Chapter 19
Genetic, Epigenetic, and Transcriptomic Studies of NeuroAIDS

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Core Message

HIV-associated neurocognitive disorders (HAND) remain highly prevalent among those with HIV-1 infection. Risk for HAND appears to vary as a function of host genotype, in particular with regard to immune- and dopamine-related genes. Because HAND is a dynamic syndrome within individual cases, gene expression and epigenetic processes are also informative about HAND pathogenesis and potential treatment targets. Additional topics arising at the intersection of genomics and HAND include the iron dysregulation, HIV-associated central nervous system impairment in children, and leveraging epigenetic changes to study the effect of HIV on biological aging in the brain. Finally, several shortcomings of current HAND phenotypes are explored, as are promising alternatives.

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19.1 Introduction

Modern combination antiretroviral therapy (cART) has markedly improved the clinical outcomes of patients with human immunodeficiency virus-1 (HIV-1) infection. Severe neurological complications, such as HIV-associated dementia and HIV encephalitis, are now rare. However, milder forms of HIV-associated neurocognitive disorders (HAND) are increasingly prevalent. The reasons for this continue to be investigated and include chronic immune activation, interactive effects with biological aging, and antiretroviral drug toxicities, among others. In addition, some individuals appear to have inherent resistance not only to infection but also to HAND and other HIV-related complications. In this chapter, we review previous human genetic studies of HAND risk. In addition, because HAND is such a dynamic syndrome, we explore studies employing transcriptomic screening, as well as those investigating epigenetic processes associated with HAND. We also explore specific topics at the intersection of HAND and genomics. This includes the influence of iron dysregulation, mediated in part by genetic variants of iron-regulatory and mitochondrial-related genes; HIV-associated central nervous system (CNS) impairment in children, with a focus on host genetic variants that provide protection or added risk; the influence of aging on HAND risk and the genes that modify this risk; and research into a particularly exciting epigenetic measure of biological aging and its application to HAND. Finally, we address the difficulties of using current HAND phenotypes in genetic studies and offer some alternatives.

19.2 Genetic, Transcriptomic, and Epigenetic Studies of Hand in Humans

cART has resulted in a largely beneficial change in the course and clinical complications associated with HIV infection. In regard to complications involving the CNS, cART has resulted in a marked decrease in the frequency of severe conditions such as HIV-associated dementia (HAD) and underlying neuropathological conditions such as HIV encephalitis (HIVE) and microglial nodules [1–3]. However, in their place are milder forms of neurocognitive impairment, including asymptomatic neurocognitive disorder (ANI) and mild neurocognitive disorder (MND) [4]. The increasing prevalence of these milder forms is due to a variety of factors, including chronic immune activation, amplification of aging processes, and antiretroviral drug toxicities [5–10]. HIV-associated neurocognitive disorders (HAND), which include ANI, MND, and HAD, are diagnosed in 40–50% of unselected, chronically HIV-infected (or HIV+) individuals in the cART era who are able to complete neuropsychological testing [1, 11]. HAND is a public health concern, as it has adverse effects on medication adherence [12], activities of daily living [1, 13], employment, and overall quality of life [5].

The neuropathogenesis of HAND remains incompletely understood; it may overlap that of other common neurodegenerative diseases in which genetics has a role and with which HAND shares certain similarities [14–18]. Studies of genomic factors underlying symptoms and disease have led to helpful insights about HAND neuropathogenesis and identified potential treatment targets. In particular, recognition of the critical importance of neuroinflammation, reflected by elevated expression of inflammation or immune activation biomarkers in the brain, CSF, and plasma [19–21], the central role played by mononuclear phagocytes [16, 22, 23], and the possible role of dopaminergic dysfunction [24, 25], has provided a framework for studies of the role of host genomics in HAND.

However, while the application of genomic and other "omics" approaches coupled with bioinformatics and systems biology is promising, it faces a serious hurdle, the lack of a reliable phenotype for HAND. How can we, without a reliable neurocognitive, neuropathological, or neurophysiological biomarker for HAND, apply these methods in an effective manner? In this review, we present the current state of research involving human genetic, gene expression, and epigenetic data to understand HAND neuropathogenesis. We employ the term HAND to include all HIV-related neurocognitive deficits and their putative neuropathological causes. The benefits and limitations of these methods as applied to HAND are discussed. Finally, we propose potential solutions to overcome the primary obstacle in this research area, namely, a shift from behavioral to biological phenotypes and the application of systems biology as a path toward understanding the complexities of this disease process.

The purpose of this first section is to summarize the current state of understanding of host genomic, transcriptomic, and epigenetic factors that predispose individuals to HAND, with an emphasis on recent studies and reasonable conclusions that may be drawn from this rapidly growing volume of data. We then cover a number of special topics related to host genomic studies of HAND, including a focused examination of mitochondrial and iron-related genes, pediatric neuroAIDS, complement activation pathway polymorphisms, and the intersection of aging and genomics. We also discuss the acute challenge of omic studies of HAND due to lack of validated biomarkers and shifting definitions. We then discuss future directions for research in this field, suggested by the current state of knowledge, including innovative statistical methods, emerging genomic technologies, and therapeutic areas of promise.

19.2.1 Genetic Studies of HAND

19.2.1.1 Candidate-Gene Studies

The field of neurogenetics has long been interested in the role of genes in relation to psychiatric and neurologic characteristics and disease. However, in the case of HAND, there are no heritable neurocognitive deficits or neuropsychiatric symptoms

that would provide a foothold from which to explore genetic contributors of disease. Instead, the focus has been variants of genes involved in various biological processes that significantly impact risk of neurocognitive impairment, course of the disease, response to antiretroviral medications (ARVs), and also those associated with putative biomarkers of HAND. As such, genetic association studies in the realm of HAND serve both as a means to identify risk factors and to help delineate the neuropathogenesis. In this section, the focus is on studies of neurocognitive dysfunction; other HAND-related phenomena are covered later in the chapter. By and large, candidate-gene association studies have focused on immune-related genes and dopamine-related genes, as both the immune system and dopaminergic system are implicated in HAND pathogenesis. A comprehensive list of gene association studies is shown in Table 19.1.

19.2.1.2 Immune-Related Genes

There is a wide variety of immune factors that have been implicated in the chronic neuroinflammatory state leading to HAND, primarily involving cytokines, chemokines, and their cell surface receptors [71–73], as well as other immune factors such as human leukocyte antigen [58] and mannose-binding lectin-2 [50]. Genetic variants of these immune factors can affect HAND neuropathogenesis via numerous routes. For example, because HIV requires chemokine co-receptors to enter cells [74, 75], structural changes in receptors or expression levels of ligands can affect HIV replication [76] and disease progression [77, 78]. Chemokines also affect macrophage activation and chemotaxis of monocytes and other cells across the blood-brain barrier [79, 80], thereby leading to increased inflammation and viral entry into the CNS. Further, chemokines can affect neuronal signaling with subsequent disturbance of glial and neuronal functions [81, 82]. Several candidate-gene association studies have characterized how specific genetic variants of immune-related genes modify risk for HAND [17, 45, 47]. The most widely cited studies are discussed below. A comprehensive list that includes important study information such as sample description, phenotype, and results is provided in Table 19.1.

C-C chemokine receptor type 5 (*CCR5* gene). CCR5 is the most common HIV co-receptor, at least during the early course of infection. CCR5 mediates gp120 neurotoxicity [83]. A 32-base-pair deletion in the *CCR5* gene, resulting in the *CCR5-Δ-32* allele (rs333), leads to structural changes within the HIV co-receptor that confers high resistance to HIV infection among those who are homozygous at this locus [84, 85]. More recently, evidence for neurocognitive improvement among patients treated with CCR5 antagonists lends support for the potential role of variants of this gene in HAND risk [86]. Early genetic association studies suggested that this allele conferred protection against HIV-associated dementia. For example, Boven and colleagues [30] found that not a single case among their samples of European-American individuals diagnosed with HAD had a *CCR5-Δ-32* allele, which normally occurs in 10–20% of individuals with northern European ancestry. Although these findings were validated by another group shortly thereafter [31],

Table 19.1 Summary of candidate-gene and genome-wide association studies of HIV-related neurocognitive disorders

Study 1st author (year)	Sample description	Phenotype	Findings*
Dunlop et al. (1997) [26]	132 adult AIDS patients from Norway; majority were male Postmortem study of the basal ganglia, frontoparietal cortex, cerebral white matter, cerebellum, brain stem, thoracic spinal cord, and hippocampus Study compared the relationship of ApoE genotype with dementia and HIVE	HIV dementia was rated on a graded scale by a physician (no dementia, possible dementia, clinical dementia); however, the criteria were not specified. Determination of HIVE appeared to be based on presence of multinucleated cells, microglial noduli, and/or diffuse damage of white matter. Authors referred to a 1995 paper [27]	No association between *APOE* genotype (rs429358 and rs7412) and either HIV dementia or HIVE
Corder et al. (1998) [28]	44 HIV+ adults; majority were male and Caucasian Participants evaluated for neurological and other symptoms twice yearly for up to 10 visits	Dementia (described as predominantly mild). Criteria were not specified. Reported use of a battery of neuropsychological tests	*APOE ε4* allele carriers were twice as likely to be diagnosed with dementia during the study. The combination of the *ε4* allele and low CD4+ T cells increased risk for dementia over time. *ε4* allele carriers were also more likely to have peripheral neuropathy
Sato-Matsumura et al. (1998) [29]	44 AIDS patients with autopsy-verified HIVE or HIV leukoencephalopathy, 30 AIDS patients without these neuropathologies	HIVE and/or HIV leukoencephalopathy	*TNF-α* genotype (rs# unspecified) did not differ between the two groups
Boven et al. (1999) [30]	9 clinically demented AIDS patients; 8 non-demented AIDS patients; 6 HIV− control patients; age and ethnicity not provided; none of the AIDS patients were being treated with antiretroviral therapy Postmortem tissue specimens from the frontal cortex analyzed	AIDS Dementia Complex/HIV-associated dementia determined by a physician. Dementia cases had a score of 2 or higher on the Memorial Sloan-Kettering scale. Diagnoses were confirmed by postmortem neuropathological examination (methods not specified)	The *Δ32* deletion variant in the *CCR5* gene (rs333) was not found at the expected frequency among ADC patients. In vitro study showed heterozygosity of the *Δ32* deletion was associated with lower viral replication

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Table 19.1 (continued)

Study 1st author (year)	Sample description	Phenotype	Findings*
Van Rij et al. (1999) [31]	49 patients with AIDS Dementia Complex (ADC); 186 AIDS patients who died of AIDS with no ADC; age and ethnicity not provided; none received triple antiretroviral therapy	AIDS Dementia Complex – criteria were not specified	Lower frequency of the *Δ32* deletion variant in the *CCR5* gene (rs333) among ADC patients. *CCR2* 64I (rs1799864) genotype did not differ between ADC and non-ADC patients
Quasney et al. (2001) [32]	16 HIV+ adults with dementia, 45 HIV+ adults without dementia, and 231 healthy adult controls; 45–56% were Caucasian	HIV dementia per AAN criteria. The Memorial Sloan-Kettering criteria was used to classify severity [33]	*TNF-α-308* (rs1800629) A allele was significantly overrepresented among those with HIV dementia
Gonzalez et al. (2002) [34]	(1) 1151 HIV+ ethnically diverse adults (55% European-American, 36% African-American, 6% Hispanic-American, and 3% others). The majority was male (94%). Sample was followed prospectively with a median follow-up time of 5.9 years (2) 592 Argentinean children perinatally exposed to HIV (322 HIV+, 270 HIV-); drawn from a larger prospective study with a median follow-up time of 4.08 years	HIV-associated dementia. Criteria were derived from the Center for Disease Control [35] and included neuropsychological testing and neuroimaging	In Caucasian adults, homozygosity for the *MCP1 G* allele at rs1024611 was associated with 4.7-fold greater risk of HAD. Further analysis found this allele to be associated with greater transcriptional activity, enhanced protein production, increased serum MCP-1 levels, and increased monocyte infiltration of tissues

Singh et al. (2003) [36]	1049 HIV+ children; median age was 2.4 years; majority (59.7%) was non-Hispanic black Participants followed for up to 36 months	Neurological deterioration, a decline in neurocognitive test scores, or brain growth failure was considered evidence of disease progression between baseline and up to 36 months	Heterozygote carriers of the *Δ32* deletion in the *CCR5* gene (rs333) exhibited delayed disease progression, lower frequency of cognitive impairment at baseline, and lower frequency of either impairment at baseline or a decline in neurocognitive status (trend level) when compared to homozygous wild-type carriers. Among homozygotic carriers of the *Δ32* deletion, the most rapid disease progression was associated with A/A genotype at rs1799987 (*CCR5*) A/A genotype at rs1801157 on the *SDF1* gene was also associated with faster disease progression, including neurocognitive impairment over time. This was relatively uncommon, occurring in <2% of children studied. Modest or little effects were documented for rs1799864 (*CCR2*) or two SNPs on *CCR5* locally designated as 59,356 and 59,353
Cutler et al. (2004) [37]	10 HIV+ African-American males This was a postmortem study of brain tissue comparing sphingolipids and sterols in the medial frontal cortex, parietal cortex, and cerebellum of HIV dementia patients	HIV-associated dementia (determined by the presence of encephalitis in brain tissue and premortem Memorial Sloan-Kettering scale >1)	The *APOE ε4* allele was associated with dysregulated lipid and sterol metabolism, as well as elevations of sphingomyelin, ceramide, and cholesterol in the medial frontal cortex, parietal cortex, and cerebellum. The *ε4* allele was not related to astrocytes or activated microglia
Diaz-Arrastia et al. (2004) [38]	270 HIV+ persons who died from AIDS complications (unknown demographics); two separate cohorts assessed (one cohort whose members died during the monotherapy era, another whose members died during the dual therapy era)	HIVE as well as the presence of any of the following pathologies: microglial nodules, multinucleated giant cells, myelin pallor, and vacuolar myelopathy	No association between pathological findings and the *APOE ε4* allele (rs429358 and rs7412), *TNF-α*, *IL1B*2*, and *ILIRN*2*
Singh et al. (2004) [39]	121 HIV+ cognitively normal participants; the majority were Caucasian, non-Hispanic (68%) and male (88%) Prospective study with a median follow-up of 3.9 years and cognitive retesting every 6–12 months	Neurocognitive impairment, defined as a Clinical Rating score of 5 or higher and based on comprehensive neurocognitive testing [40]	At baseline, none of the alleles examined (*APOE Δ32* deletion, *MCP1-2518*, *CCR2*-V64I) were associated with neurocognitive impairment rates. In the longitudinal analysis, possession of one or two *CCR2*-64I alleles at rs1799864 was associated with earlier progression to neurocognitive impairment from study entry or from estimated time of seroconversion. Null findings: *Δ32* deletion at rs333 (*CCR5*) and rs1024611 (*MCP1–2518-G/A*)

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Study 1st author (year)	Sample description	Phenotype	Findings*
Valcour et al. (2004) [41]	182 HIV+ adults ($N = 85 < 40$ years, $N = 97 \geq 50$ years); sample was 54% Caucasian, 32% Asian or Pacific Islander, and 14% others	HAD (AAN, 1991), as determined via standardized neuropsychological testing, brain MRI, and serum tests	A significant association was observed between the *APOE ε4* allele (rs429358 and rs7412) and HAD among older (age ≥ 50 years) but not younger (<40 years) participants
Shiramizu et al. (2006) [42]	Repository CSF specimens from 27 prenatally infected with HIV	HIV-associated encephalitis	*MCP1 2578G* allele at rs1024611 was significantly more common in children with high HIV DNA in CSF. This allele was also associated with higher levels of supernatant *MCP1* in the CSF
Burt et al. (2008) [43]	1267 HIV+ adults; ethnically diverse (54% Caucasian); majority were male; they were followed prospectively with a median follow-up time of 5.9 years	HIV-associated dementia. Criteria not specified; however, the cohort appears to be the same as Gonzalez et al. (2002) [34], in which criteria were derived from the Center for Disease Control [35] and included neuropsychological testing and neuroimaging	*APOE ε4* allele was not associated with time to development of HAD, though it was associated with accelerated disease progression and time to death
Pomara et al. (2008) [44]	41 non-demented HIV+ adults, predominantly African-American (63%) and male (63%) This study assessed the effect of the *APOE ε4* allele on memory following acute lorazepam administration	Performance on verbal learning/ memory and psychomotor tests	The *APOE ε4* allele (rs429358 and rs7412) was associated with better immediate and delayed verbal recall at baseline assessment only
Pemberton et al. (2008) [45]	56 Caucasian HIV+ adults with HAD/ ADC, stage ≥1 and CD4 count <500 cells/ μL; other demographics unknown Data were also combined with genetic data from participants of other studies for a meta-analysis. This included HIV+ and HIV− controls	ADC/HAD, stage ≥1 (moderate to vegetative HAD) per [46]	HAD was more common among individuals who were homozygous for *A* allele at rs1800629 (*TNF-α-308*). When this data was combined with previously published data, possession of just one *A* allele was associated with HAD. No differences in allele frequencies were found for rs3783525 (*IL1A-889*), IL1B + 3953, and *IL12B* 3'UTR. *APOE* genotype (rs429358 and rs7412) did not differ between HAD patients and HIV+ controls in this study or when the data was combined with previously published studies

Levine et al. (2009) [47]	143 HIV+ adults (primarily Caucasian and African-American) who were either neurologically normal ($N = 117$) or who met criteria for HIV-associated dementia ($N = 26$) per established AAN criteria	HIV-associated dementia (HAD) per AAN criteria. Diagnosis was established via standardized neuropsychological testing and neuromedical exam	*TT* genotype at rs1130371 within the *CCL3* gene was associated with a twofold greater risk of HAD. Depression was associated with a fivefold greater risk of HAD. There was no association between HAD and any other of the other polymorphisms studied: rs1024611 (*MCP1*), rs1719130 (*CCL5*), rs17561 (*IL-1a*), rs1800872 (*IL-10*), rs1800629 (*TNF-α*), rs1801157 (*SDF1*)
Bousman et al. (2010) [48]	192 sexually active men with and without methamphetamine dependence (METH+/METH−) and/or HIV infection (HIV+/HIV−). Ethnicity was 71% Caucasian, 15% African-American, and 14% Hispanic	Executive functioning domain Deficit Score	There was a main effect of executive functioning but not of *COMT Val158Met* (rs4680) genotype on the total number of sexual partners. There was an interaction between rs4680 and executive functioning on total number of sexual partners and insertive anal sex (among *Met/Met* and *Val/Met* but not *Val/Val* carriers)
Joska et al. (2010) [49]	144 HIV+ young adults just entering care in South Africa, where Clade C is more common; the majority was female (74%)	HAND based on Frascati criteria (Antinori et al. 2007) using standardized neuropsychological testing	Null findings between *APOE* genotype and level of HAND severity. When comparing just HAD to non-HAD, the *ε4* allele (rs429358 and rs7412) was less common in HAD
Spector et al. (2010) [50]	201 Chinese HIV+ adults predominantly (93%) co-infected with hepatitis C	Global Deficit Score based on standardized neuropsychological testing. Considered both cross-sectional comparisons and rates of changes in neurocognitive status over the 12-month study period	A higher percentage of *APOE ε4* carriers (rs429358 and rs7412) were cognitively impaired at baseline. *MBL2* genotype (based on rs1800450, rs1800451, and rs5030737) was associated with neurocognitive changes over a 12-month period: 53% of those with *O/O* genotype declined whereas 23% of those with A/A genotype declined. No significant differences in baseline neurocognitive ability or change over time were observed for rs1799987 or rs333 (*CCR5*), *CCR2-180-G/A*, rs1801157 (*SDF1*), IL4-589-C/T, rs1024611 (*MCP1*), *CX3CR1-745-G/A*, and *-849-C/T* SNPs or for the *CCL3L1* copy number variant
Sun et al. (2010) [51]	44 HIV+ male adults; 11 HIV− adults; 62% Caucasian; all males. Ethnicity included 62% Caucasian and 22% African-American	Neuropsychological impairment (>1.5 SD below normative mean in two domains on a comprehensive test battery)	*APOE* genotype (rs429358 and rs7412) was not associated with neurocognitive outcomes

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Table 19.1 (continued)

Study 1st author (year)	Sample description	Phenotype	Findings*
Andres et al. (2011) [52]	48 HIV+; 39 HIV−; majority were male; largest ethnic groups were Caucasian (40%), Native or part-Native Hawaiians (28%), and Asians (13%)	Global Cognitive Score, based on the average of several domain *Z*-scores from a comprehensive neurocognitive test battery. The HIV Dementia Scale [53] was also examined as an outcome	Significant interactions were found between *APOE* genotype and HIV serostatus, with HIV+ ε4 allele carriers (rs429358 and rs7412) performing significantly worse than HIV-seronegative *ε4+* controls and seronegative *ε4-* controls on Global Cognitive Score and several specific cognitive domains. Among the HIV+ individuals, ε4 carriers performed worse than noncarriers on the HIV Dementia Scale
Chang et al. (2011) [54]	69 HIV+ adults of mixed ethnicity; predominantly male 70 HIV− adults of mixed ethnicity; predominantly male	Domain *Z*-scores and a Global *Z*-score were determined based on a comprehensive neurocognitive test battery	The *APOE ε4* allele (rs429358 and rs7412) was associated with poorer neurocognitive functioning (verbal fluency, executive function, learning, and memory) and smaller brain volume in HIV+ participants. This allele demonstrated a positive effect among HIV− individuals Further analysis by age group (older vs. younger), HIV serostatus, and *APOE* genotype indicated that the *ε4* allele had a deleterious impact on younger HIV+ individuals and a positive effect among younger HIV− individuals
Gupta et al. (2011) [55]	310 ethnically diverse males separated into four groups: 56 HIV−/methamphetamine nonusers, 77 HIV−/ methamphetamine users, 84 HIV+/ methamphetamine nonusers, 93 HIV+ methamphetamine users	Neurocognitive impairment, defined by a Deficit Score cutoff of ≥0.5. Based on a battery of neuropsychological tests	The C allele at rs6280 of the *DRD3* gene was associated with greater rates of neurocognitive impairment only among HIV+/ methamphetamine users
Singh et al. (2011) [56]	572 HIV+ children (206 progressors and 366 nonprogressors) enrolled in the Pediatric AIDS Clinical Trials Group study. Ages 3 months–18 years	Progression-free survival (defined as either time to first clinical HIV-related disease or death) and CNS-free survival (defined as time to deterioration in brain growth, psychological function, and/or neurological status)	The *B-27* allele was associated with complete protection against disease progression and CNS impairment over a median follow-up period of 26 months. The *Cw-2* allele protected against disease progression and the *A-24* allele was associated with more rapid CNS impairment. The HLA Class II *DQB1-2* allele was associated with a delayed disease progression and CNS impairment

Bol et al. (2012) [57]	86 HAD cases and 246 non-HAD AIDS patients as controls. All cases were from the Netherlands	Diagnosis of HAD was determined in various ways due to the retrospective nature of the data. This included DSM, AAN, and Frascati criteria	The *Δ32* deletion in the *CCR5* gene (rs333) was associated with HAD in cases that developed AIDS prior to 1991, but not after. *PREP1* genotype (rs2839619) differed between cases and controls irrespective of year of AIDS diagnosis. Null findings for: rs429358 and rs7412 (*APOE*), rs1130371 (*CCL3*), rs1799864 (*CCR2*), rs12483205 (*DYRK1A*), rs1024611 (*MCP1*), rs1046099 (*MOAP1*), rs12909130 (*PDE8A*), rs17519417 (*SPOCK3*), rs1800629 (*TNF-α*), and rs2905 (*UBR7*)
Schrier et al. (2012) [58]	Baseline sample of 203 HIV+ and 198 HIV- adults from a rural area of Anhui, China. HIV+ (N = 192) participants were reassessed at 12 months. HLA genotype data was analyzed for 178. 61% were male. Average amount of formal education was 5.5 years. 94% were HCV antibody positive	Global Deficit Score based on standardized neuropsychological testing. Considered both cross-sectional comparisons and rates of changes in neurocognitive status over the 12-month study period	*HLA-DR**04 was associated with a higher rate of baseline neurocognitive impairment, neurocognitive decline at 12 months, and HIV RNA in plasma. HLA Class I alleles (*B*27, 57, 58,A*03,33*) were associated with less impairment at baseline, at 12 months, and with less neurocognitive decline in the interval. The *HLA-DR*04* allele reduced the neuroprotective effect of the Class I alleles and, when present with the *APOE-ε4* in the same individual, had a synergistic negative effect on cognition
Brown et al. (2012) [59]	262 HIV+ individuals; 60% African-American	HIV dementia severity as determined by the Memorial Sloan-Kettering (MSK) classification	There were no differences in *CCL3L1* copy number in relation to HIV dementia severity
Levine et al. (2012) [60]	184 HIV+ adults (primarily Caucasian and African-American). All cases were diagnosed as neurologically normal or with mild cognitive/motor disorder or HIV-associated dementia per established AAN criteria or subsyndromic HIV-related neurocognitive impairment equivalent to asymptomatic neurocognitive impairment as per 2007 Frascati criteria	Neuropsychological domain T-scores (working memory, processing speed, learning, memory, motor). Scores were standardized based on entire NNTC cohort	Regression analysis found that *COMT Val158Met* (rs4680), *BDNF Val66Met* (rs6265), or *DAT1* (3′-UTR 40 bp) genotypes did not predict neurocognitive functioning after controlling for disease severity, depression, and demographic variables

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Table 19.1 (continued)

Study 1st author (year)	Sample description	Phenotype	Findings*
Soontornniyomkij et al. (2012) [335]	Brain tissue from an ethnically diverse sample of 160 HIV+ and 22 HIV− adult persons. The majority were male	HAND and Aβ plaques. HAND (mild cognitive/motor disorder or HAD per AAN criteria or subsyndromic impairment equivalent to asymptomatic neurocognitive impairment per 2007 Frascati criteria). Evaluation included comprehensive neurocognitive testing and neuromedical examination	*APOE-ε4* allele (rs429358 and rs7412) and older age (≥50) were independently associated with increased likelihood of cerebral Aβ plaque deposition. Although the *APOE-ε4* allele did not increase risk of HAND independently, ε4 carriers with Aβ plaque deposition had a higher risk of HAND
Morgan et al. (2013) [61]	466 HIV+ adults; 50% Caucasian; 78.8% were male	HAND based on 2007 Frascati criteria	This cross-sectional study found no effect of *APOE-ε4* allele (rs429358 and rs7412) on HAND or HAD in particular. No interaction between *APOE-ε4* allele and age, ethnicity, substance use disorders, duration of infection, or nadir CD4 on risk for HAND was observed
Panos et al. (2013) [62]	259 HIV+ ethnically diverse adults (55.2% Caucasian)	HAND (mild cognitive/motor disorder or HAD per AAN criteria or subsyndromic HAND equivalent to asymptomatic neurocognitive impairment as per 2007 Frascati criteria). Evaluation included comprehensive neurocognitive testing and neuromedical examination	94% of older *APOE-ε4* allele carriers had HAND compared to 56% noncarriers among older participants (age ≥ 50 years). No association between ε4 and HAND was found for younger (<50 years) participants Analysis by cognitive domain revealed the combination of advanced age, and the ε4 allele was associated with poorer executive functioning and information processing speed

Singh et al. (2013) [63]	1049 HIV+ symptomatic pediatric patients; predominantly of minority status (60% African-American, 26% Hispanic). Participants were enrolled prior to combined antiretroviral therapy availability Longitudinal study with median follow-up time of 18.6 months	CNS impairment defined as time from to deterioration in brain growth, psychological function, and/or neurological status. Neurocognitive decline was defined as the absence of any increase in raw scores or a decline in normalized scores by 2 SD for children <30 months of age or by 1 SD for older children. Deterioration in neurologic function was defined as the loss of previously documented motor skills, reflexes, or behavior	*APOBEC3G-H186R* (rs8177832) *G/G* genotype was associated with CNS impairment compared with the wild-type *A/A* or *A/G* genotype. Both additive and dominant models found the *APOBEC3G-F119F-C* allele (rs5757465) to protect against CNS impairment
Hoare et al. (2013) [64]	24 HIV+ individuals with at least one *APOE-ε4* allele were compared to 19 HIV+ without *ε4* allele. Participants were young, mostly females, and of Xhosa origin. This study was conducted in South Africa, where Clade C is more common; the majority was female (74%)	Group comparisons in neuropsychological functioning and diffuser tensor imaging were conducted	The *ε4* group had poorer immediate and delayed verbal memory and decreased fractional anisotropy in the corpus callosum
Morales et al. (2013) [65]	Cross-sectional study of 20 HIV+ and 16 HIV- women from the Hispanic-Latino Longitudinal Cohort of Women	AAN criteria (1996)	HIV+ women who were heterozygous at the rs4790084/rs1204828 loci in the *YWHAE* gene were three times more likely to exhibit reduced cognitive functioning, to have been diagnosed with HAND, and to express less YHWAE protein as compared to homozygotes. HIV+ women with HAND expressed 4.5 times less YWHAE in CSF compared to HIV+ neurocognitively normal women

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Table 19.1 (continued)

Study 1st author (year)	Sample description	Phenotype	Findings*
Levine et al. (2014) [66]	Longitudinal study of 952 individuals enrolled in the Multicenter AIDS Cohort Study. 952 individuals (914 Caucasian/non-Hispanic and 38 Caucasian/Hispanic). Roughly half were HIV+. All cases must have had 2+ neurocognitive evaluations between November 1985 and May 1995, a time frame chosen to avoid the possible confounding effects of HAART	Domain T-scores based on a comprehensive neurocognitive battery	No four-way interactions were found, indicating that HIV and stimulant use do not interact over time to affect neurocognitive functioning as a function of genotype. Numerous three-way interactions were found, but only rs1024611 (*CCL2*) and rs1719134 (*CCL3*) affected HIV+ individuals specifically. Specifically, for *CCL2* HIV+ cases with *TT* genotype improved at a faster rate than the HIV+ with *CC* or *CT* genotype. For *CCL3*, memory functioning declined in HIV+ individuals with *AA/AG* genotype for *CCL3*. Dopamine-related genetic variants generally affected HIV− individuals only
Chang et al. (2014) [67]	Cross-sectional study of 177 primarily of white or mixed race individuals. 80 were HIV+ and 97 HIV-. Mean age in the mid-40s. Most were men	Metabolite concentrations using MRS. Seven neurocognitive domain scores based on a comprehensive neurocognitive battery	Frontal white matter myoinositol was elevated in HIV+ participants across the age span. An age-dependent increase in HIV− participants was observed, most notably in those with the *APOE-ε4* allele. Only HIV− participants with the *APOE-ε4* allele showed elevated myoinositol in parietal cortex. All participants with *ApoE-ε4* had lower total creatine in basal ganglia. All HIV+ participants had poorer neurocognitive functioning, with *APOE-ε4* carriers having the poorest. In addition, higher myoinositol levels were associated with poorer motor function across all participants, slower speed of information processing in *APOE-ε4* participants, and poorer fluency in HIV+ participants with *APOE-ε4* genotypes
Sundermann et al. (2015) [68]	Two cross-sectional studies. A behavioral study involving 54 HIV+ and 33 HIV− women and an imaging study involving 23 HIV+ and 13 HIV− women	For the behavioral study, an N-back test for assessment of working memory. For the imaging study, the same test in conjunction with fMRI	HIV+ participants performed worse on the N-back test. An interaction between serostatus by *COMT* genotype was found, with *Val/Val* HIV+ women demonstrating poorer performance on the N-back test compared to HIV− women with the same genotype. Analogous findings resulted from the imaging study, with a serostatus X genotype interaction: HIV+ *Val/Val* carriers had greater prefrontal activation compared to HIV− *Val/Val* carriers. Conversely, HIV− *Met* carriers had greater prefrontal activation compared to HIV+ *Met*

Becker et al. (2015) [69]	Cross-sectional and longitudinal analysis of 2846 participants from the Multicenter AIDS Cohort Study who had *APOE* genotyping and neurocognitive data available for analysis. Age range 22–87, all male	Domain *T*-scores derived from a comprehensive neurocognitive battery. Overall cognitive functioning was characterized as follows: (1) within normal limits if one or fewer domains had *T*-scores 1 SD or more below the mean (i.e., $T \leq 40$), (2) mild impairment if two or more domains had *T*-scores $\leq$40, and (3) severe cognitive impairment if two or more domains had *T*-scores $\leq$30 or one domain had a *T*-score ≤ 25	*APOE* genotype was not significantly associated with time to death. The *APOE-ε4* allele was not related to incident cognitive impairment. No significant interactions between *APOE*, HIV status, and age on neurocognitive impairment were found
Villalba et al. (2015) [70]	Cross-sectional study of 267 HIV+ adults from urban areas of Miami-Dade County, Florida. Ages 18–60. All participants had history of alcohol use disorder	Three neurocognitive tests were used to characterize executive ability, cognitive flexibility, and visual memory	Significant associations between rs6277 (*DRD2*) and executive functioning and cognitive flexibility were found. When stratified by race and gender, significant results were seen in males and in African-Americans. The *VNTR* 7-allele (*DRD4*) was significantly associated with executive dysfunction

*When possible the Research SNP (rs) number is provided

more recent studies have not replicated this association [39, 50], possibly due to the changing pathogenesis of HAND. For example, Bol et al. [57] observed that the *CCR5-Δ-32* genotype was associated with HAD in individuals who developed AIDS prior to 1991, but not after, which was interpreted as reflecting the waning effect of this genotype on viral load set point. However, Singh and colleagues [36] found that children heterozygous for the *CCR5-Δ-32* allele had slower disease progression and less cognitive impairment than homozygous wild-type individuals. The phenotype in that study was neurocognitive functioning rather than HAND diagnosis.

Monocyte chemoattractant protein-1 (*MCP1* or *CCL2* genes). MCP-1 is a chemokine that recruits monocytes and other immune cells into the CNS and is therefore believed to be responsible in part for the neuroinflammatory response. In vitro HIV infection of human leukocytes results in increased transmigration across the BBB in response to MCP-1, which in turn increases expression of MCP-1 [87]. Levels of MCP-1 are elevated in the brain and CSF of patients with HIVE and HAD as compared to controls [19, 20], and MCP-1 in CSF is associated with pathologic magnetic resonance spectroscopy (MRS) indicators [71]. The HIV protein Nef induces MCP-1 expression in astrocytes with subsequent infiltration of infected monocytes into the brain [88]. The most commonly studied polymorphism in the context of HAND is SNP rs1024611, resulting in the *MCP1-2578* allele. This allele is associated with increased levels of MCP-1 in serum [89] and CSF [90] and has been linked to accelerated disease progression and a 4.5-fold increased risk of HAD [34]. However, this finding has not been consistently replicated [47, 50]. Also, while a recent study found evidence of a statistically significant rate of working memory ability change over time among carriers of this allele compared to noncarriers and HIV-uninfected individuals, the practical change was negligible [66]. Further, Thames et al. [91] reported that this polymorphism affects levels of inflammatory factors in CSF, which in turn affect neurocognition; however, no direct association between *MCP1* genotype and neurocognitive functioning was found in that study. Other polymorphisms outside this gene that affect the impact of MCP1 on neurocognitive functioning in HIV have been examined. For example, a recent study found a significant difference in *PREP1* allele distribution among HAD cases and non-HAD HIV+ controls [57]. Prep1 is a transcription factor with preferential binding to the promoter region of the *MCP1* gene. In addition, a polymorphism within the minor HIV co-receptor CCR2, the natural target receptor for MCP-1, may result in slower HIV disease progression [92]. Specifically, individuals heterozygous for the *CCR2-V64I* allele exhibited slower disease progression and developed AIDS 2–4 years later than those who were homozygous for the wild-type allele. Still another study found *CCR2-V64I* to be associated with slower progression to neurocognitive impairment [39].

Macrophage inflammatory protein 1-alpha (*CCL3* gene). CCL3, or MIP-1α, is a chemokine and natural ligand of the HIV co-receptor CCR5. MIP-1α expression is increased in the brains of those with HIVE and is released by both microglia and astrocytes [93]. SNP rs1130371 within the *CCL3* gene was previously associated with HIV disease progression [94] and was found to be associated with a twofold

greater risk for HAD [47] in the National NeuroAIDS Tissue Consortium (NNTC) cohort. An interactive effect was found between another SNP (rs1719134) and HIV status on changes in learning ability over time, such that HIV+ individuals demonstrate less improvement over multiple assessments as compared to their HIV-negative counterparts; however, the difference was negligible from a practical standpoint [66].

*HLA-DR. HLA-DR*04* genotype was identified as a predictor of HAND, low CD4+ T-cell responses to HIV, and low plasma HIV RNA levels in a US cohort. It was hypothesized that low CD4+ T-cell activation may lead to poor immune control of HIV in the CNS, predisposing to HAND, but it may also provide fewer targets (activated CD4+ T cells) for HIV replication. To assess the consistency of these HLA Class II associations in a new cohort and extend analysis to HLA Class I, HLA types, neurocognitive, and virologic status were examined in a cohort of former plasma donors in China [58]. In this study, 178 HIV+ individuals in Anhui, China, were HLA typed and underwent assessment of neurocognitive function (using locally standardized norms) and neuromedical, treatment, and virologic status at baseline and 12 months. *HLA-DR*04* was associated with a higher rate of baseline neurocognitive impairment ($p = 0.04$), neurocognitive decline ($p = 0.04$), and lower levels of HIV RNA in plasma ($p = 0.05$). HLA Class I alleles (*B*27,57,58,A*03,33*) that specify a CD8+ T-cell response to conserved HIV sequences were neuroprotective, associated with less impairment at baseline ($p = 0.04$) and at month 12 ($p = 0.01$) and less neurocognitive decline ($p = 0.02$) in this interval. Consistent with the theory that effective CD8+ T-cell responses require CD4+ T-cell support, the *HLA-DR*04* allele reduced the neuroprotective effect of the Class I alleles. The presence of *HLA-DR*04* and the Alzheimer's disease-associated allele *APOE-ε4* in the same individual had a synergistic negative effect on cognition ($p < 0.01$). Despite major background differences between US and Anhui, China, cohorts, *HLA-DR*04* predicted neurocognitive impairment and lower plasma HIV RNA levels in both populations. HLA Class I alleles associated with CD8+ T-cell control of HIV were associated with protection from HAND.

APOE-ε4 and MBL-2 genes [50]. For the above Chinese individuals, among 43/201 patients with the *APOE-ε4* allele, 58% were cognitively impaired, compared with 31% without the *APOE-ε4* allele ($p < 0.01$, odds ratio 3.09, 95% confidence interval 1.54–6.18). The mean global deficit score (GDS, a composite score derived from a battery of neurocognitive tests) for *APOE-ε4* carriers on antiretroviral drugs for 12 months was 0.88 [standard deviation (SD) = 0.55] compared with 0.63 (SD = 0.54) for *APOE-ε4* noncarriers [p = .05, 95% confidence interval (CI) -0.004 to 0.51]. For the *MBL-2* gene, 52% of patients with the *O/O* genotype experienced cognitive decline over 12 months, compared with 23% with *A/A* genotype [odds ratio (OR) 3.62, 95% CI 1.46–9.03, $p < 0.01$)]. The *APOE-ε4* allele was associated with increased risk for cognitive deficits, whereas the *MBL2-O/O* genotype was associated with increased risk for progressive cognitive decline in Chinese individuals infected with HIV through contaminated blood products.

19.2.1.3 Dopamine-Related Genes

In recent years, there have been numerous reports of polymorphisms within dopamine (DA)-related genes, resulting in measurable differences in neurophysiological and neurocognitive functioning in non-HIV cohorts. Among the most commonly examined are the catechol-O-methyltransferase (*COMT*) *val158met* allele [95–103], the dopamine transporter-1 (*DAT1*) 3'-UTR variable tandem repeat [104–109], and the brain-derived neurotrophic factor (*BDNF*) *val66met* allele [110–117]. While the effects of these variants on neurocognitive phenotypes have been small, it is conceivable that among HIV+ individuals, in whom DA functioning may already be compromised [107, 118–121], the effects will be additive or synergistic. Despite this plausible hypothesis, cross-sectional studies to date have not found reliable evidence that DA genotype modifies risk of HAND [60]. For example, Levine et al. [60], examining cross-sectional data from the NNTC, did not detect any interactive effect of disease severity (as measured by CD4+ T-cell count) and *COMT, DAT1,* or *BDNF* genotypes described above upon a number of neurocognitive domains in an exclusively HIV+ sample. Bousman et al. (2010) reported interactive effects of *COMT val158met* genotype (rs4680) and executive functioning on sexual risk taking in both HIV+ and HIV− individuals [122]. While no differences in executive functioning were noted between groups, they did find that among *Met* allele carriers, those individuals with greater deficits in executive functioning reported a greater number of sexual partners and other risky sexual practices. Very recently, Sundermann et al. [68] examined interactive effects of *COMT* rs4680 genotype and HIV on executive functioning and frontal cortex metabolism among two samples of women enrolled in the Women's Interagency HIV Study Consortium. While vastly underpowered for a genetic association study, they found that HIV+ *Val/Val* carriers performed significantly worse on working memory tests compared to uninfected *Val/Val* carriers and that HIV+ *Val/Val* carriers also showed greater prefrontal activation compared to uninfected *Val/Val* carriers during the task.

The additive or synergistic effects of DA-related alleles and stimulants such as methamphetamine and cocaine in HIV+ cohorts have also been examined. Gupta et al. [123] investigated the impact of a SNP (rs6280) within the dopamine receptor-3 gene (*DRD3*) upon neurocognitive functioning in four groups, stratified for HIV status and methamphetamine use. The biological connection between DRD3 and HAND is especially interesting, as macrophages are more likely to be infected by HIV in the presence of both methamphetamine and increased extracellular DA individually, and this process is mediated by DA receptors expressed on macrophages, including DRD3. As the authors hypothesized, only the HIV+ methamphetamine users were found to have genotype-related neurocognitive alterations.

Analyzing longitudinal neurocognitive data from the Multicenter AIDS Cohort Study (MACS), Levine et al. [66] examined the longitudinal interaction between HIV status, stimulant use, and DA-related genetic variants in a very large cohort ($N = 952$) that included both HIV+- and HIV-uninfected individuals. *COMT* genotype (rs4680) was found to influence the longitudinal neurocognitive functioning of uninfected individuals, but not HIV+ cases. Other DA-related genetic variants,

including those in genes for *BDNF* (rs6265), dopamine-β-hydroxylase *(DBH)* (rs1611115), dopamine receptor-2*/ANKK1 (DRD2)* (rs1800497), and *DRD3* (rs6280), did not affect the longitudinal neurocognitive functioning of HIV+ individuals.

19.2.1.4 Genome-Wide Association Studies

Genome-wide association studies (GWAS) have become increasingly affordable and a practical means to study disease pathogenesis. Several such studies have identified additional risk variants associated with HIV disease progression (including so-called rapid progressors), viral set point, and other disease-related phenotypes, as previously reviewed [124, 125]. GWAS have also proven valuable for the study of already relatively well-characterized neurologic diseases, such as Alzheimer's disease [126–129]. In the context of HAND, for which the cause remains poorly understood, GWAS also hold promise because of the potential to identify common genetic variants that contribute to neuropathogenesis. This potential to lead to improved mechanistic understanding of HAND and ultimately identification of pharmaceutical targets is tempered by the need for very large sample sizes to achieve the necessary power to detect those variants that influence phenotype. To date, only one GWAS focusing on HAND has been published [130]. The study sample consisted of 1287 Caucasian adults enrolled in the MACS, leaving it vastly underpowered by general standards. However, by leveraging a MACS protocol that includes serial neurocognitive testing and neuromedical examinations, several neurocognitive phenotypes were examined for their association with over 2.5 million SNPs. The phenotypes decline in processing speed or executive functioning over time, prevalent HAD, and prevalent neurocognitive impairment based on a comprehensive neuropsychological battery. Two SNPs within the *SLC8A1* and *NALCN* genes had p-values just below the strict GWAS threshold in association with change in processing speed over time. These genes, involved in sodium transport across cellular and intracellular membranes, support the role of mitochondrial dysfunction in HAD [131–133]. In the future, additional GWAS with larger samples will be possible by encouraging collaborative efforts across cohorts.

19.2.1.5 Summary

Targeted candidate-gene association studies are valuable for investigating HAND neuropathogenesis, in part because HAND is a syndrome that is many degrees separated from its molecular causes. However, a requisite for such studies is that the genes under investigation meet a standard of biological plausibility. Accordingly, genetic association studies have implicated a variety of immune-related genes for their role as risk or protective factors for HAND. However, very few of these associations have been replicated across studies. As discussed further below, there are several reasons for this, including lack of a reliable and consistently applied

phenotype for HAND (or more accurately a consistent definition or valid biomarker), focus on a narrow range of polymorphisms, and study design issues, such as failing to consider population stratification and admixture, Hardy-Weinberg equilibrium, and other factors. Going forward, investigators of genetic associations with HAND are encouraged to follow the strengthening the reporting of genetic association (STREGA) studies guidelines [134], which set standards for reporting and transparency of such studies. In particular, recruitment methods and statistical strategies must be especially rigorous. With regard to GWAS of HAND, collaborations across cohorts with the goal of increasing the statistical power to detect common variants contributing to neuropathogenesis will be necessary, and supplemental strategies to follow up GWAS analysis may also be useful for revealing associations that were undetected initially [135].

19.2.2 Transcriptomic Studies of HAND

Gene expression alterations have been widely studied in the context of neuroAIDS, including studies in human tissue and cells, animals, and in vitro models. For the purposes of this chapter, we limit our discussion to those studies that employed genome-wide microarrays (i.e., transcriptomic studies).

19.2.2.1 Brain-Based Gene Expression Studies

Most transcriptomic studies of neuroAIDS have taken advantage of genome-wide microarrays, allowing surveillance of virtually the entire transcriptome. The next-generation sequencing method, RNA-seq, is also available but has not to our knowledge been used for a published study of HAND. RNA-seq has several advantages, including increased coverage of the genome and the ability to assay miRNA, transfer RNA (tRNA), and other RNA in addition to mRNA. Regardless of the method, once expression data is generated, it must be evaluated using bioinformatics and systems biological methods that make it possible to discern biological networks associated with a chosen phenotype [136, 137]. There have been several transcriptomic studies of HAND or related phenotypes. Some focus on specific brain cells in vitro [136, 138–140], using methods such as laser capture microdissection. However, most transcriptomic studies to date have utilized brain tissue from HIV+ humans. Early studies focused on gene expression changes of frontal gray matter associated with HIVE and generally found altered regulation of genes involved in neuroimmune functioning; they also implicated neurodegenerative pathways based on dysregulation of genes involved in synaptodendritic functioning and integrity [141], toll-like receptors [142], and interferon response [143]. Findings from human microarray studies have been partially replicated in simian immunodeficiency virus (SIV) models, especially with regard to interferon-related and neuroinflammatory-related genes [144–146], providing some degree of validation. The overlap between animal and human brain transcriptomic studies was recently reviewed [147].

In perhaps a more relevant model to contemporary HAND, Gelman et al. [148] analyzed transcriptome data derived from multiple brain regions of HIV+ individuals diagnosed premortem with HAND alone or with both HAND and HIVE (as found postmortem). That analysis led to the discovery of different transcriptome profiles between the groups, implicating two distinct etiological pathways to HAND [148]. Specifically, HIVE with concomitant HAND was associated with high RNA viral load in brain tissue, upregulation of inflammatory pathways across all brain regions, and downregulation of neuronal transcripts in frontal neocortex. In contrast, HAND without HIVE was characterized by low brain viral RNA burden without evidence of increased inflammatory response and without downregulation of transcripts in frontal neocortical neurons. Only transcripts characteristically expressed by vascular- and perivascular-type cells were consistently dysregulated in HAND without HIVE. These data were recently reexamined by Levine et al. [149] using a systems biologic analysis method and weighted gene coexpression network analysis (WGCNA) [150]. While standard gene expression studies such as the study by Gelman et al. [148] utilize a group comparison approach, WGCNA enables a more systematic and global interpretation of gene expression data by examining correlations across all microarray probes, identifying biologically meaningful modules that are comprised of functionally related genes and/or correspond to cell types [151]. These modules can be examined for their association to clinical or biological variables of interest. Levine et al. [149] found a number of biologically meaningful gene expression modules that were correlated with a global neuropsychological functioning index and CNS penetration effectiveness (CPE). While the WGCNA largely validated the findings from Gelman et al., it also identified meta-networks composed of multiple gene ontology categories as well as oligodendrocyte and mitochondrial functioning. Levine et al. [149] also identified genes that were commonly associated with neurocognitive impairment in Alzheimer's disease and HIV (Table 19.2). Specifically, common gene networks dysregulated in both conditions included mitochondrial genes, whereas upregulation of various cancer-related genes was found. An earlier meta-analysis by Borjabad and Volsky (2012) compared global transcriptomes derived from frontal gray and/or frontal white matter from individuals with HIVE (regardless of HAND status) to those derived from various brain regions of individuals who had Alzheimer's disease, without consideration of NCI [152]. Both diseases (as well as multiple sclerosis) were associated with upregulation of a wide range of immune response genes, and HAND and AD also shared down-modulation of synaptic transmission and cell-cell signaling. However, there were several methodological differences between the studies, making it difficult to compare the results.

Transcriptomic studies have also been helpful in understanding the interaction of antiretroviral drug use and HAND. Borjabad et al. were the first to examine the relationship between cART use and global brain gene expression [153]. They found that the transcriptomes in cART-treated cases more closely resembled those of HIV-seronegative cases and had 83–93% fewer dysregulated genes, compared to untreated individuals if they were taking cART at the time of death. However, both cART-treated and untreated HIV+ brains were found to have approximately 100 dysregulated genes related to immune functioning, interferon response, cell cycle,

Table 19.2 Mechanisms and biomarkers of relevance to HAND

	Mechanisms of relevance to HAND	Biomarkers
Mitochondrial and iron	Basic metabolic processes: DNA, RNA, protein synthesis Maintenance of mitochondrial membrane potential Mitochondrial electron transport chain function Calcium homeostasis Lipid homeostasis Numerous other metabolic reactions Epigenetic modifications	Markers of oxidative and nitrosative damage to DNA, RNA, proteins, and lipids: 8-oxo-DG F_2-isoprostanes (specific) and neuroprostanes Isofurans, neurofurans Malondialdehyde (less specific) Protein carbonyls 8-nitroguanine (RNA, DNA) Oxysterols Aβ and α-synuclein aggregations
Iron transport	Mitochondrial biogenesis Iron-sulfur cluster biogenesis Neuronal repair/remyelination Myelination Monoamine neurotransmitter synthesis (dopamine, 5-HT) Cellular glutamate uptake/excitotoxicity Amyloid protein processing Calcium signaling Heme-oxygenase-1 deficiency Hypoxia-response pathways Macrophage-monocyte polarization/activation Macrophage-monocyte cytokine release HIV replication Endoplasmic reticulum (ER) stress	

and myelin pathways. Of note, gene expression in the HIV+ brains was not correlated with brain viral RNA, suggesting that even high CPE [154], which has been shown to reduce CSF viral load [8], may not reverse transcriptomic dysregulation. This finding is supported by a study by Levine et al. that showed no association between CPE and brain transcriptome utilizing both standard differential expression analysis and WGCNA [149]. These findings might help to explain the equivocal findings regarding CPE and HIV-related neurocognitive dysfunction to date [155–159].

19.2.2.2 Blood-Based Gene Expression Studies

Focus on peripheral (i.e., outside the CNS) mononuclear cells rather than brain tissue in studies of HAND necessitates different hypotheses and careful interpretation of results. By examining transcriptome changes in peripheral blood mononuclear cells (PBMCs), it is possible to identify biomarkers of HAND or anticipatory

cellular changes. These cells can be assayed easily, allowing for investigation of cellular events that occur considerably farther upstream to HAND onset. Due to their central role in HAND pathogenesis [160–166], monocytes have been the cells of choice for blood transcriptomic studies of HAND.

Using an in vitro model, Buckner et al. (2011) examined dynamic transcription changes in monocytes derived from healthy donors [167]. The cells were infected with HIV to produce a CD4 + CD16 + CD11b + Mac387+ monocyte subpopulation capable of crossing a laboratory model of the BBB. Gene expression analysis revealed upregulation of chemotactic- and metastasis-related genes but not inflammatory genes. Dynamic changes were also observed as the monocytes matured into macrophages, including an increase in the expression of enolase-2, followed by a decrease once the cell was fully differentiated. Osteopontin was also observed to have increased expression in the maturing monocytes.

Sun et al. (2010) reported the first study in which blood monocyte global transcription was associated with neurocognitive functioning in HIV+ individuals. More specifically, the authors examined whether or not monocyte gene expression and other peripheral factors (CD4+ T-cell numbers, *APOE* genotype, viral load, lipopolysaccharide, and soluble CD14 levels) were associated with neurocognitive functioning in a group of 44 HIV+ individuals on cART and 11 HIV-seronegative controls [51]. Monocyte gene expression, which showed a chronic inflammatory profile in the HIV+ participants with high viral load, was not correlated with neurocognitive impairment. The other blood markers were also not found to be associated with neurocognitive functioning. More recently, the same group of researchers focused their analysis on a neurophysiological phenotype rather than HAND [168] by examining whether peripheral immune activation and monocyte gene expression were associated with brain metabolite concentrations, as measured by MRS. Thirty-five HIV+ individuals on cART and 8 HIV-seronegative adults were examined. Among the HIV+ participants, an interferon-*alpha*-induced activation transcriptome phenotype was strongly correlated with N-acetyl aspartate in the frontal white matter. Notably, interferon-gamma inducible protein-10 (IP-10 or CXCL-10) was strongly correlated with plasma protein levels, and plasma CXCL-10 was inversely correlated with N-acetyl aspartate in the anterior cingulate cortex. This study is remarkable, as it is the first to connect transcription changes with neurophysiological changes in the context of neuroAIDS. As discussed below, we believe that this tactic holds the greatest promise for elucidating the neuropathogenesis of HAND.

Levine et al. [169] utilized the Illumina HT-12 v1 Expression BeadChip to analyze monocyte-derived transcriptome data from 86 HIV+ individuals enrolled in the MACS. Examining all HIV+ samples, the standard differential expression analysis identified a number of individual gene transcripts that were significantly correlated with global neurocognitive functioning. Of the 16 genes identified, many implicated oxidative stresses, including those encoding interleukin-6 receptor *(IL6R)*, casein kinase 1-alpha-1 *(CSNK1A1)*, hypoxia upregulated-1 *(HYOU1)*, low density lipoprotein receptor-related protein-12 *(LRP12)*, and Kelch-like ECH-associated protein-1 *(KEAP-1)* [170–185]. The *KEAP-1* findings are especially interesting, as they support a recently described role for *nuclear factor E2-related factor-2 (nrf-2)* in

HAND [186]. There has been some interest in recent years by neuroAIDS researchers of factors that modify the activity of nrf-2 (e.g., GSK3-β inhibitors [187] and curcumin [188]) or that are modified by it [189]. As such, members of this pathway deserve further investigation as potential pharmacological targets during early stages of HAND or even as potential prophylactic agents.

19.2.2.3 Summary

Genome-wide transcriptomic studies have implicated numerous genes and biological pathways in the neuropathogenesis of HAND. Some results of human studies have been replicated in simian and murine models. One limitation of previous studies is the use of homogenized brain tissue, which contains mRNA from numerous cell types [141, 145, 190, 191], thus making it difficult to determine cell-specific molecular processes. In addition, most studies describe gene expression from one brain region (e.g., frontal lobe), and those regional disease-related transcription changes may not reflect the disease-related transcription changes occurring in brain regions also commonly implicated in HAND (e.g., basal ganglia). Also, most in vivo studies utilizing brain tissue have sought to understand alterations in gene expression in brain tissue of humans or animals that expired in an advanced state of disease (i.e., HIVE or HAD). Therefore, it is unclear if the findings of those studies will generalize to contemporary HAND. In tandem with studies of brain tissue, there have been investigations of monocyte transcriptome, which may provide clues about the earlier stages of HAND pathogenesis. Finally, the interpretation of transcriptome data utilizing systems biological methods such as WGCNA [150] may point the way to novel therapeutic targets.

19.2.3 Epigenetic Studies of HAND

19.2.3.1 MicroRNA Studies

MicroRNAs (miRNAs) are small RNA molecules that modify transcription and translation via interactions with mRNA and which regulate a variety of cellular processes, including within the CNS. A small number of studies have examined the role of miRNA in HAND. The first study evaluated the impact of Tat upon expression of candidate miRNAs in primary cortical neurons in vitro [192]. Tat was found to upregulate mir-128a, which in turn inhibited expression of SNAP25, a presynaptic protein. A second study involved examination of postmortem caudate and hippocampal tissue of rhesus macaques with or without simian immunodeficiency virus encephalitis SIVE as well as caudate tissue from HIV-uninfected cases and humans with both HAND and HIVE [193]. Three miRNAs were found to be elevated in both SIVE and HIVE (miR-142-5p, miR-142-3p, and miR-21). miR-21, linked to oncogenesis, was significantly upregulated in both HIVE and SIVE.

miR-21 also induced stimulation of N-methyl-D-aspartate (NMDA) receptors, leading to electrophysiological abnormalities. Further, miR-21 was found to target the mRNA of myocyte enhancer factor 2C (MEF2C), a transcription factor crucial for neuronal function and a target of miR-21, ultimately reducing mRNA expression. In a third study, Noorbakhsh et al. conducted miRNA profiling in the frontal lobe white matter of four HIV-negative and four HIVE cases who were matched by age and sex [131]. Several miRNAs were found to be differentially expressed between the groups, using a standard twofold cutoff. Bioinformatics analysis revealed that most of the upregulated miRNAs targeted genes involved in immune response and inflammation, followed by nucleotide metabolism and cell cycle. A fourth study by Tatro et al. used both global mRNA and miRNA expression analysis in order to identify changes in miRNA expression in the frontal cortex of HIV+ individuals, determine whether miRNA expression profiles could differentiate HIV from HIV with concurrent major depressive disorder (MDD), and develop a method for integrating gene expression and miRNA expression data [194]. Their sample consisted of HIV-negative controls, HIV+, and HIV+ with concurrent MDD. miRNAs from three individuals within each group were pooled and used for the miRNA profiling, and mRNAs for three individuals from both HIV+ groups were used for non-pooled mRNA profiling. Importantly, neurocognitive functioning was not considered in this study, ages varied widely between groups, and one of the HIV+/MDD brains had pathology consistent with HIVE. With these caveats in mind, the HIV+/MDD group showed a greater number of downregulated miRNAs compared to the HIV+ group. Further, the miRNAs tended to cluster more tightly around the same chromosomal regions. After identifying mRNAs that were significantly differentiated in the HIV+/MDD group, and then identifying miRNAs that were dysregulated by at least a twofold change relative to the HIV-only group, the authors employed a target bias analysis to determine the relationship between miRNA dysregulation and target gene dysregulation. Using this method, they identified miRNAs belonging to four categories: (1) those with many dysregulated mRNA targets but of marginal statistical significance, (2) those with fewer dysregulated target genes but with high statistical significance, (3) those with numerous dysregulated gene targets that were of high statistical significance, and (4) those that did not have a significant number of dysregulated targets. The authors also identified a small number of genes with 3′-UTR miRNA target sequences. Those genes were considered to be "hubs" for miRNA activity, and the authors outlined their biological roles and association with neuropsychiatric illnesses.

A fifth study examined the impact of HIV viral protein R (Vpr) in a human neuronal cell line in order to investigate the mechanisms underlying the altered expression of cytokines and inflammatory proteins in CNS cells resulting from HIV infection. Both miRNA and gene expression assays of human neurons (primary cultures or cell lines) treated with recombinant Vpr proteins were used. Vpr was found to deregulate several miRNAs and their respective mRNAs [195]. As one potential mechanism for neuronal dysfunction, they found that expression of both miR-34a and one of its target genes (*CREB*) was dysregulated in the presence of Vpr. This study was the first to demonstrate a miRNA-dependent pathway through which Vpr damages neurons.

Most recently, Kadri et al. [196] sought to identify an epigenetic marker of HAND by screening over 750 miRNAs assayed from plasma in a group of 30 HIV+ adults who had been classified as neurocognitively impaired or normal based on a battery of cognitive tests. Utilizing a miRNA pairwise analysis to analyze the array data [197], the authors identified ten miRNA pairs that were differently expressed between impaired and unimpaired HIV+ cases and then validated their findings with qRT-PCR. The miRNA pairs that best differentiated impaired from non-impaired samples were miR-495-3p in combination with miRNA let-7b-5p, miR-151a-5p or miR-744-5p, and the pair miR-376a-3p/miR-16-532-3p. Sensitivity was further improved through the combination of two microRNA pairs: miR-495-3p/miR-744-5p and miR-376a-3p/miR-532-3p. Of note, none of these miRNA pairs were associated with other clinical characteristics. As the authors point out, it was also of interest that these miRNAs are all either enriched in brain tissue or have known neuronal functions.

19.2.3.2 Histone Modification Studies

Chromatin structure, and therefore gene expression, can be modified by the acetylation and deacetylation of histone proteins, a process that is mediated by histone deacetylases (HDACs) [198]. HDAC inhibitors have been shown to improve cognitive ability and may be candidates for treating a variety of neurologic diseases [199, 200]. We are aware of only one study examining histone modification in the context of HAND neuropathogenesis. Saiyed et al. examined the influence of Tat upon expression of HDAC2 in neuronal cells in vitro and the subsequent effect of HDAC2 modification on regulating genes involved in synaptic plasticity and neuronal function [201]. HDAC2 expression was negatively correlated with expression of *CREB* and CaMKIIa genes, which were reported to be involved in neuronal regulation.

19.2.3.3 DNA Methylation Studies

Genome-wide methylation platforms are now readily available (e.g., Illumina Infinium 450 K); however, very few studies have employed this method for the study of HAND. In addition to revealing information about cellular processes involved in HAND pathogenesis, whole-genome DNA methylation technology has been leveraged to create bioinformatics tools that can be used to study aging and HAND. DNA methylation levels are particularly promising biomarkers of aging, since chronological age profoundly affects them in most human tissues and cell types. The recently developed biomarker of aging (referred to as epigenetic clock [202]) was recently applied to the study of accelerated biological aging due to HIV in brain and peripheral blood mononuclear cells [203]. It was found that the brains of HIV+ adults exhibited age acceleration of 7.4 years compared to uninfected controls according to the epigenetic clock, whereas the age of PBMC was accelerated by 5.2 years. This

marker of aging may also be clinically relevant in HIV+ individuals. As recently shown by Levine et al. (2015), brain samples from individuals diagnosed with HAND within 1 year of death also showed an age acceleration of 3.5 years compared to samples from HIV+ neurocognitively normal individuals [204].

19.2.3.4 Summary

Epigenetic studies of HAND neuropathogenesis are relatively recent, with most studies focusing on miRNA pathways in infected tissue or cells. A variety of miRNAs have been implicated, lending validation to previously identified neuropathogenic mechanisms, such as increased caspase-6 and mitochondrial dysfunction. *CREB* has been implicated in both miRNA and histone studies. Improved neuropsychological performance was recently associated with global DNA demethylation, and a new biomarker of aging (the epigenetic clock) based on several hundred CpGs revealed that HIV infection accelerates aging in brain tissue and PBMC.

19.3 Special Topics

19.3.1 Roles for Iron and Mitochondrial Dysmetabolism in Neuro-HIV/AIDS

19.3.1.1 Overview

Accumulating data provides evidence of altered systemic iron metabolism in HIV infection, with sequestration of iron within reticuloendothelial cells and elevated circulating levels of the pro-inflammatory, master iron-regulatory hormone, hepcidin [205–207]. Furthermore, altered iron status (with or without anemia) has been associated with morbidity and mortality, even after accounting for potential disease-related confounding factors, such as CD4+ T-cell count [208–210]. Hepcidin levels increase, and plasma iron falls, as part of the inflammatory cascade triggered during acute HIV infection, and hepcidin subsequently remains high in untreated individuals. Hepcidin levels decline as the virus is suppressed with cART, but longitudinal studies have shown that they remain elevated compared to HIV-negative persons, even when the virus becomes undetectable. Intracellular HIV replication requires iron, and hepcidin levels measured early in HIV infection appear to predict subsequent plasma viral load set point [207, 211]. Studies of the role of hepcidin and iron transport in determining CSF HIV RNA concentrations in HIV+ persons are ongoing. By blocking gut iron absorption as well as the release of iron from cells of the monocyte-macrophage (M/M) lineage to erythroid and other metabolically active cells, hepcidin synthesis by the liver represents an important iron-withholding mechanism, leading also to anemia of chronic inflammation [212]. In addition, regional brain iron distribution may be abnormal in chronic HIV infection, based on brain imaging studies [213–215].

Over the past decade, considerable advances have taken place in understanding the roles of iron and mitochondrial metabolism in neurodegenerative disorders. It is increasingly recognized that these processes are interconnected and that iron and mitochondrial dysregulation may go hand in hand. Therefore, not to include a discussion of recent studies implicating iron and mitochondrial dysfunction in non-HIV-related inflammatory neurocognitive disorders would be to sidestep a burgeoning area of research with clear relevance to neuro-HIV/AIDS.

19.3.1.2 Insights from Studies of Iron in Non-HIV-Related Neurodegenerative Disorders

Disrupted iron homeostasis in the brain has been a long-recognized feature of both common and rare neurodegenerative disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington disease, multiple sclerosis (MS), and the so-called "neurodegeneration with brain iron accumulation" (NBIA) disorders [216–221]. However, due to the ubiquitous nature of iron and its participation in so many fundamental metabolic processes, the independent contribution of iron to neurodegenerative disease pathogenesis has been difficult to discern from its possibly innocent bystander role. Substantial evidence linking iron homeostasis with mitochondrial function, lipid homeostasis, and energy metabolism implicates systemic and/or CNS iron dysregulation in neurocognitive disorders [222–224]. Iron accumulates in some areas of the brain with healthy aging, but the distribution and total amounts are abnormal in many neurodegenerative diseases, exceeding what is observed with normal aging. Inflammation is frequently also present in these disorders in areas of regional iron excess and neuronal cell death [225]. Iron redistribution has the potential to promote oxidative injury in areas of increased iron deposition as well as possible functional iron deficiency due to low bioavailable iron in other brain regions. Both eventualities may contribute to disease pathogenesis [226, 227].

Iron is a required cofactor for numerous essential metabolic enzymes and a critical component of cytochromes and flavoproteins of the mitochondrial electron transport chain [228–230]. Relevant to the CNS, iron is required for myelination, neuronal repair, and monoamine (dopamine and serotonin) neurotransmitter synthesis, an imbalance of which contributes to mood disturbances, oxidative stress within the brain, and excitatory neuronal loss [231–234]. Access to a steady supply of iron in non-oxidatively reactive forms is therefore essential for maintenance of cellular health and metabolism. However, iron is also a biological hazard due to its efficiency in catalyzing free radical reactions via Fenton chemistry. Most studies of the role of iron in CNS disorders have highlighted regional iron excess in the brain as the abnormality of interest and emphasized that iron accumulation is synonymous with oxidative stress, although there is relatively little direct evidence for this assertion [235, 236]. Mitochondria also contain high concentrations of iron in the presence of oxygen and superoxide radicals, yet they function normally under these conditions for extended periods. Both iron excess and iron deficiency can lead to

mitochondrial and cellular dysfunction and oxidative injury. Organisms have therefore evolved mechanisms to maintain tissue iron concentrations within a narrow physiologic range, tightly compartmentalize it within cells, and transport it in a soluble, nonreactive state, bound to a variety of large and small transporters and gatekeeper proteins [206]. Hence, iron-related mechanisms of neurocognitive impairment other than direct oxidative injury due to increased brain iron content also deserve consideration in evaluating the impact of iron.

As in the periphery, cells within the CNS tightly regulate iron homeostasis via iron-responsive expression of select proteins required for iron efflux, cellular import, transport, and storage. Recently, proteins directly implicated in pathogenesis of the most prevalent neurodegenerative diseases, such as amyloid-β precursor protein, tau, α-synuclein, prion protein, and huntingtin, have been linked to neuronal iron homeostatic control. This literature suggests that disrupted expression, processing, or location of these proteins may result in a failure of their cellular iron homeostatic roles and augment the common underlying susceptibility to neuronal oxidative damage that is triggered in neurodegenerative disease [237, 238]. Despite intensive research over the past two decades, mechanisms regulating brain iron transport and egress of iron from the brain are still only partially understood. Iron enters the brain mainly by transport across the BBB, a process expected to be tightly regulated under normal circumstances in order to buffer the brain from systemic iron fluctuations [239]. Hepcidin and the iron export protein ferroportin-1 (FPN-1, encoded by the *SLC40A1* gene) are linked to iron efflux and transport into the brain and, along with astrocytes, play key roles in iron release [238]; turnover of FPN-1 is controlled by hepcidin [240]. In vitro studies replicating the BBB, which is comprised of brain microvascular endothelial cells and underlying astrocytes, and studies in nonhuman primates, have recently suggested a model in which brain endothelial cells, far from being a passive conduit for iron, play an active role in regulating iron transport into the brain [241]. Additional in vitro studies of human brain microvascular endothelial cells suggest that expression of iron-transport proteins such as FPN-1, the copper ferroxidase ceruloplasmin (CP), transferrin (TF) and its receptor (TFR), and heavy chain ferritin are critical in regulating this process [242–244]. Other iron transporters such as iron-regulatory protein-2 (IRP-2) also play key roles in brain iron metabolism; *IRP-2* knockout mouse models show altered tissue iron distribution and mild behavioral and neurological impairments, despite showing no specific areas of neurodegeneration in the brain [245].

A substantial literature, albeit not entirely consistent, associates iron-regulatory gene variants with healthy aging and with altered incidence and age of onset of common neurodegenerative diseases like AD and PD [233, 246–255]. Polymorphisms in the iron-loading *HFE* gene (*H63D* SNP in particular) have been best studied in this regard, with in vitro studies implicating a multitude of mechanisms for the increased risk of AD associated with this SNP: increased iron accumulation, disruption of mitochondrial membrane potential, increased influx of intracellular Ca^{2+}, increased cellular glutamate uptake, increased secretion of pro-inflammatory MCP-1, increased endoplasmic reticulum stress, oxidative injury, β-amyloid (Aβ) peptide-mediated mitochondrial toxicity, and decreased activity of PIN1, which contributes

to tau phosphorylation seen in AD [222]. Recent studies of Friedreich's ataxia, NBIA disorders, and AD have also highlighted the interrelatedness of iron metabolism, mitochondrial function, autophagy (the cellular clearance of senescent or dysfunctional organelles), and lipid metabolism, processes which are critical to CNS and peripheral nervous system function [248, 256–258]. These findings demonstrate that *H63D-HFE* expression promotes processes that can influence pathways in neurons that ultimately lead to impaired cognition, such as lipid homeostasis, neurotransmission, and myelination. Iron, like other transitional metals, not only alters processing of β-amyloid, but soluble amyloid precursor protein has been shown to stimulate iron efflux from brain microvascular endothelial cells [243]. While changes in iron homeostasis may not be the primary triggering event that initiates the pathological cascade leading to HAND, disrupted iron transport may be an important factor in altering metabolism of structural proteins like β-amyloid, thereby promoting neuroinflammation and progression of these disorders (Table 19.2).

Disrupted iron metabolism has recently been confirmed in postmortem brain tissues from patients with AD [259], in their cerebrospinal fluid (CSF) [260], and using R2*-based magnetic resonance imaging of the brain in AD patients [261]. A meta-analysis of over 2500 studies of iron in AD suggested that the weight of evidence favors a role for iron dyshomeostasis in the serum and CSF, as well as in the brain in AD [262], but the literature is not entirely consistent in this regard [263, 264]. Very recent studies by Ayton et al. strongly implicate iron dysregulation in the association of the *APOE*-ε4 genetic variants with AD risk; and *APOE* genotype may modulate CSF ferritin levels, which in turn are associated with AD outcomes [260, 265]. Baseline CSF ferritin levels were inversely associated with cognitive performance during a 7-year follow-up in normal, mildly impaired and AD subjects and also predicted conversion from mild cognitive impairment to AD. CSF ferritin and CSF APOE levels were strongly correlated, and CSF levels of ferritin were elevated in individuals harboring the *APOE-ε4* allele. In PD, decreased brain iron levels have also been noted in individuals at postmortem [266]. Some studies even indicate abnormal systemic iron status in AD and PD, as well as in ALS [223, 267, 268]. Whether iron dysregulation in the brain in AD, PD, ALS, and other disorders is related to or induced by systemic iron dysregulation, or vice versa, remains unclear.

Taken together, these findings favor the concept that maldistribution of iron in the brain is not an epiphenomenon; rather that iron dysregulation is a likely pathogenic mechanism common to many types of neurocognitive disorders, including HAND. Furthermore, interactions between brain iron transport and systemic iron status are likely to be dynamic and complex: the current concept that increased regional brain iron leads to neurocognitive impairment via oxidative injury may be a gross oversimplification.

19.3.1.3 Links Between Iron Levels and HAND

Indications that HIV dysregulates systemic iron metabolism were first reported in the pre-cART era: HIV disease progression was found to be associated with altered iron metabolism in vitro, and increasing ferritin concentrations were linked to disease

progression [205, 269]. CSF ferritin was first measured in HIV+ individuals with AIDS Dementia Complex, individuals with CDC stage II HIV infection, and uninfected controls and found to be a possible marker of neurologic disease. Ferritin levels were detectable in CSF only in those with AIDS Dementia Complex, and it was speculated that the source of ferritin in CSF in these patients was activated macrophages involved in promoting neuroinflammation [270]. In HIV+ individuals, CSF ferritin levels were shown to be elevated during acute neurological episodes but were considered non-specific for HAD [271]. In another study, limited proteomic profiling of serum in a small study of HAD cases and non-demented HIV+ controls identified CP as a possible biomarker of HAD [272]. Interpretation of iron-related biomarkers remains somewhat challenging in HIV infection, due to elevation of these proteins in acute and sometimes chronic infection; CP is also an acute-phase molecule.

Neuroimaging studies have also provided evidence that brain iron deposition in HIV+ persons is abnormal; neuropathological evaluations have correlated T2 shortening (hypointensity) in magnetic resonance imaging (MRI) studies of the basal ganglia (putamen) with premature perivascular HIV-related iron deposition, but the significance of this observation was unclear [213]. A larger MRI study followed, showing significantly greater deposition of iron in the basal ganglia (globus pallidus and caudate nucleus) of HIV+ persons than in HIV-negative controls [214]. More recently, multi-contrast, high-field MRI has detected subtle structural defects in the brains of HIV+ individuals with mild neurocognitive disorder (MND) on ART, including loss of structural integrity (myelin and cellular macromolecules) and micro-edema in global white, cortical gray matter, thalamus, and basal ganglia. These subcortical changes were also found to significantly influence executive function in patients with MND, compared to those without MND, who were similar with regard to baseline demographic and HIV-related factors. Iron-sensitive imaging using susceptibility-weighted imaging (SWI) was performed in a subset of study participants. Although linear discriminant analysis incorporating T1, magnetized transfer ratio (MTR) and SWI data provided valuable information for distinguishing MND from non-MND individuals, T2* data which is most sensitive to brain iron content did not add appreciably to the model. Although there were longer T2* relaxation times in the caudate of non-MND patients vs. MND individuals, this was also observed in HIV+/non-MND subjects compared to controls and did not reach statistical significance; T1 relaxation times also suggested possibly lower iron content in HIV+ than in HIV− controls [215]. These findings clearly require further follow-up and study using iron-sensitive neuroimaging techniques, such as R2* MRI, functional MRI, susceptibility-weighted imaging (SWI), and the newer technique of quantitative susceptibility mapping [273].

19.3.1.4 Impact of Iron on HAND in the Setting of Substance Abuse/Dependence

Iron-storage and transport proteins may have multiple functions within the brain. Pitcher et al. [274] reported elevated levels of ferritin heavy chain (FHC) in cortical neurons from individuals with HAND premortem and in opiate abusers. Prior

studies showed that FHC levels increase in neurons in response to mu-opioid agonists. In neuroinflammation, the chemokine CXCL12 and its receptor CXCR4 perform many essential functions, and signaling through this receptor promotes neuronal survival and neuronal-glial communication [274]. CXCR4 is an HIV coreceptor which has been implicated in HIV neuropathogenesis, including elevated levels of excitotoxic mediators, synaptodendritic loss or "simplification," and release of inflammatory cytokines. Among HIV+ persons, opiate abuse, particularly intravenous opioids, may accelerate development of HAND. FHC regulates CXCR4 signaling by inhibiting its activation and downstream, pro-survival signaling pathways. FHC also functions to bind, oxidize, and sequester iron in nonreactive forms for stable storage within the cell, and its production is regulated in response to changing iron levels and the inflammatory milieu; of particular importance to HAND is the ability of inflammatory cytokines to regulate FHC levels. Pitcher and colleagues showed that opiate abuse may exacerbate NCI in HIV through FHC-dependent disruption of neuronal CXCL12-CXCR4 signaling. They determined that this signaling pathway increases dendritic spine density and that HIV+ persons with NCI had increased neuronal levels of FHC that correlated with reduced CXCR4 activation. These results were further confirmed in a SIV-infected nonhuman primate model with morphine administration. In vitro, transfection of a CXCR4-expressing human cell line with an iron-deficient FHC mutant resulted in increased FHC expression and dysregulated CXCR4 signaling, independent of iron binding. Furthermore, studies of neurons showed that FHC contributed to morphine-induced dendritic spine loss, suggesting that HIV (and SIV) infection independently dysregulate neuronal FHC, which in turn may actively contribute to neurocognitive decline in HIV infection among opioid abusers.

Synthesis of monoamine neurotransmitters requires iron; therefore, iron dysregulation may also impact the neurobehavioral outcomes of HIV infection, particularly in the setting of substance abuse [275, 276]. In animal models, iron deficiency has been shown to disrupt brain synthesis and metabolism of monoamine neurotransmitters and to contribute to memory deficits [277]. Specific stimulant drugs of abuse like methamphetamine and its metabolites are believed to contribute to cognitive impairment via increased dopaminergic and serotonin signaling in the brain, excitotoxicity to neurons, mitochondrial damage, and oxidative brain injury [234].

19.3.1.5 Potential Effects of Altered Iron Transport on HIV Replication and Neuroinflammation

HIV replication within the CNS is consistently associated with an increased risk of neurocognitive impairment, and much research has been aimed at reducing CSF viral load and viral reservoirs [278]. Increasing evidence has confirmed that elevated iron stores are positively associated with viral load and mortality in people infected with HIV, but interpretation of existing studies of HIV and iron must be interpreted with some caution, as most studies to date have been undertaken in

largely cART-naïve populations or those without access to modern combination antiretroviral regimens. Some work has suggested that *both* extremes of iron status are detrimental to HIV outcomes [209]. Insufficient attention has been given to the potential impact of functional iron deficiency to mitochondrial dysfunction, immune activation, and antiretroviral toxicity to mitochondria and neurons. Functional neuronal iron deficiency is suggested in some dementing disorders (e.g., AD) characterized by deposition of β-amyloid, which is often observed in HAND and exhibits altered metabolism in the presence of HIV.

Taken together, data from studies of HIV-infected individuals thus far point to independent effects of both HIV and aging on extracellular amyloid-β (specifically Aβ42) deposition [279]. Drakesmith et al. showed that HIV Nef downregulates the HFE expression on the surface of M/M, leading to iron accumulation and increased intracellular viral replication [280]. In addition, HIV infection in vitro alters cellular iron levels, which promotes viral replication, and several antiretroviral drugs alter the expression of iron-regulatory genes, increasing cellular iron content independent of HIV infection [281]. Over the past 15 years, research has also revealed close links between the iron-hepcidin-ferroportin axis and M/M-mediated inflammation, M/M polarization (M1 vs. M2 states) and activation, and regulation of innate immune responses [282]. The central role of M/M and microglia in mediating neuroinflammation and HAND has been consistently borne out by genetic, transcriptomic, and epigenetic studies to date, and it therefore begs further exploration of the role of cellular iron content in M/M polarization/activation in this phenotype. M/M with increased iron content produce more pro-inflammatory mediators and are more likely to have an activated M1 phenotype [283]. HIV replication within M/M in the CNS is associated with deficiency of heme-oxygenase-1, a neuroprotective enzyme produced by M/M, oxidative stress, and glutamate toxicity [189]. Recent ultrastructural studies have identified so-called dark microglia, which become abundant in areas of the brain affected by chronic stress, aging, depression, and AD pathology, and these cells play a role in remodeling of neuronal circuits, particularly at synapses [284]. These microglia exhibit signs of increased oxidative stress, with increased phagocytosis of synaptic elements as is seen in mouse models of HAND [285] and extensive engulfment of axonal terminals and dendritic spines. Hence, effects of iron in activating M/M or microglia, or in altering their polarization, may promote HAND.

19.3.1.6 Anemia and Erythrocyte Morphology as Predictors of HAND

Anemia, which is always associated with abnormal iron transport and occasionally with systemic iron deficiency, has been a consistently poor prognostic indicator in HIV infection in both retrospective and prospective studies [212]. Its relationship to dementia in HIV+ individuals was reported previously in the MACS and in another study [286, 287], and hemoglobin <12 mg/dl was one of the components of the Veterans Aging Cohort Study (VACS) index associated with neurocognitive impairment status in a cross-sectional analysis of the VACS, which was not designed to

evaluate anemia and therefore understandably did not include adjustment for comorbid conditions and ethnicity [288]. Until recently, its impact on milder forms of HAND prevalent in the cART era has therefore remained unclear. We conducted the first cART era prospective study in CHARTER which was designed to address the impact of anemia and erythrocyte indices in predicting milder forms of HAND. This study in >1200 individuals enrolled in the CHARTER study included a time-dependent analysis of anemia as a predictor of GDS-defined impairment and a cross-sectional analysis of erythrocyte indices in association with HAND defined by either Frascati criteria or GDS impairment. Anemia, defined as a hemoglobin <11.5 mg/dL in women and <13 mg/dL in men, was associated with incident GDS-defined HAND independent of numerous potential confounding factors, including zidovudine (ZDV) use and contributing comorbidities [adjusted hazard ratio (HR) 1.55, $p < 0.01$] [210]. Similarly, in a recently published longitudinal sub-study of CHARTER, current hematocrit emerged as an independent predictor of neurocognitive decline in multivariable analyses [289]. In addition, the Kallianpur et al. study determined that routinely available red blood cell indices such as the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly and positively associated with the GDS-defined HAND, GDS as a continuous measure, and with HAND by Frascati criteria. These measures were also associated with milder forms of HAND (i.e., ANI and MND) in addition to HAD when these phenotypes were evaluated in separate models. Erythrocyte indices are often abnormal in HIV infection, commonly in association with ZDV use, protease inhibitors, or other cART, and they have also been shown to correlate with systemic mitochondrial dysfunction in human and animal studies [290, 291]. Erythrocyte indices may therefore indicate subclinical mitochondrial dysfunction and/or lipid dysmetabolism in HIV infection, which promotes HAND. Red cell membrane fatty acid composition was found to correlate with brain volumes [292]. To date, few studies have examined the value of evaluating and treating anemia in HIV infection, but in light of these findings, such studies would seem to be of value in potentially ameliorating HAND and preventing its progression [293]. Initiation of cART improves iron utilization, coincident with decreased immune activation. Fuchs et al. further demonstrated associations between anemia (low hemoglobin levels) and immune activation markers such as serum neopterin and β_2-microglobulin in HIV+ subjects; interestingly, the latter is also a protein important in regulation of HFE protein stability and expression in M/M [294, 295].

19.3.2 Iron-Related Genetics, RNA Expression, and Associated Biomarkers in HAND

19.3.2.1 Overview

Investigation of the role of altered iron transport in HIV infection on neurocognitive function is challenging due to the fact that many iron transporters are also acute-phase proteins and increased in acute inflammatory states. Until very recently, no

studies had been undertaken to measure iron and the levels of iron-transport proteins in CSF among HIV+ persons. CP and haptoglobin (HP), an iron-binding chaperone protein which serves as a ligand for the M/M scavenger receptor molecule CD163, were recently measured in CSF in CHARTER study participants [296]. Higher levels of CP were associated with an increased risk of GDS-defined HAND in multivariable regression analyses [adjusted odds ratio (OR) 1.8, $p < 0.05$], and higher levels of both CP and HP were associated with GDS in persons with only minimal comorbidities (ORs 2.4 and 2.1, respectively, both $p < 0.05$). In this subgroup, CSF CP and HP levels were also associated with GDS impairment and HAND in individuals with undetectable plasma HIV RNA (ORs 5.6 and 3.0, respectively, both $p < 0.01$). These associations were not merely due to inflammation, as only very weak correlations were observed between iron biomarkers and concurrently measured IL-6, CXCL-10, and TNF-α.

In neuroimaging studies of CHARTER participants who had minimal comorbidities, we identified five highly significant SNPs in iron-regulatory genes including *TFRC*, *CP*, and *SLC11A1*, which predicted alterations in brain imaging traits that are commonly associated with HAND. These traits included subcortical gray matter volume, frontal gray matter *N*-acetyl aspartate (a marker of neuronal integrity), abnormal white matter volume, and basal ganglia choline (a marker of neuroinflammation) [297]. Additional iron-related genes whose expression in monocytes has been associated with neurocognitive impairment also include: *NRF-2* (regulator of heme metabolism), the "hemoglobin complex" gene module, heme-carrier protein-1 (*HCP1)*, *CD163*, *IL6R* (required for hepcidin regulation of iron metabolism), and *BOLA2* (which binds iron-sulfur clusters in glutaredoxin and may play a role in sensing cellular iron status) [169].

In a collaboration with the NNTC, expression of transferrin receptor (*TFR*) messenger RNA in brain tissue (frontal neocortex) was evaluated for association with HAND; levels of *TFR* RNA were, surprisingly, unrelated to brain HIV burden but were significantly associated with all HAND (adjusted OR 5.2, $p < 0.05$) and for milder forms of HAND. Perhaps most interesting was the negative association seen between *TFR* RNA levels in the neocortex and specific domains of executive neurocognitive function, such as speed of information processing, and working memory [298]. Studies such as these support roles for altered iron regulation in HAND.

19.3.2.2 Iron and Vascular Disease in HIV Infection

HIV is associated with a significantly increased risk of atherosclerotic vascular disease, which may contribute significantly to HAND in older HIV+ individuals [6, 299]. Therefore, while elevated iron stores and resulting hepcidin-mediated degradation of the macrophage iron exporter FPN-1 may increase HIV replication within M/M and resulting immune activation, hepcidin may also increase development of foam cells and lipid peroxidation, destabilizing atherosclerotic plaques. Disorders of vascular remodeling such as pulmonary arterial hypertension (PAH) also occur with increased frequency in HIV+ individuals, and there is a well-described increase in prevalence of iron deficiency among persons suffering from PAH [300, 301].

Studies of the regulation of iron-sulfur clusters in PAH and HIV have revealed a role for epigenetic factors (hypoxia-related miRNAs or hypoxamirs) in the pathophysiology of vascular remodeling in PAH [302, 303]. Few studies of PAH in HIV infection have been performed [304]. The hypoxia-regulated response is modulated by iron-regulatory and RNA binding proteins [305]; studies in this area may help to elucidate potential common mechanisms linking iron-related chronic immune activation and vascular complications, which in turn promote neurocognitive decline.

19.3.2.3 Mitochondrial Genetics and Biomarkers in HAND

Evidence continues to accumulate supporting a role for abnormal mitochondrial metabolism in the pathogenesis of neurodegenerative disorders in non-HIV-infected persons, implicating defects in iron-sulfur cluster biogenesis. Iron-sulfur cluster dysfunction may contribute to cognitive decline and mitochondrial dysfunction with normal aging, as well as in specific neurocognitive diseases associated with premature aging like HAND, with brain iron accumulation and maldistribution in the basal ganglia [256]. Future studies will need to determine how iron-sulfur cluster defects may participate in the natural history of these disorders and whether targeted interventions can interrupt neuronal damage.

Until recently, few studies of mitochondrial genomic variation in HAND had been undertaken. The mitochondrial DNA (mtDNA) is a separate chromosome comprised of 37 genes that encode mitochondrial proteins; the remaining proteins required for mitochondrial function are encoded by nuclear genomic DNA. We performed a mtDNA haplogroup analysis in 1027 HIV+ participants from the CHARTER study, approximately two-thirds of whom were receiving cART and 46% of whom were diagnosed with HAND [306]. In this study, ancestry was genetically defined using principal components from genome-wide genetic data and categorized as European, African, or admixed Hispanic ancestry. Recent work in CHARTER has shown a consistently higher risk of HAND and, in longitudinal studies, a higher risk of neurocognitive decline over time, among self-reported Hispanic individuals. A reduced frequency of HAND among individuals of self-reported African ancestry in CHARTER has also been noted previously [210, 289]. The reasons for these differences have been widely debated. However, analyses within CHARTER have carefully accounted for differences in race/ethnicity and education, as well as for practice or learning effects in repeat neurocognitive assessments. The study by Hulgan et al. confirmed that individuals with genetically defined admixed Hispanic ancestry had a higher risk of neurocognitive impairment or HAND, as defined by the GDS, than did persons of European or African ancestry. The study also identified a subgroup of persons of admixed Hispanic ancestry with mitochondrial haplogroup B as having significantly reduced risk of GDS-defined impairment (adjusted OR, 0.16, $p < 0.01$) compared to other admixed Hispanic haplogroups. No other significant haplogroup associations were observed among CHARTER participants of European or African ancestry. Hence, mtDNA variation may constitute an ancestry-specific factor that influences risk of neurocognitive

impairment in HIV+ individuals [306]. Mitochondrial haplogroups have been shown in cytoplasmic hybrid (cybrid) studies to be associated with differing levels of systemic inflammation and ROS production [307, 308].

Samuels et al. recently evaluated a new measure of mtDNA copy number in PBMCs, estimated using genome-wide microarray data and comparisons of probe fluorescence intensities of mtDNA SNPs relative to all nuclear DNA SNPs, with neurocognitive impairment in CHARTER participants. Lower predicted mtDNA copy number per cell by this measure was associated with longer duration of cART, higher platelet count, and higher hemoglobin levels but surprisingly was not significantly associated with age. Adjusting for these factors as well as age, mtDNA copy number was inversely associated with neurocognitive performance (GDS impairment or HAND by Frascati criteria) in study participants (Samuels et al., *manuscript submitted for publication*) [309]. Higher mtDNA content may therefore indicate increased mtDNA replication in response to systemic mitochondrial dysfunction in HIV+ individuals, although further studies are needed to replicate these associations and clarify underlying mechanisms.

The role of cART in mitochondrial toxicity within the CNS in HIV infection has been extensively debated but remains understudied as a possible contributor to aging-related neurocognitive impairment [310]. Nevertheless, long-term systemic and brain mtDNA depletion and damage may occur after exposure to some nucleoside reverse-transcriptase inhibitors (NRTIs), and mitochondrial host genomics may interact with antiretrovirals in potentiating injury; this requires further study [311, 312]. Higher levels of oxidant damage to nuclear and mtDNA in the brains of HAND patients at autopsy suggest the possibility that mtDNA damage in these persons may promote chronic neuroinflammation and neuronal apoptosis during HIV infection [313]. These findings are supported by in vitro studies and studies in nonhuman primates [310], which show that cART can generate increased oxidative stress and lead to neuronal death. Measurement of biomarkers of oxidative damage to DNA and proteins (e.g., oxidatively modified DNA and protein carbonyls measured in CSF) may be helpful in clarifying these issues.

Extracellular (cell-free) mtDNA, which contains CpG motifs that act as immunogenic toll-like receptor-9 ligands and damage-associated mitochondrial patterns, has emerged as a biomarker of neuroinflammation and mitochondrial damage in HIV infection and HAND. Cell-free mtDNA is released during cellular injury and as part of the innate immune response to viral pathogens, and it may also relate to altered autophagy, the process by which cells under stress conditions recycle and dispose damaged organelles. Recent studies by Mehta et al. (Mehta et al., *manuscript submitted for publication)* evaluated relationships between mtDNA in CSF, neurocognitive impairment, and biomarkers of neuroinflammation and immune activation in HIV infection [314]. In a cross-sectional analysis of 28 HIV+ individuals, cell-free mtDNA levels measured in CSF by droplet digital PCR were strongly associated with CSF levels of CXCL-10 and with severity of neurocognitive impairment in impaired individuals, but not with neurocognitive impairment itself. In five individuals who participated in a longitudinal treatment interruption study, mtDNA levels rose in CSF preceding the onset of CSF pleocytosis and the rise in CSF HIV

RNA. In the first neuropathological study to evaluate mitochondrial injury in the brain in HIV infection, Var et al. compared levels of mtDNA (copies per cell) and the relative proportion of the mitochondrial common deletion (a 4977-bp deletion associated with mtDNA damage) in autopsy brain tissues from HIV+ persons with and without a history of methamphetamine (METH) use and in HIV-negative controls; all decedents in this study had undergone premortem neurocognitive characterization as NNTC participants [315]. Individuals with known AD were excluded from analyses. While no regional differences were seen in mtDNA copy number per cell in gray matter, higher mtDNA levels were seen in certain white matter regions (e.g., Brodmann's area), and a significantly higher abundance of the common mtDNA deletion mutation was also observed in these regions. Higher levels of mtDNA were observed in specific brain regions in HIV+ METH users compared to METH nonusers and HIV-negative controls, but mitochondrial injury, as evidenced by the abundance of the common deletion, was lower in the HIV+ METH users. MtDNA levels per cell were not associated with age in brain tissues, although the abundance of the common deletion was associated with age, as anticipated. In the HIV + METH+ group, a higher abundance of the "common deletion" was associated with lower GDS ($p < 0.01$); however, in the HIV + METH group, higher abundance of the "common deletion" was associated with higher GDS ($p < 0.01$). Finally, in both HIV+ groups, mtDNA injury was associated with HIV DNA levels in the brain, but not with mtDNA content. Nor were levels of extracellular (cell-free) mtDNA in CSF associated with GDS, inflammatory markers, or METH use.

19.3.2.4 Novel Iron-Modifying and Mitochondrial-Targeted Therapeutics

As noted by other investigators, there is a need to carefully balance trophic and toxic properties of iron in the CNS when designing and testing iron-modulating therapies [316]. The evidence suggests that treatment of HAND should be based on a combination of anti-inflammatory, regenerative, and neuroprotective strategies. Boelaert et al. [205] first discussed the possibility that iron chelators may have a role in treating the iron dysregulation of HIV infection. Iron chelators have shown considerable promise in AD and PD and need to be investigated in HAND. Future studies evaluating and treating even milder forms of anemia in HIV+ persons hold promise for reducing the impact of HIV on cognition. Studies of erythrocyte membrane and other properties of whole blood may provide a clue the pathophysiology of cognitive decline in this population [317].

Data linking brain iron redistribution and increased extracellular iron accumulation with dysregulation of calcium transport, abnormalities of NMDA receptors, and malfunction of voltage-operated calcium channels suggest that mitochondrial targeting of therapeutic agents could be a fruitful strategy for addressing HIV-induced iron-mitochondrial dysmetabolism in the brain [318].

Recent studies in nonhuman primate SIV models of neurologic disease have suggested that fluconazole and paroxetine are protective against HIV gp120- and Tat-mediated neurotoxicity. As evidenced by neurofilament light chain levels in CSF,

amyloid precursor protein accumulates in axons and calcium in the frontal cortex, although markers of neuroinflammation and plasma or CSF viral loads were not impacted. This study points out that neuroprotection is possible even in the face of viral replication and neuroinflammation [319]. Drugs such as paroxetine and deferoxamine (an iron chelator) may modulate iron levels in the brain and suppress iron-mediated Aβ accumulation in the CNS, with significant neuroprotective potential in HIV+ individuals [320]. A recently completed randomized, double-blind, placebo-controlled clinical trial showed particular promise for paroxetine [321]. Naturally occurring iron-containing molecules, including mitochondrial ferritin and (H)-ferritin, may also hold therapeutic promise in neurocognitive disorders like HAND [322]. Mitochondrial ferritin is a relatively recently identified iron-storage protein unique to mitochondria and important for proper iron partitioning between mitochondria and the cytosol. It is primarily expressed in the brain and structurally similar to (H)-ferritin. Mitochondrial ferritin is upregulated in AD and its overexpression attenuates β-amyloid neurotoxicity; its neuroprotective effects also include maintaining mitochondrial iron homeostasis and preventing dopaminergic cell death [322]. Chelation of extracellular iron released from activated macrophages and microglia may also be a way to address iron accumulation in certain brain regions and oxidative injury to the brain in chronic inflammatory disorders of the CNS, such as HAND [225, 323]. Neurons and glial cells may export iron via a glycophosphatidylinositol-anchored form of CP, and CP levels may promote iron deposition in certain parts of the brain. As in vitro and animal studies have suggested that excess iron in the brain can be chelated and that iron chelators hold promise in the treatment of neurological disorders like AD and PD, such interventions may have therapeutic benefit in HAND [323]. Ongoing studies addressing iron-mitochondrial dysregulation in the CNS promise new interventions to evaluate in clinical trials soon, providing hope for improving quality of life for this growing population of individuals surviving (and aging) with HIV infection.

19.3.3 Genetic Factors in CNS Impairment in HIV+ Children

19.3.3.1 Introduction

HIV infection has different effects on neurocognitive function in pediatric HIV disease as compared to adults. The natural history of HIV disease also differs between children and adults. Children generally do not have the same confounding factors such as drug abuse encountered in HIV+ adults. Since children's immune systems are immature, they are unable to fight HIV infection, and there is a risk of more rapid disease progression. Also, normal CD4+ T-lymphocyte counts are higher in young children than in adults, dependent on age; hence, these cell counts need to be interpreted differently from CD4 cell counts in adults. However, children also have higher potential for immune system reconstitution, as they have higher numbers of CD4+ T cells than adults.

HIV crosses the BBB and enters the brain early in infection. Compared to HIV infection in the CNS of adults, there have been fewer studies in children. Absence of coinfections or confounding factors in the CNS of children with HIV infection has allowed better understanding of the brain damage and lesions associated with primary HIV brain infection. In a comparison of primary HIV infection of the brain in children and adults, it was observed that children had more florid inflammation, more frequent multinucleated giant cell localization in the cerebral cortex, and more basophilic mineralization compared to adults. In contrast, adults had more perivascular brown pigment and more obvious white matter changes [324]. These neuropathological observations support the presence of more fulminant CNS disease in HIV-infected children, due to increased virulence of HIV in the immature CNS.

HIV+ infants may manifest early, catastrophic encephalopathy, with loss of brain growth, motor abnormalities, and cognitive dysfunction [325]. HIV-infected infants score lower than seroconverters on developmental measures, particularly language acquisition. Symptoms similar to adult HAD are occasionally seen in adolescents with advanced AIDS, including dementia, bradykinesia, and spasticity. The risk of HIV encephalopathy increases with very early age of infection and with high viral loads.

In a study of the French Perinatal Cohort of children born to HIV+ mothers and followed from birth with the French SEROCO Cohort of adults with a known date of infection, early encephalopathy in infants had a different pathophysiologic mechanism from that occurring in children, which showed similarities with mechanisms observed in adults [326]. Early encephalopathy was probably related to the occurrence of pathologic events during late fetal life.

Another study evaluated neuropsychological development, prevalence of neurological impairment, and neuroimaging in nine HIV-infected children for a period of 10 years using electroencephalography every 6 months and computed tomography/MRI once a year, which were very informative tools to follow the course of neuropsychological problems of HIV+ children [327].

Antiretroviral agents can improve or even reverse the course of neurological impairment in children due to various degrees of CNS drug penetration. Addition of the protease inhibitor ritonavir to nucleoside analogue therapy has been reported to delay disease progression and prolong life in adults with moderate to advanced HIV disease [328].

As part of the neurodevelopmental examination of children, the Clinical Adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS) detected neurodevelopmental differences between HIV+ and uninfected children at 12 and 18 months of age [329].

In a study of HIV-related encephalopathy in 50 pediatric patients, born to HIV-seropositive mothers or infected by contaminated blood, 17 pediatric patients with HIV-related neurological impairment, 16 cases of encephalopathy, and 1 case of neurotoxoplasmosis were observed, demonstrating a high frequency of neurological impairment in HIV+ infants and children [330].

In a follow-up study of 784 HIV+ Argentinian children infected by vertical transmission, 311 developed neuroAIDS [331]. Also, antiretroviral treatment showed complete remission or noteworthy improvement of progressive and nonprogressive encephalopathy, conversion of the most severe cases of progressive encephalopathy (severe developmental delay, acquired microcephaly, spastic quadriparesis, and fatal progression) into a more moderate phenotype (less developmental delay, normal head growth, spastic paraparesis, and chronic evolution of the disease), and reversion of acquired microcephaly observed in the first years of the epidemic. Another study recently summarized the spectrum of neuro-HIV in children, the neurocognitive and behavioral sequelae, the effects of treatment on the primary neurologic effects of the disease, and the specific challenges of identifying and managing these problems in resource-limited contexts, such as those found on the African continent [332].

19.3.3.2 Effects of Host Genetic Variants on CNS Disease in HIV+ Children in the United States

Several studies in HIV+ children from two US cohorts are described here; 1053 children with symptomatic HIV infection from Pediatric AIDS Clinical Trial Group (PACTG) protocols P152 [333] and P300 [334] were studied. P152 and P300 were multicenter, prospective, randomized, double-blind, placebo-controlled protocols that assessed the efficacy of single or combination NRTI treatment regimens in symptomatic HIV+ children in the United States, prior to the availability of cART. Important eligibility criteria included an age range of 3 months to 18 years with symptomatic HIV infection for P152 [333], an age range of 42 days to 15 years with symptomatic HIV infection for P300 [334], and meeting the requirements that the original Centers for Disease Control (CDC) classification system had established to diagnose HIV infection in children at the time of these studies [335]. In these two protocols, CD4+ T-lymphocyte count and percentage and HIV RNA concentration were measured at entry, prior to initiation of therapy and baseline CD4+ T-cell count, and HIV RNA data were used as dependent variables in analyses to determine their associations with host genetic variants. Of the 1053 subjects, 1045 had baseline CD4+ T-cell counts, and 871 had baseline HIV RNA data.

The primary endpoints of the analyses were progression-free survival (PFS) and CNS impairment. PFS was defined as either time from study randomization to progression to first clinical HIV-related disease outcome or death, whichever occurred earlier. The disease outcomes included weight growth failure, ≥ 2 opportunistic infections, malignancy, CDC clinical disease category C, and/or abnormality of the CNS (e.g., neurological deterioration, decline in neurocognitive test scores, and/or brain growth failure). The CNS impairment endpoint, a subset of PFS, was defined as time from randomization to deterioration in brain growth, psychological function, and/or neurological status.

19.3.3.3 Chemokine and Chemokine Receptor Polymorphisms in CNS Disease in HIV+ Children

The prevalence of chemokine and chemokine receptor polymorphisms in symptomatic HIV+ children in the United States was determined first [336]. Furthermore, the genetic influence of *CCR5*, *CCR2*, and *SDF1* variants on HIV-related disease progression and neurological impairment in children with symptomatic HIV infection was studied [36]. Variants including *CCR2-V64I, CCR5-wt/Δ32, CCR5-59029-G/A, CCR5-59353-T/C, CCR5-59356-C/T,* and *SDF1-3'-G/A* were evaluated. Children with the *CCR5-wt/Δ32* genotype experienced significantly delayed disease progression, including less neurocognitive impairment.

The presence of genetic polymorphisms in the *CX3CR1* gene, a minor chemokine co-receptor of HIV, predicted HIV disease progression in children independently of CD4+ lymphocyte count and HIV RNA load [337]. Children with the *CX3CR1 I/I249* genotype experienced more rapid disease progression (*I/I249* vs. *V/V249*, HR 2.19 (95% CI, 1.30–3.68), $p < 0.01$; *I/I249* vs. *V/I249*, HR 1.77 [95% CI, 1.00–3.14], $p = 0.05$) and a trend toward more CNS impairment (*I/I249* vs. *V/V249*, HR 2.19 [95% CI, 1.00–4.78], $p < 0.05$; *I/I249* vs. *V/I249*, HR 2.02 [95% CI, 0.85–4.83], $p = 0.11$). Children with the *V249-T280* haplotype experienced significantly less disease progression (HR 0.42 [95% CI, 0.24–0.73]; $p < 0.01$) and CNS impairment (HR 0.39 [95% CI, 0.39–0.22]; $p < 0.01$). Of note, these effects remained significant after adjusting for CD4+ T-lymphocyte count and plasma HIV RNA load at baseline and in a longitudinal, multivariable analysis. *CX3CR1* genotypes and haplotypes impacted HIV disease progression independently of CD4+ T-lymphocyte count and plasma HIV RNA load, suggesting that the fundamental role of CX3CR1 in the alteration of disease progression might be the recruitment of immunomodulatory cells responsible for the control of HIV. MCP-1 is the ligand for CCR2, a minor co-receptor of HIV. The *MCP1 2518-G* allele was marginally associated with CNS impairment at study entry [338]. Alone or in combination with *CCR2-64I*, the *MCP1 2518-G* allele did not alter disease progression or subsequent CNS impairment. These findings differ from studies in adults and suggest that MCP-1-CCR2 protein interactions may play a different role in HIV immunopathogenesis in children. The interleukin *(IL)4 589-C/T* polymorphism has been reported to protect against HIV-related disease progression in white adults. The *IL4 589-T* allele was more prevalent in Hispanic and in black, non-Hispanic children, compared with white, non-Hispanic children. We found that the *IL4 589-C/T* polymorphism does not affect the risk of HIV-related disease progression or CNS impairment in children, and this result did not differ by race/ethnicity [339]. These findings suggest that the *IL4 589-C/T* polymorphism is not an important determinant of HIV disease progression in children.

19.3.3.4 Polymorphisms in Intracellular Antiviral APOBEC3G Gene Alter HIV Disease and CNS Impairment in Children

Apolipoprotein B mRNA-editing catalytic polypeptide 3G (APOBEC3G) protein is incorporated into nascent virus particles and mediates cytidine deamination (C-to-U) of first-strand reverse transcripts of HIV in target cells, resulting in *G*-to-*A* hypermutation of the coding strand and premature degradation. Genetic variants in the *APOBEC3G* gene were found to be associated with HIV-related disease progression and CNS impairment in children [63]. *APOBEC3G-H186R* homozygous *G/G* genotype was associated with more rapid HIV disease progression (HR 1.69; $p = 0.01$] and CNS impairment (HR, 2.00; $p = 0.02$) compared with the wild-type *A/A* or heterozygous *A/G* genotype in a recessive model. In both additive and dominant models, *APOBEC3G-F119F-C* allele was associated with protection against disease progression (HR [additive] 0.69, $p < 0.01$, and HR [dominant] 0.60, $p < 0.01$, respectively) and CNS impairment (HR [additive] 0.65, $p = 0.02$, and HR [dominant] 0.54, $p < 0.01$, respectively). These associations remained significant in multivariate analyses controlling for baseline characteristics or previously identified genetic variants known to alter HIV-related disease in this cohort of children.

19.3.3.5 An Age-Dependent Association of Mannose-Binding Lectin-2 Genetic Variants on HIV-Related Disease in Children

Mannose-binding lectin (MBL) is part of the lectin pathway of complement activation against various pathogens; however, its role in innate immune responses against HIV infection in children is unknown. The effects of mannose-binding lectin-2 (*MBL2*) alleles on HIV disease progression and CNS impairment in children [340] were determined. Children with the homozygous variant *MBL2-O/O* genotype were more likely to experience rapid disease progression and CNS impairment than those with the wild-type *AA* genotype. The effects were predominantly observed in children younger than 2 years. In unadjusted Cox proportional hazards models, children younger than 2 years with *MBL2-O/O* experienced more rapid disease progression (*O/O* vs. *AA*, HR 1.54; 95% CI, 1.07–2.22; $p = 0.02$; *O/O* vs. *A/O*, HR 2.28; 95% CI, 1.09–4.79; $p = 0.03$). Similarly, children with *MBL2-O/O* were more likely to experience rapid progression to CNS impairment (*O/O* vs. *A/A*, HR 1.69; 95% CI, 1.06–2.69; $p = 0.03$; *O/O* vs. *A/O*, HR 2.78; 95% CI, 1.07–7.21; $p = 0.03$). These effects remained significant after adjustment for CD4+ lymphocyte count, plasma HIV RNA, and other genotypes (*MBL2-H/L*, *MBL2-P/Q*, *MBL2-X/Y*, and *CCR5-wt/Δ32−59029-G/A*, *CX_3CR1-249-V/I*, *−280-T/M*, and *SDF-1-180-G/A*). *MBL2-O/O* genotypes, which result in lower expression of MBL, were associated with more rapid HIV disease progression, including CNS impairment, predominantly in children younger than 2 years.

19.3.3.6 HLA Alleles Are Associated with Altered Risk for Disease Progression and CNS Impairment of HIV+ Children

Five hundred seventy-two HIV+ children, identified as disease progressors or non-progressors, were selected from PACTG P152 and P300 through a case-cohort sampling scheme. Study endpoints were HIV-related disease PFS and time to CNS impairment. DNA was genotyped for *HLA* alleles using a Luminex 100 platform. Weighted Kaplan-Meier methods and Cox proportional hazards models were used to assess the effects of *HLA* alleles on study endpoints [56]. The presence of the *B-27* allele ($n = 20$) was associated with complete protection against disease progression and CNS impairment over the median follow-up of 26 months ($p < 0.0001$ for both). These findings held in multivariate analyses controlling for baseline covariates, including race, gender, age, HIV viral load, CD4+ T-lymphocyte count and percent, weight-for-age z score, and treatment, and for other genotypes known to affect HIV-related disease progression. Also, the presence of the *A-24* allele was associated with more rapid CNS impairment (HR 2.01; 95% CI, 1.04 to 3.88; $p = 0.04$). The *HLA Class II DQB1-2* allele was associated with a delayed disease progression (HR 0.66; 95% CI, 0.47–0.92; $p = 0.01$) and CNS impairment (HR 0.58; 95% CI, 0.36–0.93; $p = 0.02$) in children.

19.3.4 The Interaction of Age and Host Genomics on Hand

19.3.4.1 Overview

Although cART has been highly effective at preventing AIDS-related complications, treated patients are at significant risk for a number of diseases typically associated with aging, including cardiovascular disease, osteoporosis, cancer, cognitive impairment, and frailty [341–353]. Among the aging HIV+ population, it has become evident that the incidence of HIV-associated non-AIDS (HANA) conditions is increasing [354]. HANA conditions affect virtually every organ system and have as a common theme an association with advancing age and a pathogenesis likely based on chronic inflammation. Arguably, given its somewhat liberal inclusion criteria, the most common HANA condition is HAND. Early studies indicated that older individuals were at increased risk for HAND [355]. Additional evidence that HAND is related at least partially to accelerated aging includes studies of CSF metabolomics [356], in vitro analysis in astrocytes [357], and neurophysiological studies [358, 359].

In order to effectively investigate accelerated aging in HAND, one first needs to understand what is meant by normal aging and to find a way of measuring it. Telomere length, which relates to cellular senescence, has been the most popular method to date for studying biological aging. In the context of HAND, telomere length has not been consistently associated with neurocognitive impairment or other indicators of neuro-HIV/AIDS [360, 361], and reported associations have been

weak and difficult to interpret. For example, Malan-Müller et al. [361] found a positive correlation between telomere length and learning performance in HIV+ South African women and a negative correlation between telomere length and verbal fluency among HIV+ subjects who had also experienced psychological trauma. More recently, the utility of epigenomics as a tool for studying age acceleration has been demonstrated in HIV+ individuals. A recent study examining DNA methylation derived from HIV+ and HIV-uninfected brains indicates that infection accelerates aging by as much as 9.3 years [203], based on the recently developed epigenetic clock [202]. Similar accelerated aging has been found in PBMC, with an acceleration of 14 years in HIV+ adults compared to uninfected adults [362] and 3.6 years in individuals with detectable plasma viral loads as compared to those with undetectable plasma viral loads [203]. Whether or not these changes correlate with HAND remains to be seen. However, these findings are consistent with clinicopathological studies in which age-related pathology has been observed in relatively young HIV+ cases, including reduced Aβ-42 in CSF [363] and increased amyloid-β deposits in brain tissue [364]. These findings also suggest a role for *APOE* genotype in HAND. Despite these observations, the relationship between the *APOE*ε4 allele and HAND remains equivocal. Early studies appeared to show a robust relationship between this allele and risk for HAND. For example, Corder and colleagues [28] found that twice as many individuals carrying at least one *ε4* allele were given a diagnosis of HAD over the course of the 5-year study. Subsequent studies over the last decade, however, have yielded inconsistent findings. Potential mitigating factors include the deleterious influence of the *ε4* allele on disease progression and survival rates, methodological differences between studies in the operationalization of HAND, and differences between studies in terms of the inclusion of a (HIV) seronegative control sample [43, 365, 366]. Recent studies using such a control sample and objective measures of neurocognitive functioning suggest synergistic deleterious effects of the *APOE-ε4* allele and HIV on cognition [52, 54]. Within HIV+ samples, age has been found to be a modulating factor in some studies [41, 367] but not others [50, 54, 368]. In support of a modulating factor, Valcour and colleagues [41] observed older (age ≥ 50 years) *ε4* carriers to have higher rates of HAD compared to age-matched *ε4* noncarriers. This was not observed in their younger (<40-year-old) participants. Using more broadly defined HAND as the outcome variable, similar findings were recently documented by Panos et al. [367]. Of the published longitudinal studies of *APOE* genotype and HAND [28, 43, 50, 69], none employed a design aimed at measuring individual changes on objective neurocognitive measures over time while also accounting for mitigating factors such as disease severity. Such an approach may help clarify the nature of the relationship between *APOE* genotype and HAND. Most recently, Becker et al. [69] examined time to incident cognitive impairment in 1481 HIV+ individuals who were cognitively normal at their first neuropsychological evaluation. No association between *APOE-ε4* and time to develop neurocognitive impairment was found, nor did they observe interactions between *ε4*, HIV infection, age, and either death or neurocognitive impairment. However, that study was largely limited to younger (<65-year-old) individuals.

Looking beyond behavioral phenotypes, Diaz-Arrastia and colleagues [38] and Dunlop and colleagues [26] probed associations between *APOE-ε4* and pathological findings of HIVE or HAND; no associations were observed. However, while HIVE is thought to be a common pathological substrate for HAD, the two can occur independently of one another [369]. It should also be noted that these studies were conducted with patients who died in the pre-cART era. Given the deleterious relationship between the *APOE-ε4* allele and disease severity, individuals may have died before pathological effects emerged in the brain. *APOE-ε4* is associated with faster disease progression, possibly due to enhanced HIV fusion/cell entry [43]. More recently, Cutler and colleagues [37] found evidence for lipid metabolism derangements in the brain tissue of *ε4* carriers, although their sample also consisted of patients who died in the pre-cART era. Soontornniyomkij et al. [370] found that *APOE-ε4* and older age were independently associated with the increased likelihood of cerebral Aβ plaque deposition in HIV+ adults. While the Aβ plaques in HIV brains were immunohistologically different from those in brains of individuals who died with symptomatic AD, this study is among the first to provide a link between genotype and neuropathological findings in HIV.

19.3.4.2 Host Iron-Mitochondrial Interactions with Age and Age Acceleration in HAND

Despite being only a few percent of the total body mass, the brain is responsible for approximately half of the oxygen consumption and 20% of the mitochondrial oxygen consumption [371]. In the brain, a very high metabolic requirement for iron, coupled with the high susceptibility of brain tissue to iron-generated lipid peroxidative damage, requires particularly stringent regulation of iron availability and mitochondrial iron utilization in order to preserve structural integrity and energy metabolism. Mitochondrial dysfunction leads to neuromuscular degeneration, aging, energy depletion, and free radical production; defects in iron-sulfur cluster biosynthesis are important mitochondrial mediators of aging [372]. Iron-sulfur clusters are essential components of respiratory electron transport chain complexes as well as specific tricarboxylic acid cycle enzymes, including the iron-regulated cytoplasmic and mitochondrial isoforms of aconitase, succinate dehydrogenase, and DNA repair enzymes. Iron-sulfur cluster assembly and disassembly based on ambient iron levels in cells determines the binding of IRPs to their mRNA targets [373]. Disruption of iron homeostasis may therefore have significant impact on shifts in brain energy metabolism in the brain via effects on cytoplasmic and mitochondrial enzyme function [374].

Trace metals including but not limited to iron may also interact with proteins directly or indirectly involved in the pathogenesis of HAND. In non-HIV-related neurocognitive disorders (AD, prion diseases, and Lewy body dementia), there is accumulating evidence that misfolding of disease-associated proteins such as β-amyloid and α-synuclein is effected by interactions of these proteins with iron and that these proteins are also in part responsible for the iron dysregulation seen in disorders of aging [375].

Increased brain iron has been associated with age-related decline in motor strength as well as cognitive decline, and one study recently evaluated the association between iron content in the basal ganglia and hand grasp performance in older women [376]. Higher basal ganglia iron content assessed by T2* MRI was associated in this study with an increased number of errors committed during learned handgrasp tasks with the same and contralateral hands, suggesting a direct link between brain iron content and motor dysfunction.

Finally, it is significant to note that the effects of caloric restriction, long known to be neuroprotective and to slow the aging, have recently been shown in mouse models to include downregulation of hepcidin in the brain, which would be expected to limit aging-related iron accumulation in the brain parenchyma [377].

19.3.4.3 Aging, Host Iron-Mitochondrial Genomics, and HAND

Genomic, transcriptomic, and metabolomic studies have determined that HAND is associated with global mitochondrial dysfunction and a downregulation of mitochondrial protein expression, as have studies in animal models [149, 356, 378, 379]. These studies are consistent with the concept of age acceleration and increased oxidative stress in HIV infection. Changes in iron transport and mitochondrial function contribute to systemic oxidative stress. In HIV+ men, higher circulating iron levels are associated with increased levels of oxidative stress as measured by plasma F_2-isoprostane levels, a sensitive and relatively specific measure of in vivo oxidative injury. However women, in whom iron stores are significantly lower than in men, had higher F_2-isoprostane levels than men overall; the reasons for this finding are unclear [380].

The epigenetic clock, recently reported to show accelerated aging in HIV infection, also highlights the importance of nuclear-encoded mitochondrial genes [204, 381]. Whether mtDNA-encoded genes contribute to epigenetic aging is not known, but mtDNA methylation has recently been linked to neurodegenerative disease [382, 383]. Iron metabolism regulates mitochondrial biogenesis, and iron transport is itself regulated in part by mitochondrial function [384], so iron-related genes likely play a role in mediating age acceleration and neurocognitive impairment. Ongoing studies by our group are actively investigating interactive effects of nuclear and mtDNA variants in HAND.

Although studies in the modern cART era have generally not associated *APOE* alleles with HAND, *APOE* genotype interactions with age and HIV serostatus have been postulated [54, 69, 370, 385–387]. Increased influence of *APOE-ε4* genotypes in HAND among older HIV+ persons may therefore relate to iron dysregulation.

19.3.4.4 Iron-Sensitive Neuroimaging in Aging-Related NCI

Differentiating HAND from other neurocognitive disorders of aging such as AD and PD is increasingly challenging as the HIV+ population ages, and the use of neuroimaging techniques particularly sensitive to iron deposition in the brain may be of value, assuming that the characteristic patterns of iron deposition in these

disorders will differ from those in HAND. In addition, reliable estimates of brain tissue iron concentration are likely to be important in monitoring individuals with HIV infection for physiological age-related and pathological conditions such as HAND, AD, and vascular disorders. Several imaging techniques are helpful in this regard, including T2-weighted or T2*/R2* MRI, because they can detect differences derived from changes in signal due to the magnetic properties of the major tissue iron-storage proteins, ferritin, and hemosiderin; the magnetic properties of these iron-carrier proteins shorten the relaxation time of nearby water protons, leading to signal extinction in iron-rich areas. However these methods are not altogether specific for iron, due to the potential influence of water content of the surrounding tissue, other trace metals, and myelin density. More iron-specific imaging techniques under development are relaxometry, magnetic field correlation imaging, and phase-based multi-contrast imaging (covering susceptibility-weighted imaging and quantitative susceptibility mapping) [215, 388]. Diffusion tensor imaging is also reflective of white matter integrity and fractional anisotropy changes in HIV infection that appear to be impacted by iron [246]. In the future, monitoring changes in iron storage and content may serve as sensitive biomarker for diagnosis as well as treatment monitoring.

19.3.5 Alternative Phenotypes for the Study of Hand Genomics

One possible reason for the lack of replicability of findings in the genetic association studies described above is the use of different phenotypes across studies. Earlier studies primarily used HAD or similar diagnoses (e.g., AIDS Dementia Complex) as the phenotype [30, 34, 41, 45, 47]. Others have used composite measures of global neurocognitive functioning, usually derived from a comprehensive battery of neuropsychological tests [36, 39, 389], as the determination of neurocognitive impairment is more reliable than a formal diagnosis of HAND [390]. Perhaps the greatest shortcoming of these phenotypes is that they are influenced by a number of environmental, psychometric, and endogenous factors. This might be most problematic for detecting mild neurocognitive impairment characteristic of ANI or MND, which have a relatively low threshold to meet criteria [391, 392]. Furthermore, the numerous nongenetic contributors to variance in these measures (e.g., measurement error) make them less suitable targets for genetic analysis, especially when effect sizes for genetic variants are small. Finally, the use of global measures of neurocognitive function, which have become the norm in such studies, runs the risk of missing domain-specific associations with genes of interest. One partial solution is to utilize domain-specific composite scores (e.g., memory or processing speed) composed of measures with documented heritability. However, this strategy also increased Type I error rate, necessitating more strict corrections for multiple testing.

Unlike genomic studies, the majority of transcriptomic studies have focused on encephalitis (either SIVE or HIVE) as the disease phenotype. Prior to the development cART, HAD was common, and HIVE was considered to be its

neuropathological basis [19, 71, 87, 164, 286, 393–398]. In the current era of widespread cART use, however, the vast majority of HAND cases present with milder symptoms [399, 400], and upon autopsy examination, they do not have neuropathological findings consistent with HIVE as defined in the pre-cART era [401]. Increasingly, evidence suggests that for the vast majority of cART era HAND cases, HAND is the result of potentially reversible neurodegeneration driven by chronic neuroinflammation [164, 394, 396, 401]. The relevance of this concept to transcriptomic studies was best demonstrated recently in a study comparing transcriptome changes in individuals who had premortem HAND but no evidence of postmortem HIVE to those changes in individuals with premortem HAND, who also showed postmortem HIVE [148]. Despite the similar neurobehavioral phenotype, the resulting transcriptome profiles were highly distinct, as described above. This study underscored the need to evaluate currently relevant disease phenotypes.

Due to difficulties in using neuropsychological tests or diagnoses based primarily on such tests, as phenotypic measures of HAND, some investigators have explored alternative outcome measures. Among these are various neuroimaging indices. While beyond the scope of this review, a variety of MRI and MRS markers have been associated with HIV disease progression, neurocognitive impairment, and response to putative treatments [402–407]. Several studies have employed neuroimaging in conjunction with genotyping. A recent neuroimaging study of 177 (80 HIV+ and 97 HIV-) did not find interactions between *APOE* genotype and brain metabolite levels as measured via MRS [67], despite group differences in neurocognitive measures. Sundermann et al. [68] examined interactive effects of *COMT* rs4680 and HIV on executive functioning and frontal cortex metabolism using functional MRI in two samples of women enrolled in the Women's Interagency HIV Study Consortium. In the first sample, HIV+ participants who possessed *Val/Val* genotype performed significantly worse on the N-back tests than the uninfected controls with similar genotype, whereas those with a *Met* allele performed similarly to uninfected controls. In a second sample in which the N-back task was performed during fMRI, HIV+ *Val/Val* carriers showed greater prefrontal activation compared to uninfected *Val/Val* carriers during the task, suggesting that increased cortical activation was required by HIV+ *Val/Val* carriers in order to complete the task. Conversely, uninfected *Met* allele carriers demonstrated significantly greater activation as compared to HIV+ *Met* allele carriers; however, this activation occurred outside the prefrontal cortex and therefore may not be indicative of compensatory recruitment. Importantly, because the participants who generated the imaging data did not show the serostatus x genotype effects that the behavioral sample showed, it remains unclear if the altered metabolism in the prefrontal cortex is related to working memory deficits in HIV+ *Val/Val* carriers. It is important to point out that, as with candidate-gene studies of behavioral HAND phenotypes, these imaging studies are likely to be underpowered and difficult to validate. One potential solution is to pool imaging data across cohorts. One current endeavor with great potential of linking neuroimaging biomarkers of HAND with genetic variation is the ENIGMA-HIV consortium (http://enigma.ini.usc.edu/ongoing/enigma-HIV-working-group/), which ties together several hundred participants from a growing number of cohorts.

Another promising focus for alternative phenotypes is the histopathological changes in HIV+ brains that can be quantified via immunohistochemistry. Markers of dendritic simplification [408], or a combination of synaptic and dendritic markers, appear to have the strongest relationship with the neurocognitive deficit characteristic of HAND. For example, Moore et al. found that a combined histopathological phenotype consisting of synaptodendritic neurodegeneration, as measured by synaptophysin (SYP) and microtubule-associated protein-2 (MAP2), was associated with HAND across both subcortical and cortical brain regions [393]. Another histopathological candidate is β-amyloid deposition, which has been observed in the cortex in HIV+ individuals [409–412]. In addition to these markers of neurodegeneration and abnormal protein aggregation, markers of neuroinflammation have also been shown to be associated with neurocognitive impairment or HIV-related brain dysfunction [413–415]. Indeed, macrophage proliferation, microglial activation, astrocytes activation, and increased chemokine levels have all been found in CSF and brains from HIV+ individuals [19, 17, 87, 164, 394–398]. While these may all represent candidates for neuropathogenic processes underlying HAND, new methods are necessary to determine which ones are relevant. Toward that end, an innovative approach for simultaneously determining which histopathological markers are HAND relevant and which genetic susceptibility loci influence HAND is to examine the association between host genotype, histopathological findings, and behavioral (i.e., neurocognitive) outcomes. In this scenario, neuropathological changes (or neurophysiological changes, in the case of neuroimaging) are considered *intermediate phenotypes*. To put it more simply, if HAND is considered at its most basic level to be the end result of a sequence of physiological events that commences with HIV-induced cellular changes that are modified by genetic factors, then determining the extent to which known genetic susceptibility loci for HAND perturb candidate neuropathological and neurophysiological intermediate phenotypes may reveal which ones are most contributory. The advantage of this approach is that histopathological intermediate phenotypes, like neuroimaging phenotypes, are less prone to exogenous factors and have a stronger association with genetic susceptibility loci than neurobehavioral phenotypes. This approach has been successfully employed in genetic association studies of AD [416, 417], for example, delineating the relationship between *APOE* genotype and Alzheimer's-related cognitive impairment as a function of the sequential cascade of amyloid plaque formation and neurofibrillary tangle formation [416–419]. Relevant to HAND, to our knowledge, only three studies have examined the relationship between genetic susceptibility loci and neuropathological outcomes. Sato-Matsumura et al. [29], with a sample of 44 AIDS patients with autopsy-verified HIVE or HIV leukoencephalopathy and 30 AIDS patients without these neuropathologies, did not find an association between *TNF-α* genotype at rs1800629 and either of the neuropathological conditions. Diaz-Arrastia et al. [38] assessed for HIVE or vacuolar myelopathy in the brains of 270 HIV+ individuals who died with AIDS between 1989 and 1996. Neurocognitive functioning and HAND were not considered. They determined the presence of microglial nodules,

multinucleated giant cells, myelin pallor, and vacuolar myelopathy in the brains and/or spinal cords. None of the alleles examined were associated with the presence of these markers. More recently, Soontornniyomkij et al. found that *APOE-ε4* and older age were independently associated with an increased likelihood of cerebral amyloid-β plaque deposition in HIV+ adults [370]. While the amyloid-β plaques in HIV+ brains were immunohistologically different from those in brains of individuals who died with symptomatic AD, this study is the first to provide a link between genotype and neuropathological findings in HIV infection. However, neither study considered clinical manifestations of HAND in their design.

CSF and blood-based biomarkers of HAND have been difficult to identify and replicate. Still, studies relating host genomic factors with these markers have yielded interesting results. Morales et al. [65] examined differences in the frequency of *YWHAE* polymorphisms and protein levels between HIV+ and HIV− individuals who were neurocognitively characterized. The *YWHAE* gene product (also called 14-3-3ε protein) is considered a reliable biomarker for neurodegeneration and interacts with HIV. Drawing from 20 HIV+ and 16 seronegative, randomly selected samples from the Hispanic-Latino Longitudinal Cohort of Women, they found that HIV+ participants heterozygous at rs4790084/rs1204828 had a threefold higher risk of cognitive deficits, of HAND diagnosis, and they had less CSF 14-3-3ε protein expression as compared to homozygotes. Genotype did not affect neurocognitive function in the seronegative group. Furthermore, CSF 14-3-3ε protein levels were 4.5-fold lower in women with HAND as compared to HIV+, cognitively normal women. This latter finding was at odds with studies cited by the authors in which 14-3-3ε protein was not found in the CSF of healthy individuals and occurred almost exclusively in individuals with neurological illness (e.g., CJD and HAD). The authors explained these findings by suggesting that 14-3-3ε protein expression may be elevated in the early stages of neurological illness but depleted in advanced stages. This would not seem to be an adequate explanation for their findings, however, since the women in their study did not have advanced neurologic disease. Furthermore, the conclusion of a genetic association between *YWHAE* genotype and risk for HAND was based on an extremely small sample.

Thames et al. [91] examined interrelationship between *CCL2* (rs1024611) genotype, expression of inflammatory markers in CSF, HIV disease markers, and neurocognitive functioning in 145 HIV+ adults enrolled in the NNTC, hypothesizing that carriers of the *−2578-G* allele would have higher concentration of CCL2 and other inflammatory markers in CSF and worse neurocognitive function. That analysis revealed that while there was no difference in neurocognitive function between genotype groups, carriers of the *CCL2-2578-G* allele had higher levels of CCL2 in CSF, which was in turn associated with higher levels of other pro-inflammatory markers and poorer neurocognitive function. This study is particularly useful in demonstrating the importance of CSF markers as intermediate phenotypes of HAND in genomic studies (Table 19.3).

Table 19.3 Summary of genetic, epigenetic, and transcriptomic mechanisms of relevance to HAND

Genetic factors	**Mechanisms**
Immune-related genes (CCR5, CCR2, CX3CR1, MIP1alpha, RANTES, MCP-1, MBL2, HLA)	Chemokine receptors, chemokine ligands and for viral entry, immune response macrophage activation, monocyte chemotaxis across blood-brain barrier (BBB), viral epitope recognition and CD4 and CD8 cell lysis in brain, and neuroinflammation
Dopamine-related genes (DA, DAT-1, DRD2, DRD3, DBH, COMT, BDNF)	Impairment of dopamine receptor or dopamine expression, transport, or functioning
Intracellular antiviral genes (APOBEC3G)	Intracellular viral genome editing and deactivation
Genes affecting sodium transport across cellular and intracellular membranes (SLC8A1, NALCN)	Mitochondrial dysfunction
Epigenetic factors	**Mechanisms**
miRNA (miR-21, miR-34a, miR-495-3p, miR-151a-5p or miR-744-5p, miR-376a-3p/ miR-16-532-3p)	Interference in gene transcription, translation, and viral gene expression in brain
Histone modifications (HDACS)	Modification of brain gene expression, viral gene expression in brain affecting neuronal function
DNA Methylation (DNMTs)	Modification of global brain gene expression related to neurodegeneration, DA metabolism and transport, and oxidative phosphorylation
Brain/blood transcriptomic factors	**Mechanisms**
Brain gene expression in HAND and HIV encephalitis	Altered pathways of neuroimmune functioning, synaptodendritic functioning and integrity, myelin, neurodegeneration, neuroinflammation, neuropsychological functioning, mitochondrial functioning, cell cycle, cell-cell signaling
Peripheral blood gene expression	Peripheral blood-based markers of HAND: monocytes (BBB crossover), CD4, nadir CD4, CD8, viral load, oxidative stress, hypoxia, transcription factors

19.4 Summary

In this chapter we have summarized all genetic association and epigenetic studies of HAND of which we are aware, as well as gene expression studies employing whole-genome screening (i.e., transcriptomic studies). The reader will notice the general lack of replicability across genetic association studies and, to a lesser extent, across transcriptomic studies. This may be a function of inconsistent or variable neurocognitive phenotypes but may also reflect other aspects of study design (e.g., thresholds for statistical significance). A large portion of this chapter was dedicated to iron and mitochondrial dysregulation as it relates to genetic variants. This line of research is

particularly exciting as it may open doors to novel research efforts. We have also discussed the epigenetic clock as a tool for the study of HAND, but this tool may also be useful for the study of HIV disease progression in general. In sum, genetic, transcriptomic, and epigenetic studies continue to provide important information about HAND pathogenesis, lay foundations for novel and innovative methods, and uncover potential candidates for therapeutic drug development.

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