

# OAS Gene Family Expression Is Associated with HIV-Related Neurocognitive Disorders

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**Abstract** HIV-associated neurocognitive disorders are common in HIV-infected individuals, even in the combination antiretroviral therapy (c-ART) era. Several mechanisms are involved in neuronal damage, including chronic inflammation immune activation. Mammalian 2'-5'-oligoadenylate synthetase (OAS) genes are produced in response to interferon (IFN), mainly by monocytes, and exert their antiviral functions by activation of RNase L that degrades viral and cellular RNAs. In this study, we aimed at exploring OAS gene family RNA expression in simian immunodeficiency virus encephalitis (SIVE), in HIV-associated neurocognitive disorders (HAND), and in HIV-associated dementia (HAD). We analyzed three microarray datasets obtained from the NCBI in order to assess the expression levels of OAS gene family network in brain biopsies of macaques with SIVE vs uninfected

animals, as well as post-mortem brain of individuals with HAND (on or off ART) vs uninfected controls and three brain regions of HIV-infected individuals with both neurocognitive impairment (HAD) and encephalitis (HIVE). All OAS genes were upregulated both in SIVE and in HAND. OAS expression was significantly higher in high-viremic individuals; increased expression levels persisted in cART subjects when compared to healthy controls. OAS gene network analysis showed that several genes belonging to the type I IFN pathway, especially *CXCL10* and *IFIT3*, were similarly upregulated in SIVE/HAND. Furthermore, we identified a significant upregulation of OAS gene family RNA expression in basal ganglia, white matter, and frontal cortex of HIV-1, HAD, and HAD/HIVE patients compared to healthy subjects. OAS gene family expression is increased in brain sections from individuals with HAND, HAD, and HIVE as well as macaques with SIVE. OAS family expression is likely to be induced by IFN as a consequence of viral replication in the CNS. Its long-term upregulation may contribute to the chronic inflammatory status and neurocognitive impairment we still observe in virologically suppressed individuals on c-ART.

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## Abbreviations

c-ART	Combination antiretroviral therapy
HAART	Highly active anti-retroviral therapy
AIDS	Acquired immune deficiency syndrome
CNS	Central nervous system
HIV	Human immunodeficiency virus
SIV	HIV/simian immunodeficiency virus
SIVE	Simian immunodeficiency virus encephalitis
HAND	HIV-associated neurocognitive disorders

HAD	HIV-infected with neurocognitive impairment or HIV associate dementia
ANI	Asymptomatic neurocognitive impairment
HIVE	HIV encephalitis
BBB	Blood-brain barrier
CSF	Cerebrospinal fluid
CXCL10	C-X-C motif chemokine 10
FDR	False discovery rate
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
OAS1	2'-5'-Oligoadenylate synthetase 1
OAS2	2'-5'-Oligoadenylate synthetase 2
OAS3	2'-5'-Oligoadenylate synthetase 3
OASL	2'-5'-Oligoadenylate synthetase-like
MeV	MultiExperiment Viewer
IQR	Interquartile range
BVL	Brain viral load

## Introduction

The introduction of combination antiretroviral therapy (c-ART) has transformed human immunodeficiency virus (HIV) infection in a chronic disease. Despite the dramatic reduction in the prevalence of AIDS-related morbidity and mortality, HIV-infected individuals are at increased risk of non-AIDS-related comorbidities, including cardiovascular, renal, and bone disease, as well as malignancies and neurocognitive impairment [1–6]. Though c-ART has significantly reduced the prevalence of HIV-associated dementia and central nervous system (CNS) opportunistic infections, it is estimated that up to 50% of HIV-patients have mild forms of HIV-associated neurologic disease (HAND), known as asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND) [6]. The events leading to neuronal injury are multifaceted and not only related to the direct neurotoxicity of the virus.

Monocyte/macrophages are the main cell type that infiltrates the CNS during HIV infection [7]. Blood-born infected monocytes are able to migrate to the CNS through the blood-brain barrier (BBB) and lead to neuronal damage and loss. Once migrated in the CNS, those cells can live for a long time in the brain as resident macrophages, where they are thought to contribute to the establishment of a reservoir and the promotion of the inflammatory cascade [7]. In fact, impaired neurocognition has been associated with increased production of inflammatory cytokines, such as interleukin IL-6 and interferon IFN- $\gamma$ , and proteins related with monocyte activation/chemotaxis (soluble CD14, CXCL10, CCL3, MCP-1) in the cerebrospinal fluid (CSF) of HIV-positive subjects on c-ART. The release of inflammatory cytokines promotes neuronal damage and death as well as the expression of adhesion molecules on the brain microvascular endothelium, which recruit

more monocytes to the BBB [7]. Similarly, in the setting of advanced HIV infection, c-ART-naive individuals with HAND have been reported to have higher levels of inflammatory chemokines and cytokines than those without HAND, with similar plasma viral load [8]. Globally, these findings suggest the importance of host immune responses, and in particular innate immunity, in HAND pathogenesis.

The 2'-5'-oligoadenylate synthetases (OAS) are IFN-stimulated proteins belonging to a highly conserved family, which consists of four members, OAS1, OAS2, OAS3, and OAS-like protein (OASL). OAS proteins are induced by type I IFN and have been shown to play an important role in innate immune responses. In fact, OASs have antiviral functions, as they act as nucleotidyltransferases, catalyzing the synthesis of 2'-5'-linked oligoadenylates, which leads to the activation of RNase L and finally to the degradation of viral and cellular RNAs [9, 10].

Considering the role of innate immunity in the pathogenesis of HIV-related neurocognitive disorders, in the present study, we aimed at exploring the correlation between OAS gene family RNA expression and the presence of neurocognitive disorders in the setting of HIV/simian immunodeficiency virus (SIV) infection, by analyzing three microarray datasets obtained from the NCBI.

## Methods

### Bioinformatics Analysis Tools

For this paper, we analyzed microarray datasets from the NCBI Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) databank under accession number GSE13824, GSE28160 and GSE35864, in order to define a correlation between the OAS gene family network and molecular events of neurological impairment and inflammation associated with HIV infection in the brain regions. From the GSE13824 microarray dataset, we selected the data from the hippocampal region of nine male monkeys with simian immunodeficiency virus encephalitis (SIVE) (used in biological replicate) and nine male healthy controls (used in biological replicate). The author stated that all SIV-infected monkeys had neuropathological evidence of SIVE. SIV-infected monkeys were sacrificed after development of SIVE (median time 93 days post-inoculation) (IQR 56–132). Rhesus macaques-SIV encephalitis (SIVE) is a standard model used to study human HIV-associated dementia (HAD), occurring in patients with advanced HIV. The brain viral load (BVL) was determined on a sample of frontal lobe, containing both gray and white matter, and is given in log viral genome equivalents per microgram RNA. Complete experimental details can be retrieved in the publication by Gersten et al. [11].

To confirm the results obtained with GSE13824, we also analyzed a human microarray dataset (GSE28160). From GSE28160 microarray dataset, we selected the data of post-mortem brain of c-ART-treated ( $n = 7$  and 5 biological replicate) and untreated ( $n = 8$  and 6 biological replicate) HIV-infected patients with neurocognitive disorders (HAND) and healthy subjects ( $n = 6$  and 3 biological replicates). The authors stated that the brain biopsies were obtained from the centrum semiovale (deep white matter) at the coronal level of the genu of the corpus callosum. Complete experimental details can be retrieved in the publication by Borjabad et al. [12].

We decided to extend the analysis of OAS gene expressions to the subjects affected by HIV-associated dementia (HAD). To achieve these results, we selected a human microarray dataset (GSE35864) consisting of three brain regions (basal ganglia, white matter, and frontal cortex) of 24 human subjects divided in four groups (healthy, HIV-infected, HIV-infected with neurocognitive impairment, and HIV-infected with both neurocognitive impairment and encephalitis patients). Complete experimental details can be retrieved in the publication by Gelman et al. [13].

The MultiExperiment Viewer (MeV) software was used to identify differentially expressed genes. In cases where multiple probes insisted on the same NCBI GeneID, we used those with the highest variance. By restricting the threshold level of significance to  $p < 0.01$  and  $\log_2$  fold to 1.5, for GSE13824, we identified 3426 significant upregulated and 4416 downregulated genes in hippocampus SIVE vs healthy monkeys. For GSE28160, we identified 3426 significant upregulated and 4643 downregulated genes in untreated HAND brain vs healthy subjects. As regard GSE35864, comparing all brain sections, we obtained 2285 significant upregulated and 39 downregulated genes in healthy vs HIV-infected patients (Supplementary Table 1). In order to identify genes commonly modulated in OAS gene network and hippocampus SIVE, HAD, or HAND, Venn diagrams were drawn using the Web-based utility Venn Diagram Generator (<http://www.bioinformatics.lu>). Weighted Gene Networks were built for the commonly modulated genes using STRING software (<http://string-db.org/>) [14] and GIANT software (<http://giant.princeton.edu/>) [15]. The STRING combined score was based on data from Neighbourhood in the Genome, Gene Fusions, Co-occurrence Across Genomes, Co-Expression, Experimental/Biochemical Data, and Association in Curated Databases.

The OAS gene pathway was obtained from the GIANT database (<http://giant.princeton.edu/>) setting the tissue menu in *Hippocampus*, the Network filter with *Minimum relationship confidence 0.18* and *Maximum number of genes in 50*. The relationship confidence score (Edge score) indicates how two genes are significantly coexpressed in the same tissue. Scores corresponded to edges with a tissue-

network interaction probability greater than the naive-network probability. High values of Edge score correspond to high index of coexpression in the same tissue [15].

Biological pathway, biological process, heatmap cell localization, and analysis of KEGG pathways was performed with FunRich tool (<http://www.funrich.org>) against human FunRich background database [16].

## Statistical Analysis

For statistical analysis, Prism 7 software (GraphPad Software, USA) was used. Based on Shapiro-Wilk test, almost all data were skewed, so nonparametric tests were used. Significant differences between groups were assessed using the Mann–Whitney  $U$  test, and Kruskal–Wallis test was performed to compare data between all groups followed by Dunn’s post hoc test. Correlations were determined using Spearman’s  $\rho$  correlation. All tests were two-sided and significance was determined at  $P < 0.05$ . To analyze a  $2 \times 2$  contingency table, a chi-squared with Yates correction was performed by GraphPad Prism software (<http://graphpad.com/quickcalcs/contingency1.cfm>). The association between rows (groups) and columns (outcomes) with  $p < 0.0001$  was considered to be extremely statistically significant.

## Results

### High Expression Levels of OAS mRNA in SIV-Infected Hippocampus

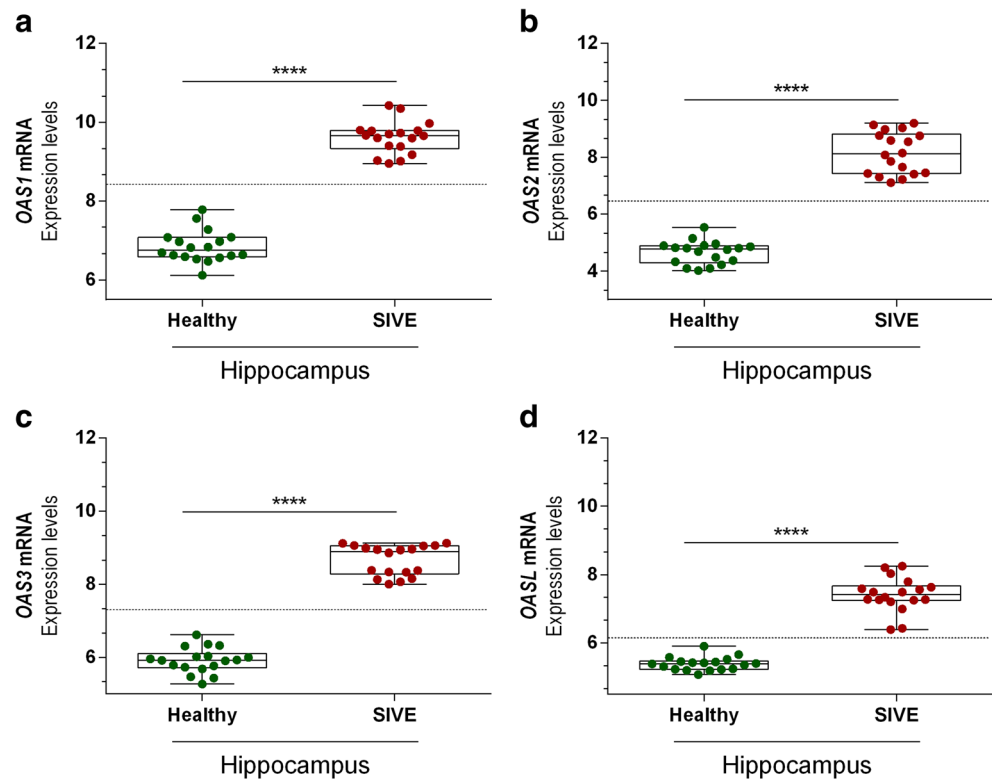
The GSE13824 analysis was performed in order to determine the expression levels of OAS gene family RNA expression in the hippocampus of monkeys with SIVE. The mRNA levels of *OAS1*, *OAS2*, *OAS3*, and *OASL* were significantly higher in animals with SIVE compared to healthy controls ( $p < 0.0001$ ) (Fig. 1). Also, we showed a significant upregulation of *RNAseL* mRNA in SIV-infected hippocampus compared to healthy control ( $p < 0.001$ ) (Supplementary Fig. 1).

We found a neat separation between the two groups, as none of the expression values of OAS mRNA in the healthy group was in the expression range of the SIVE group. No significant correlation was observed between the BVL and the OAS mRNA expression levels (Supplementary Fig. 2A). On the contrary, all the OAS mRNA were positively correlated one with the other (Supplementary Fig. 2B).

### OAS Gene Network Is Significantly Expressed in SIV-Infected Hippocampus

In order to define a “OAS gene network,” the GIANT software was interrogated. The analysis in the selected tissue (i.e., *hippocampus*) identified 50 genes significant coexpressed to

**Fig. 1** OAS genes are significantly modulated in SIV-infected monkeys' hippocampus. *OAS1*, *OAS2*, *OAS3*, and *OASL* mRNA expression levels reveal a significant upregulation ( $p < 0.00001$ ) in the hippocampus of monkeys with SIVE compared to healthy animal controls (a–d). Dataset accession number GSE13824. Data are expressed as intensity expression level and presented as vertical scatter dot plots.  $p$  values  $< 0.05$  were considered to be statistically significant ( $*p < 0.05$ ;  $**p < 0.005$ ;  $***p < 0.0005$ ;  $****p < 0.00005$ )



OAS genes (Supplementary Fig. 3A). Positive regulation of *response to virus* ( $p$  value, FDR corrected  $9.80e-9$ ) (GO-BP-0009615), *viral life cycle* ( $p$  value, FDR corrected  $1.21e-8$ ) (GO-BP-0019058), and *viral process* ( $p$  value, FDR corrected  $2.88e-8$ ) (GO-BP-0016032) was found in enriched Pathways/Processes/Diseases.

The 50 genes obtained by the GIANT analysis were compared to the 3426 genes that were significantly upregulated in SIVE hippocampus vs healthy animal control (Fig. 2a). Among these 50 genes, 33 (61%) were similarly upregulated in the SIVE group vs healthy animal controls ( $p < 0.0001$  by chi-square with Yates' correction) and 21 (38%) unequivocally belonged to the OAS gene network (Fig. 2a). No significantly common genes to the OAS gene network were observed among the list of downregulated genes (Supplementary Fig. 3b).

This list of upregulated genes included 12 genes coexpressed to the type I IFN pathway (FDR =  $8.31e-19$ ) (STRING analysis) (Fig. 2b). The most upregulated mRNA genes were two IFN-induced molecules, *CXCL10* (also known as Interferon gamma-induced protein 10) (Fig. 2c), and *IFIT3* (Fig. 2d). Both *CXCL10* and *IFIT3* mRNA were significantly correlated with the expression levels of OAS mRNA in the GSE13824 dataset (Supplementary Fig. 4A/B).

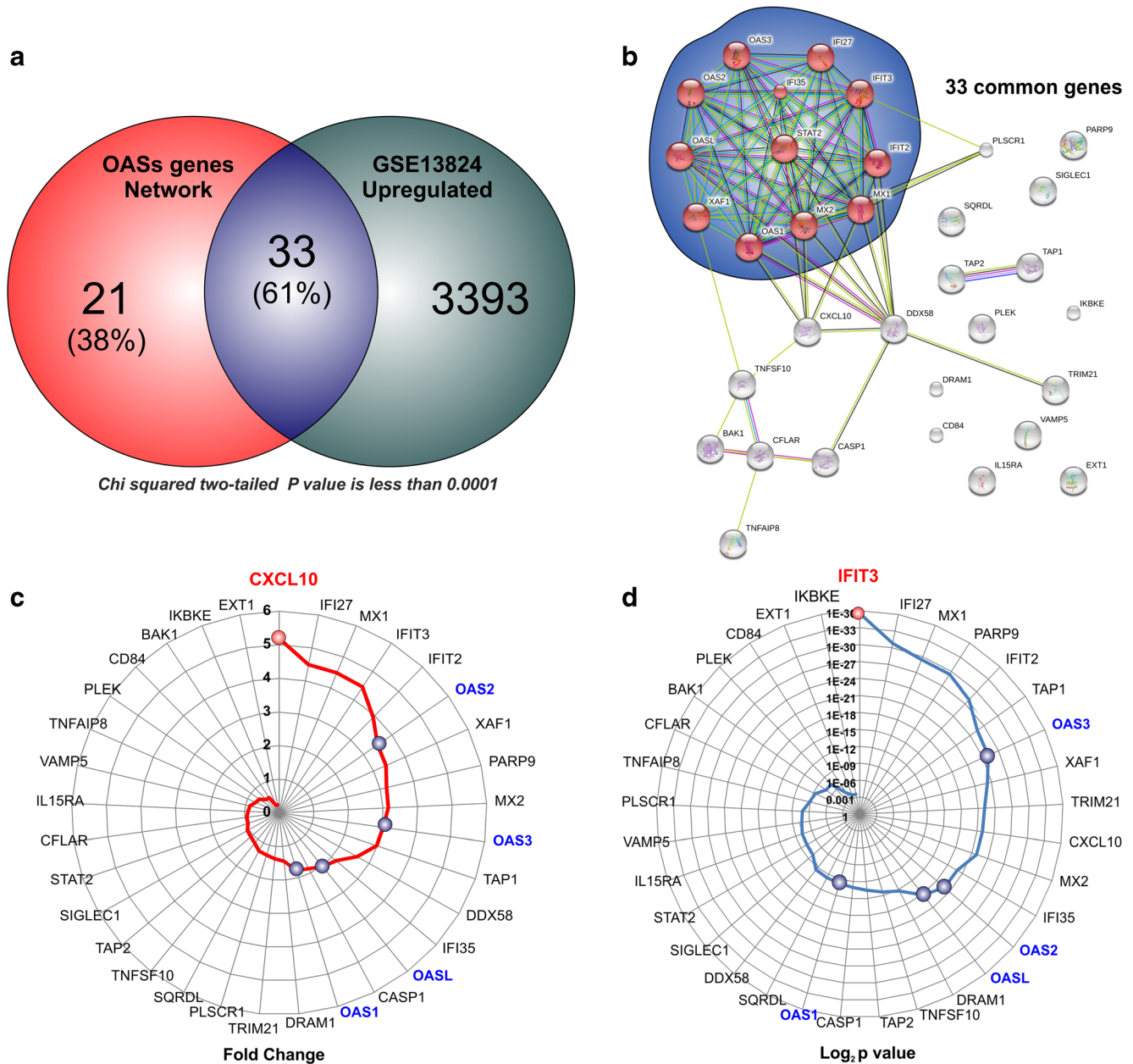
The GIANT/STRING analysis showed that *CXCL10* was only directly coexpressed to *OAS1* (combined score 0.430) without intermediate genes (Fig. 2b) (Supplementary Fig. 3A). As for *IFIT3*, we found that it

was directly coexpressed to *OAS1* (combined score 0.959), *OAS2* (combined score 0.950), *OAS3* (combined score 0.940), and *OASL* (combined score 0.961) (Fig. 2b) (Supplementary Fig. 3A).

The FunRich analysis of 33 query genes common to the OAS gene network and SIVE showed that 36.4% of these genes were significantly modulated by type I/III IFN pathways ( $p < 0.001$ ) (Fig. 3a). Two significant biological process were highlighted, the *immune response* (30.3%,  $p < 0.001$ ) and *apoptosis* (12.1%,  $p < 0.001$ ) (Fig. 3b). The tissue and cell localization analysis showed a pleiotropic expression of OAS genes in CD4 T cells, CD8 T cells, natural killer cells, and high expression in monocytes (Fig. 3c). Finally, the KEGG pathway analysis showed different viral pathways connected to the 33 query genes (influenza A 29%; measles 23%; herpes simplex 19%; hepatitis C 16%) (Fig. 3d).

### OAS Gene Family RNA Expression Are Significantly Upregulated in the Brain Sections of HAND and HAD/HIVE Patients

To validate the results obtained from the GSE13824 analysis, we used further microarrays, GSE28160, containing data on individuals with HAND. We evaluated which genes were similarly upregulated in SIVE (3426 upregulated genes, GSE13824) or HAND (3136 upregulated genes, GSE28160) vs the 50 genes belonging to the OAS gene network (Fig. 4a). The GSE28160 microarray



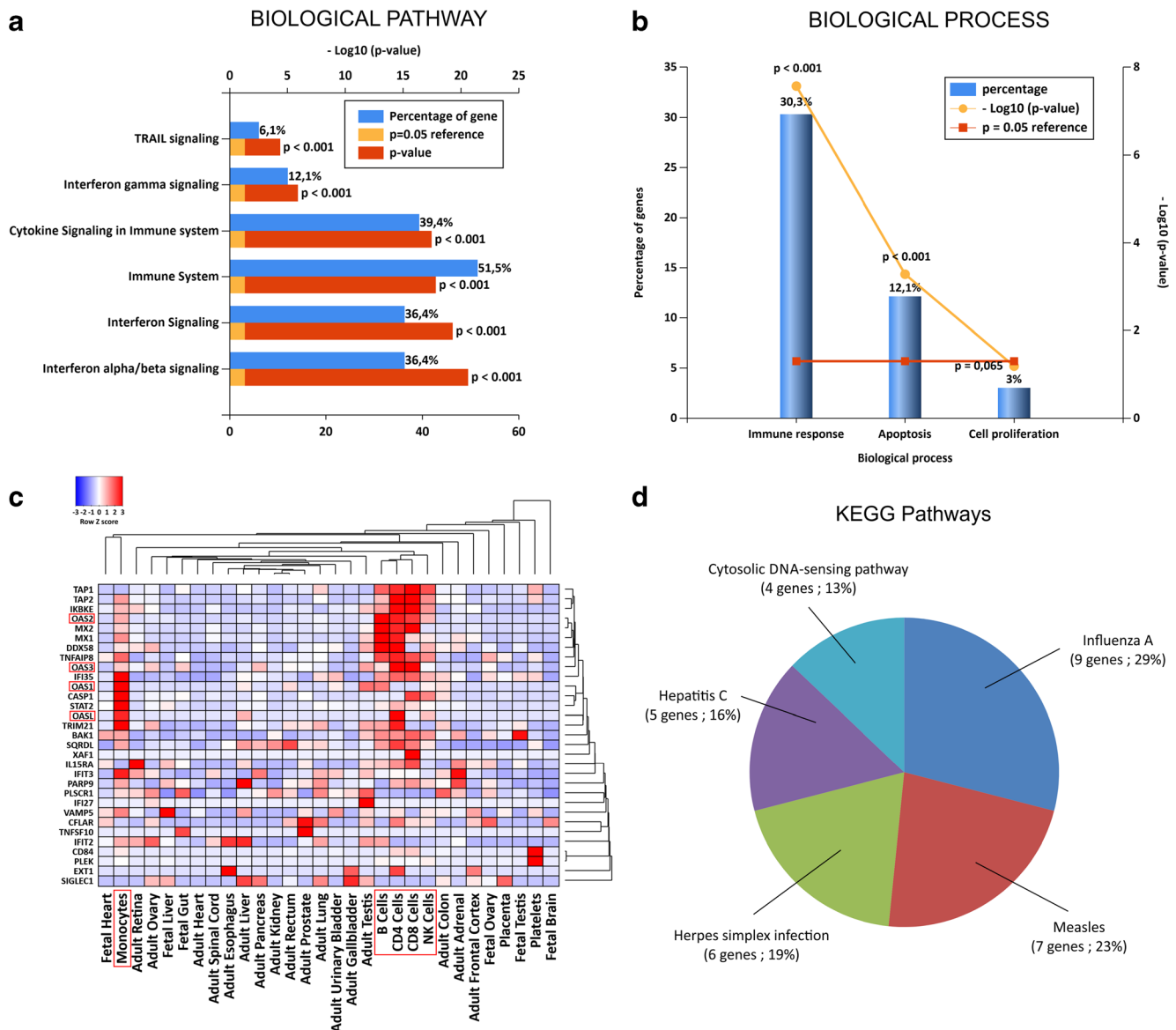
**Fig. 2** Sixty-one percent of OAS gene network is similarly modulated in the hippocampus of monkeys with SIVE. Venn diagrams were drawn using the Web-based utility Venn Diagram Generator and graphical homemade modified weighted Gene Networks were built for the commonly modulated genes using GIANT software. The 50 genes obtained by the GIANT analysis were compared to the 3426 significantly upregulated genes in the hippocampus of monkeys with SIVE. The 33

genes (61%) were in common with the SIVE group ( $p < 0.0001$ ) and 21 (38%) unequivocally belonged to the OAS network (a). We build a STRING network on 33 common genes. The genes shown in red belong to the type I interferon pathways (b). A Radar graph representation highlighted the most significant upregulated genes, *CXCL10* (fold 5.18,  $p = 1.14 \times 10^{-22}$ ) and *IFIT3* (fold 4.45,  $p = 2.14 \times 10^{-36}$ ) (C/D) (color figure online)

contained two groups of brain samples: 15 HIV-infected patients with HAND and six HIV-negative subjects with neither neurological nor neuropathological abnormalities. Seven out of 15 HIV-positive patients were receiving c-ART therapy. The Venn diagram analysis showed that among the 50 genes belonging to the OAS gene network, 27 (50%) were in common with the SIVE and HAND network ( $p < 0.0001$ ) (Fig. 4a), including *CXCL10* and

*IFIT3* (Fig. 4b). Of the OAS gene network, 81.4% (44 genes) were significantly upregulated in HAND and 61.2% (33 genes) in SIVE (as shown previously) (Fig. 4a) (Supplementary Table 1).

The analysis confirmed that *OAS1*, *OAS2*, *OAS3*, and *OASL* mRNA were significantly upregulated in the brain of patients with HAND with high blood viral load compared to healthy controls (Fig. 5). OAS gene family RNA



**Fig. 3** The interferon signalling, immune, and viral response are involved in SIVE hippocampus. The FunRich analysis of 33 query genes significantly upregulated in both OAS gene network and SIVE showed that 48% of these genes are significantly modulated by interferon type I/II pathways ( $p < 0.001$ ) (a) and two significant biological process, the *immune response* (30.3%, with  $p < 0.001$ ) and *apoptosis*

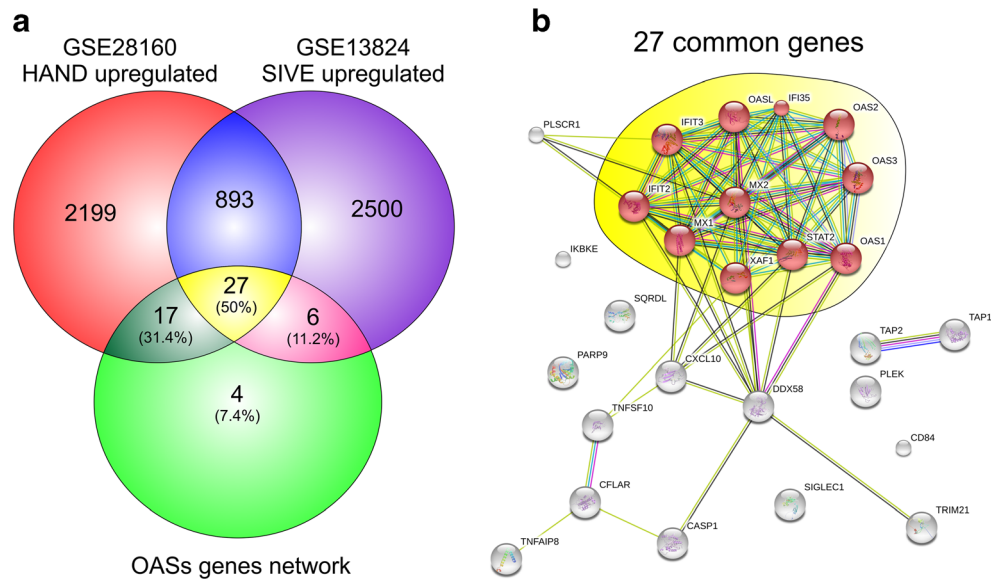
(12.1%, with  $p < 0.001$ ), were highlighted (b). The heatmap (c) showed a pleiotropic expression of OAS genes, in CD4 T cells, CD8 T cells, natural killer cells, and high expression in monocytes. The KEGG pathways analysis (d) showed different viral pathways connected to the 33 query genes

expression levels were significantly reduced in patients on c-ART therapy (Fig. 5a–d). No significant correlation was observed between OAS gene mRNA expression and blood viral load, as well as CD4 T-cell count (data not shown).

Similar results were obtained analyzing the GSE35864 dataset. During the exploration of OAS gene family RNA expression in basal ganglia, white matter, and frontal cortex of HIV-1, HAD, and HAD/HIVE patients, we identified a significant upregulation in HAD/HIVE patients compared to healthy subjects (Supplementary Fig. 5) (Supplementary Table 1). The *OAS2* mRNA expressions

levels were not significant only in frontal cortex of HAD/HIVE patients (Supplementary Fig. 5B). The Venn diagram analysis showed that among the 50 genes belonging to the OAS gene network, 34 (68%) were in common with the significant upregulated genes in HAD/HIVE vs healthy individuals ( $p < 0.0001$  by chi-square with Yates' correction) (Supplementary Fig. 6A). No genes were found comparing the significantly downregulated genes in HAD/HIVE vs healthy individuals and OAS gene network (data not shown). In addition, we showed that all OAS genes were significantly upregulated in the three GSE dataset analyzed (Supplementary Fig. 6B).

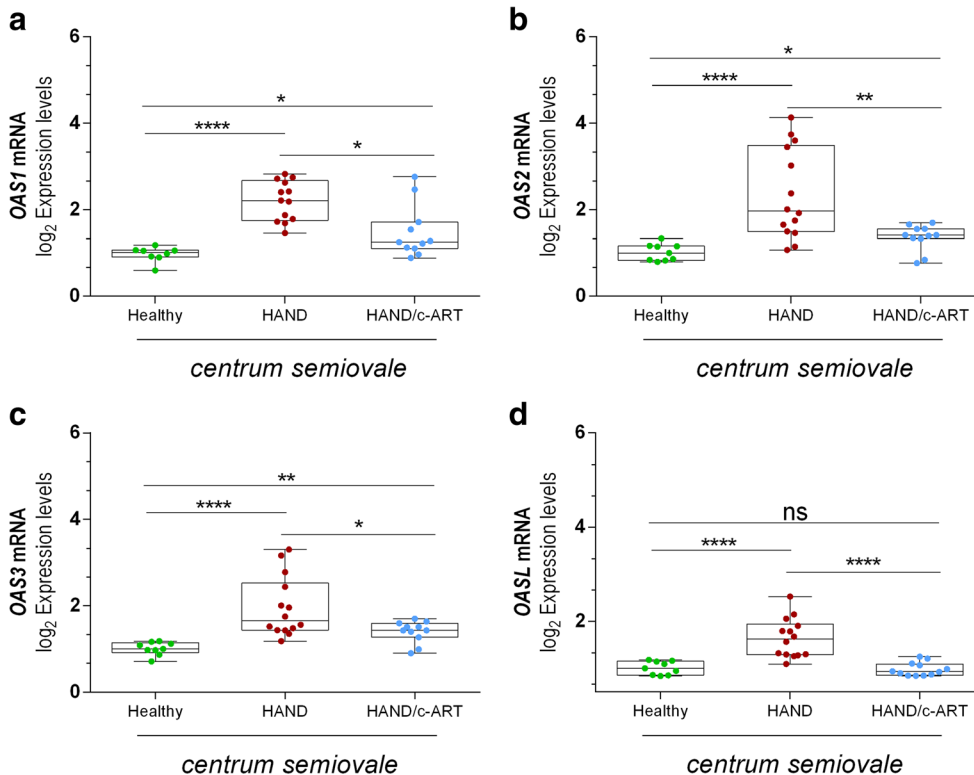
**Fig. 4** Almost 80% of OAS gene network is upregulated in brain biopsies of patients with HAND. Venn diagrams were drawn using the Web-based utility Venn Diagram Generator. We used two microarray datasets to compare the significantly upregulated genes in SIVE ( $n = 3426$ , GSE13824) and HAND ( $n = 3136$ , GSE28160) vs the 50 genes belonging to the OAS gene network. Twenty-seven genes (50%) were common to SIVE, HAND, and OAS gene network ( $p < 0.0001$  by chi-square with Yates' correction) (a). We built a STRING network of 27 common genes. The genes shown in red belong to the type I interferon pathways (b) (color figure online)



**Discussion**

In our microarray analysis, we found that OAS genes were significantly upregulated both in SIVE, HAND, and HAD/

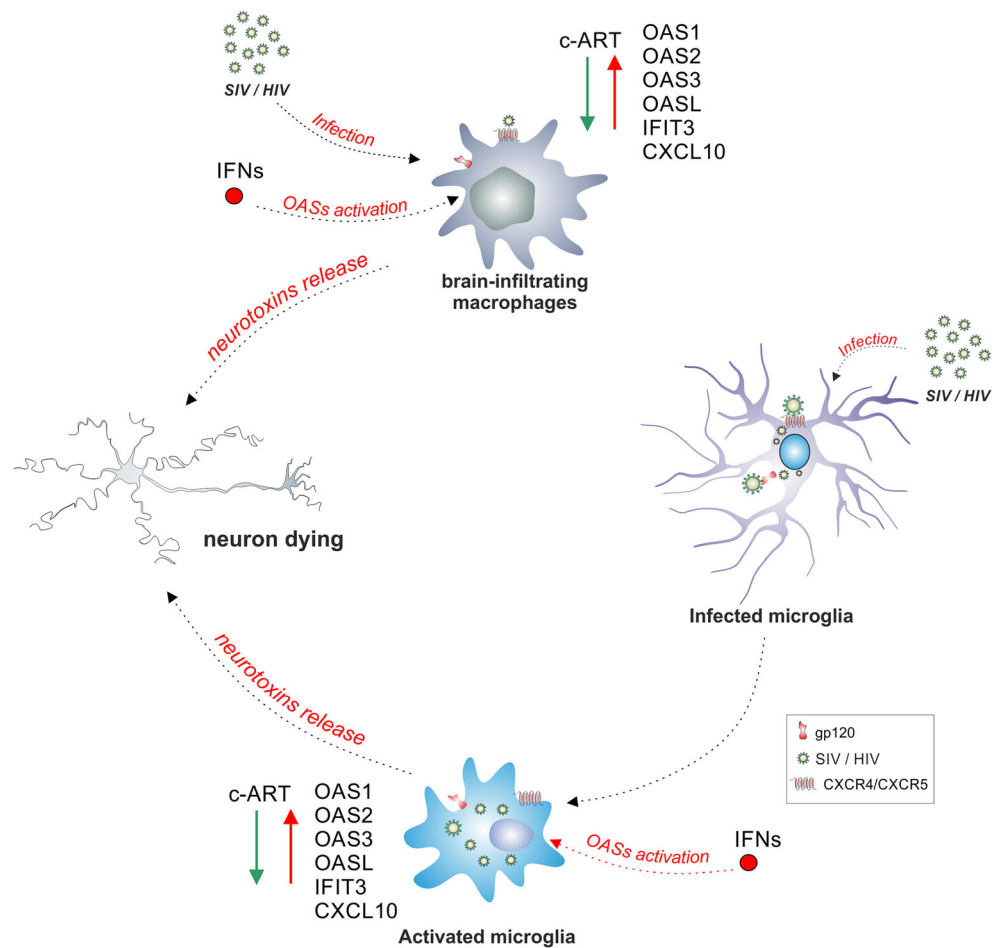
HIVE, and that several genes belonging to the OAS network, especially *CXCL10* and *IFT3*, were highly expressed in the setting of SIVE/HAND. Moreover, the OAS gene expressions were significantly higher in c-ART-naive patients with HAND



**Fig. 5** Antiretroviral therapy significantly reduces the expression levels of OAS gene family RNA expression in the brain of patients with HAND. Expression levels of *OAS1*, *OAS2*, *OAS3*, and *OASL* reveal a significant upregulation ( $p < 0.00001$ ) in HAND brain patients with high blood viral load compared to healthy controls. The brain section (centrum semiovale, deep white matter, at the coronal level of the genu of the corpus callosum)

of HAND patients on c-ART showed a significantly reduction of OAS gene family RNA expression compared to untreated patients. Dataset accession number GSE28160. Data are expressed as  $\log_2$  intensity expression levels and presented as vertical scatter dot plots.  $p$  values  $< 0.05$  were considered to be statistically significant (\* $p < 0.05$ ; \*\* $p < 0.005$ ; \*\*\* $p < 0.0005$ ; \*\*\*\* $p < 0.00005$ , ns = not significant) (a–d)

**Fig. 6** Innate immunity involvement in HIV neurocognitive disorders. OAS gene family is upregulated in HIV brain. As OAS genes are mainly produced in response to IFNs by monocytes/microglia, these findings suggest their involvement in the innate immune responses to HIV



in comparison to virologically suppressed ones. Furthermore, in HAD/HIVE patients' brain region, the OAS gene expression was significantly upregulated.

Innate immunity has been reported to play a crucial role in the development of neurocognitive disorders in HIV infection. Though the mechanisms of neuronal damage have not been fully explained, it is known that monocyte/macrophages are involved not only in early viral spread to the CNS and viral persistence in the brain, but also in the release of inflammatory cytokines and chemokines, including IFNs, which leads in turn to chronic immune activation and neurocognitive decline [7].

From this perspective, it can be hypothesized that the presence of HIV RNA in the CNS may be responsible for the release of several IFN-induced molecules, including OAS proteins. OASs are nucleotidyltransferases which exert their antiviral functions by stimulating the degradation of RNAs. OAS genes are induced by double-stranded RNAs and IFN, and they are mainly expressed by monocytes [9, 10]. In a recent study, increased expression of OAS gene family was reported in monocytes from HIV-infected high-viremic subjects [10]. Moreover, human macrophages treated with IFN- $\tau$  have been shown to increase the synthesis of OAS/RNase L [17].

Few studies have evaluated the expression of OAS gene family in HIV infection. In a report exploring immune responses to SIV in Rhesus macaques after oral infection, the authors found that *CXCL10* and OAS genes were upregulated both in the lymph nodes and peripheral blood [18]. In the peripheral blood, OAS upregulation was similar for high- and low-dose infection, while in lymph nodes increased OAS mRNA was observed only when animals were infected with high-dose virus. As expected, the authors identified CD14<sup>+</sup> monocytes as the major producers of OAS [18]. Interestingly, the same group found that increased expression of OAS and CXCL10 at the oral and rectal mucosa of macaques was associated with slower disease progression while increased expression of OAS and CXCL10 in lymph nodes and peripheral blood was associated with rapid clinical progression [19]. This discrepancy can be due to the differential increase of one or more cell types migrating to different sites and expressing these molecules. The high mucosal levels of CXCL10 and OAS may represent the attempt of the immune system to stimulate early adaptive responses, while the high levels observed in the lymph nodes can play a detrimental role, bursting immune activation and disease progression. The association between untreated infection and elevated



CXCL10 levels has been reported by several groups: Sarkar et al. have shown that macaques with high viral load have an increased frequency of CXCL10-producing cells compared to low-viremic animals [20]. Similarly, patients with HIV have increased circulating levels of CXCL10 compared to uninfected controls [21]; moreover, elevated CXCL10 have been reported in the CSF of subjects with AIDS-dementia complex [22]. As for OAS expression, a report from Read et al. has shown increased circulating OAS levels to predict the risk of progression to AIDS [23].

In the CNS, chronic IFN stimulation, with the subsequent dysregulation of IFN-stimulated genes, is likely to play a crucial role in neuropathogenesis [12, 24]. In fact, IFN- $\alpha$  is known to induce the production of IL-10 by recruiting CXCL10 and Stat3. IL-10, together with macrophage colony-stimulating factor (M-CSF), can drive the development of CD14+/CD16+ monocytes. These CD14+/CD16+ monocytes have an increased frequency in HIV-infected subjects, especially those with dementia [25]. Moreover, M-CSF may induce the differentiation of monocytes toward M2-macrophages, with potential important consequences in the induction of CNS damage, as M2-cells are preferentially infected with HIV [26]. Taken together, these results suggest IFN-induced production of antiviral molecules, including OAS proteins, represents the attempt of the immune system to contain HIV replication in the brain; however, chronic induction of these pro-inflammatory pathways is likely to have a significant impact on the development of neurocognitive impairment and immune dysfunction, even after starting c-ART, as confirmed by our finding of persistently increased OAS levels in virologically suppressed individuals compared to uninfected controls.

In conclusion, we found that OAS genes are upregulated both in SIVE and HAND. As OASs are mainly produced in response to IFN by monocytes/microglia, these findings suggest their involvement in the innate immune responses to HIV (Fig. 6). In addition, we found that OAS upregulation is reduced but not normalized after starting c-ART, suggesting that chronic induction of IFN pathways may have a role in the development of neuroimpairment. OAS molecules may potentially represent a new target for immune-modulatory strategies aiming at reducing CNS chronic inflammation and immune activation, which still occur even in the setting of effective c-ART.

#### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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