1 enzyme activity with mortality from Crimean-Congo hemorrhagic fever virus [43] and this is to our knowledge the only known link between CHIT1 and virus infection so far. The CHIT1 mRNA transcription in hippocampus infected by HIV-1 virus could be attributed to microglia/macrophage activation in the CNS. Macrophages or microglia infected by HIV release viral proteins, pro-inflammatory cytokines and chemokines, which in turn activate uninfected macrophages and microglia [44]. These activated cells may release brain neurotoxic substances over prolonged time periods inducing neuronal injury, synapse damage, and cell death [44]. There are at least three forms of microglial activation during HIV infection: M1 (during the early infection); M2a and M2c (during the intermediate and

late infection) [45]. The M1 activation pattern inhibits viral entry, assembly, and budding but it is also known that pro-inflammatory signals (TNFα, IL1β and IL6) may promote the formation of viral reservoirs with increased transcription of HIV-1 LTR (long terminal repeat) [46,47]. A transition from M1 to M2 status is associated with neuroprotection in the case of HIV-associated neurocognitive disorders, suggesting M2 may curtail the M1-HIV polarized activity resulting in tissue damage [48]. In concordance with these data, it has been seen that CHIT1 expression is upregulated in M2 macrophages [48]. HIV-1 infection increases expression and secretion of beta-amyloid (Aβ) and Aβ peptides [49]. Moreover, it has been hypothesized that chitin-like polysaccharides provide a scaffolding for amyloid-beta deposition [50]. In this context, the CHIT1 could interact with amyloid-beta via chitin-like polysaccharides. All these activation patterns and correlations provide indirect support for an association between HIV/SIV-induced microglial activation and neurodegenerative processes, which is reflected by altered transcriptional regulation of multiple players in chitinase-related gene networks.

Beside this, in our analysis we found that SLC11A1 mRNA was significantly upregulated in SIVE and there is a positive correlation with CHIT1 expression. These results are in accordance with the role played in Central Nervous System (CNS) by SLC11A1 [51]. The proton-divalent cation transporters encoded by SLC11A1 is expressed in the brain and regulate ion homeostasis from endosomal compartments. SLC11A1 also has pleiotropic effects on pro-inflammatory responses that may be important in AD [51]. Furthermore, higher concentrations of IL-33, an inflammatory cytokine expressed in the CNS and in activated microglia cells, in HIV clade B infection was showed to be associated with an increased levels of SLC11A1 [52]. All these data corroborate our findings and the possible role played by the CHIT1 network in the SIVE.

Regarding chitinases with true enzymatic activity, we found that CHIA mRNA is negatively correlated to BVL. This result could be explanation in the polarization of the macrophages. CHIA expression is linked to the M2 macrophages, occurring during the IL13 pathways activation [53]. The BVL increase indicates an active state of hippocampus infection, which should correlate with a M1 microglial activation pattern [54]. This data was confirmed by the positive correlation found between C1s (a complement molecule secreted by microglia under stimulation of pro-inflammatory cytokine) and BVL [55]. Therefore, it seems very likely that the M1 to M2 macrophage polarization determines the reduced expression of CHIA mRNA.

In 2008 Bonneh-Barkay and colleagues [25] found that the CHI3L1 expression was elevated fivefold in CSF of SIVE cases versus non-encephalitis cases but the origin and functions in the CNS are still unknown. Furthermore, they showed a positive correlation between CHI3L1 and CSF viral load. Our result partially confirmed this finding. We found a significant mRNA upregulation of CHI3L1 in SIVE hippocampus but no significant correlation was found toward BVL. This discrepancy it could be explained through the possible role played by CHI3L1 during the infection. The CHI3L1 upregulation is connected to the immuno-activation and chemotaxies [56]. Neurodegeneration is associated with increased microglial activation and the recruitment of innate immunity system [57]. In this process the CHI3L1 could play an important role during the SIVE neuronal damage. In vitro experiments showed

that CHI3L1 produces extracellular matrix (ECM) damage through macrophages activation [58]. The association between CHI3L1, inflammation, and macrophages might have particular relevance for neuroinflammation associated with dementia. The virus infection could induce CHI3L1 expression and the resulting effects in the CNS as well as innate immunity activation occur, possibly qualifying CHI3L1 as a biomarker for SIVE.

CHI3L2 is strongly expressed in the brains of MS patients, especially in astrocytes and microglial cells in the white matter plaques [28]. This data could better support our results justifying the high CHI3L2 mRNA levels detected in SIVE hippocampus. These results could be correlated to the role played by this molecule in the brain tissue. Furthermore, it has been shown that CHI3L2 could regulate the angiogenesis in the brain tumours [29] [59]. The angiogenesis dysregulation represents a new pathogenic mechanism involved in the progression of Alzheimer's and neurodegenerative diseases [60]. Moreover, it has been shown that HIV-1 Tat increases the levels of molecules involved in cell migration, angiogenesis, neurogenesis and synaptic plasticity. The CHI3L2 upregulation in SIVE could be correlated to the lysosomal immuno-activation of microglia/macrophages induced by HIV1 virus. Furthermore, our analysis showed that CHI3L2 is also positively correlated to SCL11A1, CHID1 and CHIT1 in SIVE hippocampus. These data confirm even more the chitinases family network implication in the brain degenerative process.

In conclusion, these findings support a role of chitinase family network in SIVE. The data may be extended to both neurodegenerative and neuroinflammatory diseases. The strong transcriptional changes detected in our analyses suggest that the protein products of these genes should be examined further as potential biomarkers for microglial activation in several neurological diseases.

CONFLICT OF INTEREST

The Authors declare that they have no conflict of interests.

FIGURE LEGENDS

Figure 1: Chitinase expression levels in SIVE hippocampus section.

A) Expression levels of true chitinases reveal upregulation of CHIT1 ($p=0.0009$) in SIVE hippocampus compared to control hippocampus. B) No significant regulation was observed in the CHIA expression levels ($p=0.28$). Dataset accession number GDS4214. Data are expressed as intensity expression levels and presented as box and whiskers. P values <0.05 were considered to be statistically significant (*$p<0.05$; **$p<0.005$;***$p<0.0005$).

Figure 2: Chitinases like protein expression levels in SIVE hippocampus section.

A) and B) The chitinase-like lectins, CHI3L1 and CHI3L2, were high significant upregulated in SIVE hippocampus ($p=2.66E-13$ and $p=2.53E-12$, respectively). C) CHID1 mRNA levels are unaltered in SIVE hippocampus ($p=0.27$). Dataset accession number GDS4214. Data are

expressed as intensity expression levels and presented as box and whiskers. P values <0.005 were considered to be statistically significant (*p<0.05; **p<0.005;***p<0.0005).

Figure 3: Hippocampal network analysis of chitinase-related genes

Hippocampal chitinase gene network analysis was performed using the GIANT software (http://giant.princeton.edu/). The figure shows the network hippocampus analysis for each chitinases; the relationship confidence score for the genes network referred to the chitinase; the most chitinase-related genes in SIVE hippocampus section compared to the control group. Edge thickness and colour correspond to edge strength. Dataset accession number GDS4214. Data are expressed as intensity expression levels and presented as box and whiskers. P values <0.005 were considered to be statistically significant (*p<0.05; **p<0.005;***p<0.0005).

Figure 4: Chitinase genes network statistical significance in SIVE hippocampus section

The figure shows the Radar chart of chitinases significant genes network and heatmap of medium expression levels in SIVE hippocampus section. Gene expression values are color coded from bright red (most upregulated) to dark blue (most downregulated). No significant genes are marked in red.

Figure 5: Chitinase correlation with brain viral load

A) SIV-infected monkeys BVL was determined on a sample of frontal lobe, containing both grey and white matter, and is given in $\log_{(10)}$ viral genome equivalents per microgram RNA. C1s expression levels show a strong positive correlation with BVL (B) and negative with CHIA (D). As regard the expression levels of CHIA, these were correlated negatively with BVL (C).

Figure 6: Chitinases genes network correlation

Positive correlation was found between BVL and C1s (r=0.955 and p=0.0007); CHIT1 *vs* CHI3L2 and SLC11A1 (r=0.901 and p=0.001; r=0.798 and p=0.01 respectively); CHI3L2 *vs* CHID1 (r=0.782 and p=0.013); CHID1 *vs* SLC11A1 (r=0.880 and p=0.002). Negatively correlation was found between CHIA and BVL (r=-0.829 and p=0.0060) and a positive one between C1s and CHIA (r=0.837 and p=0.0050). The genes correlated are showed in red box square box.

Graphical abstract: Chitinases expression in macrophages/microglia SIVE hippocampus.

HIV-1 shows a tropism for brain cells. The microglia cells express surface markers like CXCR4, CXCR5 and CD4. These markers are used by the HIV-1 virus to infect the microglia. Activated microglia increase the production of lysosomal vesicles as well as chitinases. CHI3L1 and CHI3L2 increase cell migration and chemotaxis. Macrophages recruited by the chitinases infiltrate the brain and here become polarized (M1/M2). The brain infiltrating macrophages now became target of HIV-1 virus. Secreted CHIT1 can interact

with beta-amyloid plaque via Chitin-like polysaccharides. All this may contribute to neuronal injury and death induced by HIV-1 infection.

Table 1: Correlation analysis between chitinase hippocampal gene networks *vs*. BVL

REFERENCES

1. Antinori, A.; Arendt, G.; Becker, J.T.; Brew, B.J.; Byrd, D.A.; Cherner, M.; Clifford, D.B.; Cinque, P.; Epstein, L.G.; Goodkin, K., *et al.* Updated research nosology for hiv-associated neurocognitive disorders. *Neurology* **2007**, *69*, 1789-1799.
2. Elbirt, D.; Mahlab-Guri, K.; Bezalel-Rosenberg, S.; Gill, H.; Attali, M.; Asher, I. Hiv-associated neurocognitive disorders (hand). *The Israel Medical Association journal : IMAJ* **2015**, *17*, 54-59.
3. Etherton, M.R.; Lyons, J.L.; Ard, K.L. Hiv-associated neurocognitive disorders and antiretroviral therapy: Current concepts and controversies. *Current infectious disease reports* **2015**, *17*, 485.
4. McArthur, J.C.; Hoover, D.R.; Bacellar, H.; Miller, E.N.; Cohen, B.A.; Becker, J.T.; Graham, N.M.; McArthur, J.H.; Selnes, O.A.; Jacobson, L.P., *et al.* Dementia in aids patients: Incidence and risk factors. Multicenter aids cohort study. *Neurology* **1993**, *43*, 2245-2252.
5. Fortunak, J.M.; de Souza, R.O.; Kulkarni, A.A.; King, C.L.; Ellison, T.; Miranda, L.S. Active pharmaceutical ingredients for antiretroviral treatment in low- and middle-income countries: A survey. *Antiviral therapy* **2014**, *19 Suppl 3*, 15-29.
6. Ellis, R.J.; Deutsch, R.; Heaton, R.K.; Marcotte, T.D.; McCutchan, J.A.; Nelson, J.A.; Abramson, I.; Thal, L.J.; Atkinson, J.H.; Wallace, M.R., *et al.* Neurocognitive impairment is an independent risk factor for death in hiv infection. San diego hiv neurobehavioral research center group. *Archives of neurology* **1997**, *54*, 416-424.
7. Kaul, M.; Zheng, J.; Okamoto, S.; Gendelman, H.E.; Lipton, S.A. Hiv-1 infection and aids: Consequences for the central nervous system. *Cell death and differentiation* **2005**, *12 Suppl 1*, 878-892.
8. Diesing, T.S.; Swindells, S.; Gelbard, H.; Gendelman, H.E. Hiv-1-associated dementia: A basic science and clinical perspective. *The AIDS reader* **2002**, *12*, 358-368.
9. He, J.; Chen, Y.; Farzan, M.; Choe, H.; Ohagen, A.; Gartner, S.; Busciglio, J.; Yang, X.; Hofmann, W.; Newman, W., *et al.* Ccr3 and ccr5 are co-receptors for hiv-1 infection of microglia. *Nature* **1997**, *385*, 645-649.
10. Jordan, C.A.; Watkins, B.A.; Kufta, C.; Dubois-Dalcq, M. Infection of brain microglial cells by human immunodeficiency virus type 1 is cd4 dependent. *Journal of virology* **1991**, *65*, 736-742.
11. Hesselgesser, J.; Halks-Miller, M.; DelVecchio, V.; Peiper, S.C.; Hoxie, J.; Kolson, D.L.; Taub, D.; Horuk, R. Cd4-independent association between hiv-1 gp120 and cxcr4: Functional chemokine receptors are expressed in human neurons. *Current biology : CB* **1997**, *7*, 112-121.
12. Pelchen-Matthews, A.; Kramer, B.; Marsh, M. Infectious hiv-1 assembles in late endosomes in primary macrophages. *The Journal of cell biology* **2003**, *162*, 443-455.
13. Henrissat, B.; Davies, G. Structural and sequence-based classification of glycoside hydrolases. *Current opinion in structural biology* **1997**, *7*, 637-644.
14. Eide, K.B.; Stockinger, L.W.; Lewin, A.S.; Tondervik, A.; Eijsink, V.G.; Sorlie, M. The role of active site aromatic residues in substrate degradation by the human chitotriosidase. *Biochimica et biophysica acta* **2016**, *1864*, 242-247.

15. Lee, C.G.; Da Silva, C.A.; Dela Cruz, C.S.; Ahangari, F.; Ma, B.; Kang, M.J.; He, C.H.; Takyar, S.; Elias, J.A. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. *Annual review of physiology* **2011**, *73*, 479-501.
16. Di Rosa, M.; Distefano, G.; Zorena, K.; Malaguarnera, L. Chitinases and immunity: Ancestral molecules with new functions. *Immunobiology* **2016**, *221*, 399-411.
17. Di Rosa, M.; Tibullo, D.; Saccone, S.; Distefano, G.; Basile, M.S.; Di Raimondo, F.; Malaguarnera, L. Chi3l1 nuclear localization in monocyte derived dendritic cells. *Immunobiology* **2016**, *221*, 347-356.
18. Varghese, A.M.; Sharma, A.; Mishra, P.; Vijayalakshmi, K.; Harsha, H.C.; Sathyaprabha, T.N.; Bharath, S.M.; Nalini, A.; Alladi, P.A.; Raju, T.R. Chitotriosidase - a putative biomarker for sporadic amyotrophic lateral sclerosis. *Clinical proteomics* **2013**, *10*, 19.
19. Harris, V.K.; Sadiq, S.A. Biomarkers of therapeutic response in multiple sclerosis: Current status. *Molecular diagnosis & therapy* **2014**, *18*, 605-617.
20. Di Rosa, M.; Dell'Ombra, N.; Zambito, A.M.; Malaguarnera, M.; Nicoletti, F.; Malaguarnera, L. Chitotriosidase and inflammatory mediator levels in alzheimer's disease and cerebrovascular dementia. *The European journal of neuroscience* **2006**, *23*, 2648-2656.
21. Rosen, C.; Andersson, C.H.; Andreasson, U.; Molinuevo, J.L.; Bjerke, M.; Rami, L.; Llado, A.; Blennow, K.; Zetterberg, H. Increased levels of chitotriosidase and ykl-40 in cerebrospinal fluid from patients with alzheimer's disease. *Dementia and geriatric cognitive disorders extra* **2014**, *4*, 297-304.
22. Olsson, B.; Malmestrom, C.; Basun, H.; Annas, P.; Hoglund, K.; Lannfelt, L.; Andreasen, N.; Zetterberg, H.; Blennow, K. Extreme stability of chitotriosidase in cerebrospinal fluid makes it a suitable marker for microglial activation in clinical trials. *Journal of Alzheimer's disease : JAD* **2012**, *32*, 273-276.
23. Choi, J.; Lee, H.W.; Suk, K. Plasma level of chitinase 3-like 1 protein increases in patients with early alzheimer's disease. *Journal of neurology* **2011**, *258*, 2181-2185.
24. Pagliardini, V.; Pagliardini, S.; Corrado, L.; Lucenti, A.; Panigati, L.; Bersano, E.; Servo, S.; Cantello, R.; D'Alfonso, S.; Mazzini, L. Chitotriosidase and lysosomal enzymes as potential biomarkers of disease progression in amyotrophic lateral sclerosis: A survey clinic-based study. *Journal of the neurological sciences* **2015**, *348*, 245-250.
25. Bonneh-Barkay, D.; Bissel, S.J.; Wang, G.; Fish, K.N.; Nicholl, G.C.; Darko, S.W.; Medina-Flores, R.; Murphey-Corb, M.; Rajakumar, P.A.; Nyaundi, J., *et al.* Ykl-40, a marker of simian immunodeficiency virus encephalitis, modulates the biological activity of basic fibroblast growth factor. *The American journal of pathology* **2008**, *173*, 130-143.
26. Bonneh-Barkay, D.; Zagadailov, P.; Zou, H.; Niyonkuru, C.; Figley, M.; Starkey, A.; Wang, G.; Bissel, S.J.; Wiley, C.A.; Wagner, A.K. Ykl-40 expression in traumatic brain injury: An initial analysis. *Journal of neurotrauma* **2010**, *27*, 1215-1223.
27. Colton, C.A.; Mott, R.T.; Sharpe, H.; Xu, Q.; Van Nostrand, W.E.; Vitek, M.P. Expression profiles for macrophage alternative activation genes in ad and in mouse models of ad. *Journal of neuroinflammation* **2006**, *3*, 27.
28. Hinsinger, G.; Galeotti, N.; Nabholz, N.; Urbach, S.; Rigau, V.; Demattei, C.; Lehmann, S.; Camu, W.; Labauge, P.; Castelnovo, G., *et al.* Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. *Multiple sclerosis* **2015**, *21*, 1251-1261.
29. Saidi, A.; Javerzat, S.; Bellahcene, A.; De Vos, J.; Bello, L.; Castronovo, V.; Deprez, M.; Loiseau, H.; Bikfalvi, A.; Hagedorn, M. Experimental anti-angiogenesis causes upregulation of genes associated with poor survival in glioblastoma. *International journal of cancer. Journal international du cancer* **2008**, *122*, 2187-2198.
30. Areshkov, P.A.; Kavsan, V.M. Chitinase 3-like protein 2 (chi3l2, ykl-39) activates phosphorylation of extracellular signal-regulated kinases erk1/erk2 in human embryonic kidney (hek293) and human glioblastoma (u87 mg) cells. *TSitologiia i genetika* **2010**, *44*, 3-9.
31. Di Rosa, M.; De Gregorio, C.; Malaguarnera, G.; Tuttobene, M.; Biazzo, F.; Malaguarnera, L. Evaluation of amcase and chit-1 expression in monocyte macrophages lineage. *Molecular and cellular biochemistry* **2013**, *374*, 73-80.

32. Ohno, M.; Tsuda, K.; Sakaguchi, M.; Sugahara, Y.; Oyama, F. Chitinase mrna levels by quantitative pcr using the single standard DNA: Acidic mammalian chitinase is a major transcript in the mouse stomach. *PloS one* **2012**, *7*, e50381.
33. Reese, T.A.; Liang, H.E.; Tager, A.M.; Luster, A.D.; Van Rooijen, N.; Voehringer, D.; Locksley, R.M. Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* **2007**, *447*, 92-96.
34. Nance, J.P.; Vannella, K.M.; Worth, D.; David, C.; Carter, D.; Noor, S.; Hubeau, C.; Fitz, L.; Lane, T.E.; Wynn, T.A., *et al.* Chitinase dependent control of protozoan cyst burden in the brain. *PLoS pathogens* **2012**, *8*, e1002990.
35. Riabov, V.; Gudima, A.; Wang, N.; Mickley, A.; Orekhov, A.; Kzhyshkowska, J. Role of tumor associated macrophages in tumor angiogenesis and lymphangiogenesis. *Frontiers in physiology* **2014**, *5*, 75.
36. Kzhyshkowska, J.; Yin, S.; Liu, T.; Riabov, V.; Mitrofanova, I. Role of chitinase-like proteins in cancer. *Biological chemistry* **2016**.
37. Kzhyshkowska, J. Multifunctional receptor stabilin-1 in homeostasis and disease. *TheScientificWorldJournal* **2010**, *10*, 2039-2053.
38. Watry, D.; Lane, T.E.; Streb, M.; Fox, H.S. Transfer of neuropathogenic simian immunodeficiency virus with naturally infected microglia. *The American journal of pathology* **1995**, *146*, 914-923.
39. Burdo, T.H.; Marcondes, M.C.; Lanigan, C.M.; Penedo, M.C.; Fox, H.S. Susceptibility of chinese rhesus monkeys to siv infection. *Aids* **2005**, *19*, 1704-1706.
40. Gersten, M.; Alirezaei, M.; Marcondes, M.C.; Flynn, C.; Ravasi, T.; Ideker, T.; Fox, H.S. An integrated systems analysis implicates egr1 downregulation in simian immunodeficiency virus encephalitis-induced neural dysfunction. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **2009**, *29*, 12467-12476.
41. Greene, C.S.; Krishnan, A.; Wong, A.K.; Ricciotti, E.; Zelaya, R.A.; Himmelstein, D.S.; Zhang, R.; Hartmann, B.M.; Zaslavsky, E.; Sealfon, S.C., *et al.* Understanding multicellular function and disease with human tissue-specific networks. *Nature genetics* **2015**, *47*, 569-576.
42. Artieda, M.; Cenarro, A.; Ganan, A.; Lukic, A.; Moreno, E.; Puzo, J.; Pocovi, M.; Civeira, F. Serum chitotriosidase activity, a marker of activated macrophages, predicts new cardiovascular events independently of c-reactive protein. *Cardiology* **2007**, *108*, 297-306.
43. Kurt, Y.G.; Cayci, T.; Onguru, P.; Akgul, E.O.; Yaman, H.; Aydin, I.; Bodur, H.; Turker, T.; Kurt, I.; Cevik, M.A., *et al.* Serum chitotriosidase enzyme activity in patients with crimean-congo hemorrhagic fever. *Clinical chemistry and laboratory medicine* **2009**, *47*, 1543-1547.
44. Kaul, M.; Garden, G.A.; Lipton, S.A. Pathways to neuronal injury and apoptosis in hiv-associated dementia. *Nature* **2001**, *410*, 988-994.
45. Lugo-Villarino, G.; Verollet, C.; Maridonneau-Parini, I.; Neyrolles, O. Macrophage polarization: Convergence point targeted by mycobacterium tuberculosis and hiv. *Frontiers in immunology* **2011**, *2*, 43.
46. Cassol, E.; Cassetta, L.; Rizzi, C.; Alfano, M.; Poli, G. M1 and m2a polarization of human monocyte-derived macrophages inhibits hiv-1 replication by distinct mechanisms. *Journal of immunology* **2009**, *182*, 6237-6246.
47. Herbein, G.; Varin, A. The macrophage in hiv-1 infection: From activation to deactivation? *Retrovirology* **2010**, *7*, 33.
48. Di Rosa, M.; Malaguarnera, G.; De Gregorio, C.; D'Amico, F.; Mazzarino, M.C.; Malaguarnera, L. Modulation of chitotriosidase during macrophage differentiation. *Cell biochemistry and biophysics* **2013**, *66*, 239-247.
49. Khan, M.B.; Lang, M.J.; Huang, M.B.; Raymond, A.; Bond, V.C.; Shiramizu, B.; Powell, M.D. Nef exosomes isolated from the plasma of individuals with hiv-associated dementia (had) can induce abeta secretion in sh-sy5y neural cells. *Journal of neurovirology* **2015**.
50. Castellani, R.J.; Perry, G.; Smith, M.A. The role of novel chitin-like polysaccharides in alzheimer disease. *Neurotoxicity research* **2007**, *12*, 269-274.
51. Jamieson, S.E.; White, J.K.; Howson, J.M.; Pask, R.; Smith, A.N.; Brayne, C.; Evans, J.G.; Xuereb, J.; Cairns, N.J.; Rubinsztein, D.C., *et al.* Candidate gene association study of solute

carrier family 11a members 1 (slc11a1) and 2 (slc11a2) genes in alzheimer's disease. *Neuroscience letters* **2005**, *374*, 124-128.
52. Yndart, A.; Kaushik, A.; Agudelo, M.; Raymond, A.; Atluri, V.S.; Saxena, S.K.; Nair, M. Investigation of neuropathogenesis in hiv-1 clade b and c infection associated with il-33 and st2 regulation. *ACS chemical neuroscience* **2015**, *6*, 1600-1612.
53. Zhu, Z.; Zheng, T.; Homer, R.J.; Kim, Y.K.; Chen, N.Y.; Cohn, L.; Hamid, Q.; Elias, J.A. Acidic mammalian chitinase in asthmatic th2 inflammation and il-13 pathway activation. *Science* **2004**, *304*, 1678-1682.
54. Zink, M.C.; Suryanarayana, K.; Mankowski, J.L.; Shen, A.; Piatak, M., Jr.; Spelman, J.P.; Carter, D.L.; Adams, R.J.; Lifson, J.D.; Clements, J.E. High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. *Journal of virology* **1999**, *73*, 10480-10488.
55. Veerhuis, R.; Janssen, I.; De Groot, C.J.; Van Muiswinkel, F.L.; Hack, C.E.; Eikelenboom, P. Cytokines associated with amyloid plaques in alzheimer's disease brain stimulate human glial and neuronal cell cultures to secrete early complement proteins, but not c1-inhibitor. *Experimental neurology* **1999**, *160*, 289-299.
56. Libreros, S.; Iragavarapu-Charyulu, V. Ykl-40/chi3l1 drives inflammation on the road of tumor progression. *Journal of leukocyte biology* **2015**, *98*, 931-936.
57. Kolson, D.L. Ykl-40: A candidate biomarker for simian immunodeficiency virus and human immunodeficiency virus encephalitis? *The American journal of pathology* **2008**, *173*, 25-29.
58. Johansen, J.S.; Jensen, B.V.; Roslind, A.; Nielsen, D.; Price, P.A. Serum ykl-40, a new prognostic biomarker in cancer patients? *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **2006**, *15*, 194-202.
59. Di Rosa, M.; Sanfilippo, C.; Libra, M.; Musumeci, G.; Malaguarnera, L. Different pediatric brain tumors are associated with different gene expression profiling. *Acta histochemica* **2015**, *117*, 477-485.
60. Jung, J.; Kim, S.; Yoon, K.; Moon, Y.; Roh, D.; Lee, S.; Choi, K.; Jung, J.; Kim, D. The effect of depression on serum vegf level in alzheimer's disease. *Disease markers* **2015**, *2015*, 742612.