Herpesviruses are a Non-genetic Driver of Alzheimer's Disease by Non-cell Autonomous Degeneration Through Secreted Factors

Executive Summary (250 words)

Occult herpesvirus infections could be the non-genetic driver causing sporadic Alzheimer's disease. Neurotropic alphaherpesviruses can infect adrenergic neurons in the locus coeruleus. Herpesvirus secreted glycoprotein B can disrupt endosomal trafficking causing the displacement of APP from the cell surface, amyloidogenesis, and tau hyperphosphorylation. Without APP for stabilization, inhibitory autofeedback α_2-adrenergic receptors are internalized, resulting in persistent norepinephrine release, and reduced astrocytic GABA release. Moreover, herpesvirus infected adrenergic cells may be entrained to the host circadian rhythm resulting nocturnal hyperactivity. The displacement of APP also impairs cell-surface stabilization of the NGF receptor, high-affinity choline transporter, and ferroportin resulting in reduced survival. Increased norepinephrine can cause insomnia, immunosuppression, vasoconstriction, and reduced insulin release and CNS glucose utilization. Increased blood glucose produces advanced glycation end-products, mediating RAGE inflammatory signaling. In blood vessels, amyloid-β and activated RAGE mediate inflammatory signaling that initiates coagulation cascades, causing microvessel occlusion and hypoxia. Inflammatory cytokines, norepinephrine, and herpesvirus-secreted factors released from adrenergic neuron terminals can attract peripheral blood mononuclear cells infected with other herpesviruses. Inflammatory cytokine-induced differentiation can lead to the reactivation of multiple herpesviruses. Most herpesvirus can infect cells of the neurovascular unit. Human cytomegalovirus and Epstein-Barr virus both encode secreted factors that can disrupt endosomal trafficking and cause non-cell autonomous degeneration. The alphaherpesviruses, accompanied by other herpesviruses, can produce Alzheimer's disease pathology by non-cell autonomous secreted factors, resulting in diffuse degeneration with relatively few infected cells. In addition, some herpesvirus secreted factors, along with norepinephrine, cause immunosuppression, resulting in opportunistic microbial infections. It appears that multiple herpesvirus types are involved in AD pathogenesis.

Keywords: herpesvirus, hypoxia, amyloid-precursor-protein, immunosuppression, opportunistic infections

Herpesviruses are a non-genetic driver of Alzheimer's disease risk. In this respect, seven major components related to AD pathology are addressed: (1) the missing herpesviruses; (2) multiple herpesviruses; (3) amyloid precursor protein (APP) deficiency-mediated α-adrenergic destabilization and norepinephrine; (4) herpesvirus-mediated immunosuppression leads to opportunistic infection; (5) insulin deficiency; (6) vascular inflammation and hypoxia; and (7) APP and Sorla deficiency.

The missing herpesviruses

Dozens of reports suggest an association between Alzheimer's disease (AD) and herpesviruses. The suspect human herpesviruses are: herpes simplex 1 and 2 (HSV-1 and -2), varicella zoster virus (VZV), human cytomegalovirus (HCMV), human herpesvirus 6A/B and 7 (HHV-6A/B -7), and Epstein-Barr virus (EBV). There are contradictory reports, however, finding no more herpesvirus DNA in the AD brain than from healthy control brain.[1–3] However, the absence of herpesvirus DNA in AD brain can be explained. First, most postmortem AD brain samples are taken from late stage AD patients. This is analogous to examining the cause of an auto accident after the drivers and wreckage have long left the scene. Also, most studies did not take samples from the brainstem, a region replete with pathology. Second, herpesvirus infections can be occult infections. For instance, HCMV brain infection in transplant and congenital reactivation patients show widespread neurodegeneration with only sparsely infected cells which are usually associated with small blood vessels.[4–6] Moreover, the lack of characteristic HCMV inclusion bodies upon histological examination in these patients heralds the absence of inclusion bodies in the AD brain. Thus, infection of very few cells makes it difficult to detect viral RNA/DNA and conclude disease association.

AD is characterized by amyloid-β (Aβ) and neurofibrillary tangles (NFT) derived from abnormal tau phosphorylation.[7] Aβ and NFT begin forming decades before AD symptoms in cognitively healthy adults. NFT first appears in the entorhinal cortex and adrenergic neurons of the locus coeruleus (LC) that project to the entorhinal cortex.[8,9] The LC is decimated in AD brains, with 70% neuron loss in late-stage disease.[10] Infection of LC adrenergic neurons with the neurotropic alphaherpesviruses HSV-1 and -2 and VZV is almost certain given that trigeminal ganglion (TG) neurons are twice as likely to test positive for HSV-1 from AD patients than from cognitive controls and all three trigeminal nerve branches send afferent fibers to sensory mesencephalic neurons intermingled among rostral LC neurons (Fig. 1A and 1B).[11–14]

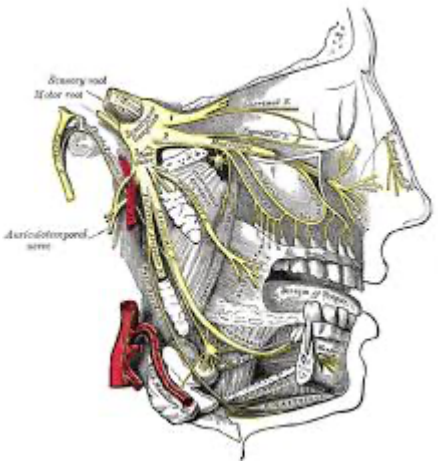

Fig. 1A. Branches of the trigeminal nerve and their anatomical sites of innervation.[11]

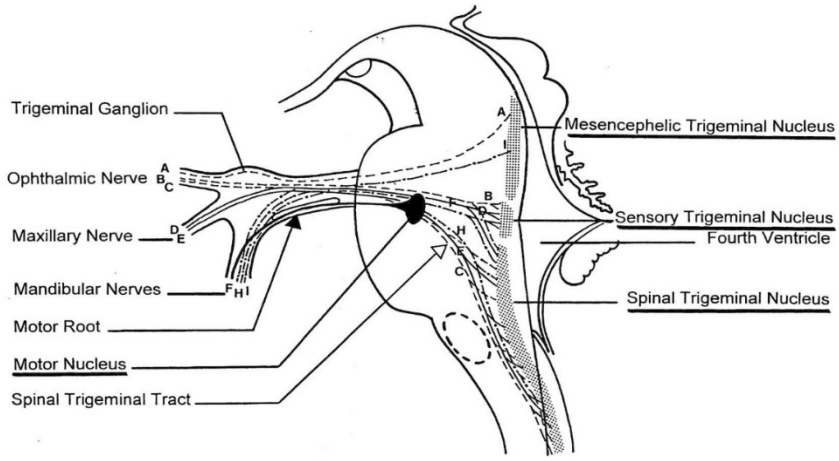

Fig. 1B. Processes pass through the trigeminal ganglion to synapse on CNS neurons in the trigeminal nuclei.[11]

The mesencephalic trigeminal neurons travel through the motor root of the trigeminal mandibular nerve to innervate widely to transmit propriosensory information from the teeth, hard palate, joint capsule, periodontium, lingual, facial, and extraocular muscles.[12,15] Further, motoneurons residing in the trigeminal motor nucleus send fibers directly through the mandibular branch of the trigeminal nerve, passing underneath the ganglion, to innervate jaw muscles. Neurons of the mesencephalic nucleus are also located directly rostral to the main LC nucleus, corresponding to the LC region with the most severe degeneration. In addition to entering the CNS at the mesencephalic nucleus, HSV-1 can travel anterograde in axons from bipolar trigeminal neurons to the three trigeminal nerve nuclei. Other pathways to the LC are through olfactory epithelium and ocular inoculation.[16–18]

With so many ways in—it is not surprising that HSV-1 is found in the brainstem of healthy human cadavers.[19–24] Fraser et al. found 6 out 11 cadavers tested positive for HSV-1 DNA by Southern blot, and using PCR, and Baringer and Pisani identified HSV-1 DNA in various brain regions in 14 out of 40 individuals, with the pons, medulla, and olfactory bulbs testing positive most often. Theil et al. detected HSV-1 DNA in multiple cranial nerve nuclei from all 5 healthy cadavers using PCR but found no latency-associated transcript (LAT) RNA expression by RT-PCR. Steiner et al. detected HSV-1 RNA expression in brainstems from all 7 cadavers examined. Using a larger probe Steiner detected HSV-1 transcripts that included LAT, ICP27, U55, and UL56 by in situ hybridization, while a smaller probe specific for just LAT was negative on northern blot. Therefore, LAT may not be expressed in all latent cell types, and this could explain why studies detecting LAT reported finding no HSV-1 RNA. Apparently, LAT expression is not necessary for latency. Nicoll et al. showed that LAT-negative HSV-1 could still enter latency in mouse TG neurons but had increased reactivation.[25] Nevertheless, what is striking about the reports by Steiner and Theil, is that HSV-1 DNA was found in the brainstem of every cadaver tested. Although these studies did not sample the LC *per se*, the proximity of LC neurons to trigeminal innervation guarantees infection. Since axons of LC adrenergic neurons project directly to the hippocampus, the hippocampus is likely to be infected as well.

Multiple Herpesviruses and non-cell autonomous degeneration

AD may start with infection of the neurotropic alphaherpesviruses (HSV-1, HSV-2, and VZV) in adrenergic neurons of the locus coeruleus (LC) and nucleus tractus solitarius. Human herpesviruses remain latent in most cells, and reactivate with stress, infection, inflammation, and immunosuppression.[26–28] Furthermore, with increasing age, epigenetic dedifferentiation lowers the herpesvirus reactivity threshold, increasing reactivation frequency.[29–32] Risk of HSV-1 infection and AD is significantly higher in ApoE4 allele carriers.[33–41] The ApoE4 allele and age enhance HSV-1 infection rate by increasing exofacial cholesterol, and decreasing flotillin-stabilized APP and sphingosine in lipid rafts.[42–44]

It is well known that EBV infects B cells, HCMV infects monocytes, and roseolovirus infects $CD4^+$ T cells. However, HSV-1 and 2, VZV, EBV, HCMV, and HHV-6A/B and -7 also infect the neurovascular unit, including endothelial cells, pericytes, smooth muscle cells, and astrocytes.[45–56] HSV infected adrenergic neurons can release host and viral chemokines that attract herpesviruses infected cells, which induces cell differentiation and subsequent reactivation of multiple herpesviruses. HCMV-mediated immune modifications appear to upregulate a secondary inflammatory response. For example, a murine cytomegalovirus (MCMV) infection produces an enhanced immune response in an experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis.[57] In EAE, the mouse is immunized with myelin oligodendrocyte glycoprotein to induce an immune response. The increased immune response may be related to $CD4^+CD28^{null}$ T cell expansion after repeated MCMV antigen stimulation. The increased inflammation in the MCMV/EAE mouse model could occur in human herpesvirus coinfections. Thus, multiple herpesvirus infections or a particular virulent strain can increase AD risk.[58]

HSV

Locus coeruleus adrenergic neurons project axons throughout the brain to control optimal circadian homeostatic activity through the release of norepinephrine and coordinated peptides.[59] HSV infected LC adrenergic neurons could be hyperactive. Notably, mouse autonomic neurons infected with an alphaherpesvirus and viewed with live calcium imaging show aberrant synchronous firing.[60] Hyperactive LC adrenergic neurons would explain why norepinephrine is significantly higher in cerebrospinal fluid from AD patients compared to age-matched controls.[61] Herpesviruses evolved to entrain viral activity with their host circadian rhythm. For instance, the HSV ICP0 transactivator has binding affinity to cellular BMAL1. In the morning, when BMAL1 level is high, it binds the viral ICPO transactivator to block herpesvirus activity. As BMAL1 falls through the day, ICPO becomes free to activate viral transcription.[62] Nocturnal HSV-mediated norepinephrine release could explain why AD patients experience sleep disorders.[63]

HSV-infected neuroblastoma cells increase production of Aβ.[64,65] HIV-infected macrophages and microglia also misprocess APP to produce toxic Aβ. In HIV-infected cells, APP associates with the HIV Gag polyprotein, restricting HIV particles from translocating to lipid rafts. However, Gag induces APP secretase cleavage for release, resulting in amyloidogenic processing.[66,67] HSV-1 may use a similar strategy as HIV to reduce APP restriction. A C-terminal sequence of HSV-1 glycoprotein B (gB) has a 67% sequence similarity to $Aβ_{(34-42)}$ in the Aβ transmembrane region.[68] The sequence similarity is enclosed in a stippled rectangle in Fig. 2A., with the mismatch designated by X. HSV-1 C-terminal gB binds Rab5 in endosomes to entrap MHC-II and prevent antigen presentation. gB induces endosome enlargement and blocks maturation of the endosomes into late Rab7-endosomes.[69] Accordingly, Rab5-mediated

endocytic pathway is dysregulated in AD, with Rab5 and Aβ are localized to enlarged endosomes.[70,71]

gB is secreted in exosomes and a cleaved gB peptide self-assembles into fibrils, accelerates Aβ fibril formation in culture, and increases APP amyloidogenic processing.[68,69,72] The gB peptide is neurotoxic to cells, causing neurite retraction, neuritic dystrophy, reduced cell size, and cell death within 12 hours of incubation.[73] Notably, HIV Tat is also capable of increasing Aβ production and accelerating Aβ multifibrillar structures.[67,74] Searching for additional Aβ sequence similarity in HSV-1 using the GenBank Basic Local Alignment Search Tool (BLAST) identified sequences at the C-terminal end of a 233-amino-acid protein—UL56 (Fig. 2A). The alignment has 9 out of 11 matches, including one conservative replacement, as shown in the solid rectangle in Fig. 2B, with the conservative match designated by an asterisk. This segment of APP associates with Sorla and contains mutations linked to early-onset autosomal dominant AD.[75] Thus, HSV-1 gB and pUL56 can theoretically compete with γ-secretase or Sorla for APP binding, effectively reducing APP γ-secretase cleavage, resulting in the toxic β-CTF C99, which induces autophagic and mitochondrial dysfunction.[76–80]

membrane protein UL56 [Human alphaherpesvirus 1]

Sequence ID: YP_009137132.1 Length: 234 Number of Matches: 1

See 6 more title(s) See all Identical Proteins(IPG)

Range 1: 219 to 230 GenPept Graphics ▼ Next Match ▲ P

Score	Expect	Identities	Positives	Gaps
24.4 bits(50)	5e-05	9/13(69%)	10/13(76%)	1/13(7%)

```
Query  8    GVVIATVIVITLV  20
            GVV A V+VI LV
Sbjct  219  GVV-AIVVVIILV  230
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Fig. 2A. Sequence similarity between APP and HSV-1 C-terminal UL56 sequence.

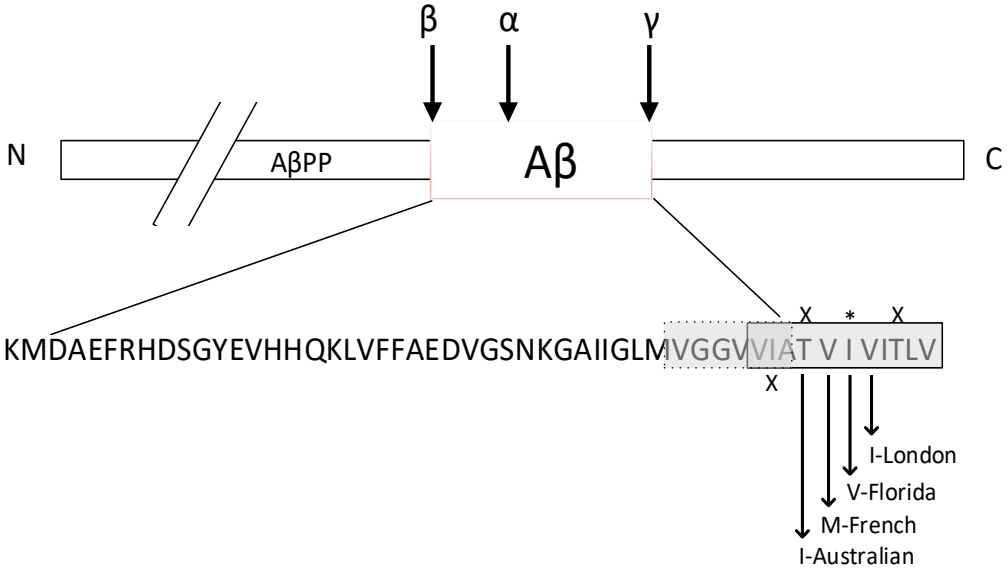

Fig. 2B. Amyloid precursor protein showing amyloid-β amino acid sequences and the locations of secretase cleavage. Stippled box shows sequence similarity to HSV-1 C-terminal glycoprotein B

sequence. Solid box shows sequence similarityto HSV-1 C-terminal UL56 sequence. The “X” designates an amino acid mismatch. The asterisk designates a
conservative amino acid replacement. Four amino acid mutations that cause early-onset AD are found in the UL56 similarity sequence. The names are based on the location of individuals or ethnic group in which the mutation was discovered.

The HSV-2 glycoprotein G is secreted as a 34kDa high-mannose cleavage product (SgG2) corresponding to the N-terminal 300 amino acids of glycoprotein G. SgG2 is further processed into a 15 amino acid peptide (gG-2p20). The peptide gG-2p20 produces monocyte and neutrophil chemotaxis and neutrophil oxidative bursts that can kill lymphocytes and NK cells and injure bystander cells.[81] HSV-1 also secretes VP22 and a 30-amino acid peptide from N-terminal glycoprotein B, both with unknown function.[82,83] Accordingly, non-cell autonomous signaling may explain why HSV-1 DNA levels do not correlate with encephalitis severity.[84] In summary, HSV gB, and UL56, can interact with APP processing factors to alter APP and endosomal trafficking. Furthermore, gB, VP22, and SgG2 is secreted and can produce non-cell autonomous AD pathology.

HCMV

During productive infection the HCMV tegument phosphoprotein pp150 is required for final nucleocapsid envelopment.[85] The pp150 protein binds to bicaudalD1 (BicD1), the effector protein of the small GTPase Rab6.[86] Binding of pp150 to BicD1 transfers Rab6 vesicles to the viral assembly compartment.[87] BicD1 binds Rab6 vesicles to the dynein-dynactin complex for intracellular movement along microtubules. Rab6 also binds the spliced isoform APP-binding, family A member 1 (APBA), previously called Mint1 826, which colocalizes with APP during vesicle transport.[88] The displacement of Rab6 vesicles to the viral assembly compartment (VAC) by pp150 disrupts APP trafficking to the cell surface. Accordingly, the upregulation of Rab6 and APP expression in AD brains is an attempt to compensate for their functional deficiency.[89]

The displacement of Rab6 vesicles to the VAC could affect neurite maintenance, since restriction of BicD1|Rab6 to the centrosome prevents anterograde transport, which inhibits neuritogenesis.[90] Pp150 could also disrupt Rab6-mediated transport of late endosomes to the lysosome.[91] Thus, the effects of pp150-mediated Rab6 vesicle displacement are consistent with AD pathology. Furthermore, pp150 is secreted, as well as 13 other HCMV viral proteins, which means that a few infected cells can cause widespread non-cell autonomous degeneration.[92–94]

Homologs to HCMV pp150 are found in the HHV-6A, HHV-7 and EBV. Figure 3A shows a graphical alignment of sequence similarity between HCMV pp150 and HHV-6A, HHV-7, and EBV from N-terminal to C-terminal. Sequence similarity is at the N-terminal of pp150, with HHV-6A pp100, encoded by U11, having 45% positives and an E-value (expected number of errors per query) of $1x10^{-20}$ and HHV-7 pp100, encoded by U11, having 42% positives and an E-value of $1x10^{-16}$ (Figs. 3B and 3C). The EBV protein gp350 has a shorter similarity sequence to pp150 located towards the C-terminal of pp150 with 49% positives and an E-value of $6x10^{-05}$ (Fig. 3D).

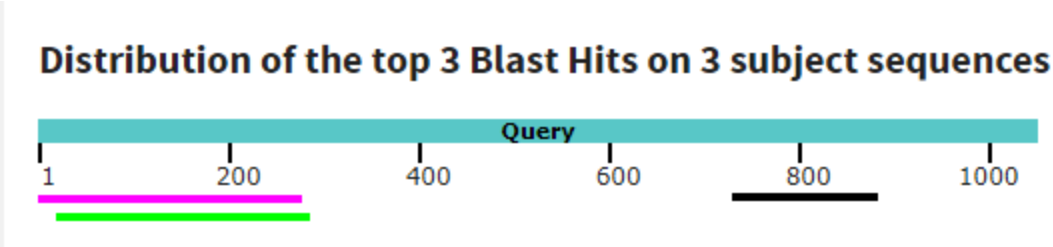

Fig. 3A. Alignment of sequence similarity between HCMV pp150 protein (Query) and HHV-6A and HHV-7 pp100 protein (N-terminal) and EBV gp350 protein (C-terminal).

```
Score:86.3 bits(212), Expect:1e-20,
Method:Compositional matrix adjust.,
Identities:70/286(24%), Positives:129/286(45%), Gaps:26/286(9%)

Query  1    MSLQ-----FIGLQRRDVVALVNFLRHLTQKPDVDLEAHPKILKKC----GEKRLHRRTV  51
            M LQ     F  L R  V  L +FL +L +  +VDL  HP +   C    G+     +T
Sbjct  1    MDLQRHPIPFAWLDRDKVERLTDFLSNLERLDNVDLREHPHVTNSCVVREGDDVDDLKT-  59

Query  52   LFNELMLWLGYYRELRFHNPDLSSVLEEFEVRCVAVARRGYTYPFGDRGKARDHLAVLDR  111
            L+N L+LWL Y+  L    PD +++ ++   +  +V          ++G   +     ++
Sbjct  60   LYNLLVLWLMYHYVLSKRKPDYNAIWQDI-TKLQSVVNEYLNSKGLNKGIFENMFT--NK  116

Query  112  TEFDTDVRHDAEIVERALVSAVILAKMSVRETLVTAIGQTEPIAFVHLKDTEVQRIEENL  171
             +F++        + RAL+      K      + T          +V+L   +   IE NL
Sbjct  117  EKFESQFSD----INRALLRLGNFIKWGSNVAIDTP--------YVNLTAEDSSEIENNL  164

Query  172  EGVRRNMFCVKPLDLNLDRHANTALVNAVNKLVYTGRLIMNVRRSWEELERKCLARIQER  231
            +   +NM      ++N     N  L+ ++NKL+Y G+L + + +SW +LE+  +++I
Sbjct  165  QDAEKNMLWYTVYNINDPWDENGYLITSINKLIYLGKLFLALTQSWSKLEKVAMSQIVIT  224

Query  232  CKLLVKELRMCLSFDSNYCRNILKHAVENGDSADTLLELLIEDFDI  277
               L   LR   +F+  Y   +L+  +  G   ++ L+++  D+DI
Sbjct  225  QNHLSGHLRRHDNFNIVYSHRVLQTPL-TGQRVESFLKIITSDYDI  269
```

Fig. 3B. HCMV (Query) pp150 protein sequence similarity alignment to HHV-6A pp100 protein (Sbjct.)

```
Score:71.2 bits(173), Expect:4e-16,
Method:Compositional matrix adjust.,
Identities:65/273(24%), Positives:116/273(42%), Gaps:24/273(8%)

Query  19   FLRHLTQKPDVDLEAHPKILKKCGEK---RLHRRTVLFNELMLWLGYYRELRFHNPDLSS  75
            F  +++  P VD+  +P IL +C  K    ++    L+N L+LW+ +++ L    PD
Sbjct  22   FFENISSLPVVDIRENPWILSQCIVKTGNSINNVKTLYNNLILWIYFHQTLCKKKPDYEE  81

Query  76   VLEEFEVRCVAVARRGYTYPFGDRGKARDH--LAVLDRTEFDTDVRHDAEIVERALVSAV  133
            V +E  +  V    + Y      R    D+  L   ++  F+T+ ++   + +  L
Sbjct  82   VWQE--ILKVQKILKDY---LEQRQMITDYSSLTSFNKVGFETEFKN---VAKDLLKLGS  133

Query  134  ILAKMSVRETLVTAIGQTEPIAFVHLKDTEVQRIEENLEGVRRNMFCVKPLDLNLDRHAN  193
             L   +V          T    +V+L   E   I ENL+  + NM       +    + N
Sbjct  134  FLRWGTV----------THAADYVNLTTEERAEIGENLQKAKNNMLSFTIYQIVDPWNEN  183

Query  194  TALVNAVNKLVYTGRLIMNVRRSWEELERKCLARIQERCKLLVKELRMCLSFDSNYCRNI  253
               V  +N+L+Y G L++ +  SW  +E+  L  I E+   ++K +    +F S Y   I
Sbjct  184  GYYVTNINRLLYLGNLLITLHGSWMNMEKLALNTINEKKNAILKAIENNKNFVSIYSYQI  243

Query  254  LKHAVENGDSADTLLELLIEDFDIYVDSFPQSA  286
            L   +       +  ++L EDFD+   S    A
Sbjct  244  LSLPL-TSHRVTSFFKILTEDFDVITKSLELHA  275
```

Fig. 3C. HCMV (Query) pp150 protein sequence similarity alignment to HHV-7 pp100 protein (Sbjct).

```
Score:35.0 bits(79), Expect:6e-05,
Method:Compositional matrix adjust.,
Identities:51/159(32%), Positives:79/159(49%), Gaps:19/159(11%)

Query  728  SLGHTTPSSDYNNDVISPPSQTPEQSTPSRIRKAKLSSPMTTTSTSQKPVLGKR-----V  782
            +LG T+P+S     V +P   TP  ++P+  + +  S+  T T  +  P LGK      V
Sbjct  552  TLGKTSPTSA----VTTP---TPNATSPTLGKTSPTSAVTTPTPNATSPTLGKTSPTSAV  604

Query  783  ATPHASARAQTVTSTPVQGRLEKQVSGTPSTVPATLLQPQPASSK-TTSSRNVTSGAGTS  841
             TP  +A   TV  T  Q       G  S  P    QP+ A+S  TT   N+TS   +S
Sbjct  605  TTPTPNATGPTVGETSPQANATNHTLGGTSPTPVVTSQPKNATSAVTTGQHNITS---SS  661

Query  842  SASSARQPSASASVLSPTEDDVVSPATSPLSMLSSASPS  880
            ++S + +PS++   LSP+  D    +TS + +L+SA P+
Sbjct  662  TSSMSLRPSSNPETLSPSTSDN---STSHMPLLTSAHPT  697
```

Fig. 3D. HCMV (Query) pp150 protein sequence similarity alignment to EBV gp350 protein (Sbjct).

HCMV antibody titers are significantly higher in AD patients and even higher in patients with the human leukocyte antigen (HLA) BW15.[95] HLA-BW15 is adjacent to HLA-DR15 in the major histocompatibility region with few recombination hotspots, indicating linkage equilibrium.[96] The HLA-DR15 haplotype DRB1*15:01 is associated with AD risk[97]. HCMV antibody levels are significantly associated with the cortical density of neurofibrillary tangles, cognitive function, and AD risk.[98–100] Peripheral mononuclear blood cells from AD patients have significantly higher interferon-γ levels in response to the HCMV protein pp65 compared to non-demented controls.[101] Both AD patients and HCMV seropositive subjects have significantly elevated inflammatory cytokines, such as IL-6 and TNF-α.[102] In summary, an occult HCMV infection can secrete pp150 and other viral factors to cause diffuse non-cell autonomous degeneration resembling AD.

Roseolovirus genus

Like HSV, HHV-6 A and B also contains the “VGGVV” motif at the C-terminal of conserved gB (Fig. 4). Thus, HHV-6 A and B gB may also bind Rab5 vesicles to impair endosome function. The HHV-6 and -7 protein pp100 has N-terminal alignment to HCMV pp150 (Figs. 3B and 3C). However, transfection of a pp150 mutant indicates that the conserved N-terminal pp150 is critical for capsid binding, whereas the carboxy terminal sequence is important for virion maturation.[103] Moreover, BicD1 binds pp150 at the C-terminal. Thus, the pp100 alignment with pp150 is unlikely to be significant.

```
     SP|P06437|GB_HHV1K --------------------
GGVVSAVSGVSSFMSNPFGALAVGLLVLAGLAAAFFAFRY 797
     SP|P03188|GB_EBVB9 --------------------
GLFSSLVSGFISFFKNPFGGMLILVLVAGVVILVISLTRR 755
     SP|P06473|GB_HCMVA --------------------
GAVASVVEGVATFLKNPFGAFTIILVAIAVVIITYLIYTR 773
     SP|Q9J3M8|GE_VZVO  AIEERGFPPTAGQPPATTKPKEITPVNPGTSPLLR--
YAAWTGGLAAVVLLCLVIFLICT 560
     SP|P36320|GB_HHV6Z --------------------
GALGDIVGGVVSFLKNPFGGGLMLILAIVVVVIIIVVFVR 711
     SP|P28864|GB_HHV6U --------------------
GALGDIVGGVVSFLKNPFGGGLMLILAIVVVVIIIVVFVR 711
```

```
     SP|P52352|GB_HHV7J --------------------
GALGDVVNGVFSFLKNPFGGALTILLTLGVIGLVIFLFLR 706
     SP|P24994|GB_HSV2S --------------------
GGVVSAVSGVSSFLSNPFGALAVGLLVLAGLAAAFFAFRY 782
```

Fig. 4. Like HSV-1 and -2, HHV-6 A (Z) and B(U) also contains the “VGGVV” motif at the C-terminal of conserved gB

The HHV-6A and B gene U94 encodes a protein called RepH6. RepH6 expression is required for latent infection but is also expressed during productive infection.[104] RepH6 increases the expression of both soluble and membrane bound human leukocyte antigen G (HLA-G) isoforms through the upregulation of the transcription factor ATF3.[105] Increased ATF3 expression is associated with increased expression of proinflammatory response and oxidative stress genes.[106] Soluble HLA-G1 inhibits angiogenesis by binding directly to the CD160 receptors on endothelial cells and inducing apoptosis.[107,108] Accordingly, Wiendl and colleagues found increased HLA-G expression in brain tissue from 2 AD patients compared to healthy controls.[109] Thus, HHV-6, through RepH6 upregulation, may secret soluble HLA-G, preventing microvessel regeneration.

A meta-analysis pooling data from five studies showed that HHV-6 status significantly increased the risk of AD, with pooled odds ratio of 2.23 (95% CI .95-5.33).[110] In addition, a survey of IgG reactivity to various herpesviruses showed that AD patients have significantly lower HHV-6 IgG compared to controls, indicating that impaired HHV-6 immunity may increase the risk of AD.[111] These findings were recently corroborated in a study showing that AD brains contain significantly more HHV-6A and HHV-7 RNA transcripts compared to controls, and while HSV-1 transcripts were also more abundant in AD, they were much less prevalent than HHV-6A and HHV-7.[112]

EBV

EBV infects primary cultured human brain microvessel endothelial cells using the entry receptors CD21 and CD35.[47,48,113,114] EBV may also infect erythrocytes. Erythrocytes express CD35 and are metabolically anomalous in AD patients.[115] And receiving a washed erythrocyte cell transfusion significantly increases AD risk.[116] EBV has also been found to infect neurons and astrocyte cell lines.[55,117] Although, immunolabeling of CD35 is restricted to astrocytes in the brain.[118–121,121] Early onset autosomal dominant AD can be caused by mutations in APP, SORL1, and the γ-secretase subunits, PSEN1 and PSEN2.[122] SORL1 encodes the sortilin-related receptor 1 (Sorla) protein. Sorla is an APP chaperone that is decreased in AD brain and is required for transporting APP from the Golgi to the cell surface. If EBV is involved in AD, it may encode factors that disrupt APP or Sorla expression.

Indeed, EBV DNA sequence similarity with SORL1 was identified in a BLAST of Herpesviridae family DNA (Table 1). The highest sequence similarity identified a sequence at the C-terminal end of SORL1, with an e-value of $5x10^{-116}$, matching 610/634 nucleotides. The same GenBank BLAST found 10 EBV isolates with a 35-nucleotide sequence repeated in multiple SORL1 intron locations and in an HSV-2 isolate with a 48-nucleotide SORL1 sequence similarity.

Table 1. EBV DNA sequence similarity with SORL1 was identified in a BLAST of Herpesviridae family DNA

HHV type - isolate	HHV isolate Accession	E value	Percent identity	Virus Position	Virus region	SORL1 region	SEQ ID No.	Similarity Sequence
HHV 4 - HKHD40	MH590409.1	5.00E-116	96	158635	intergenic	intron	3	GTTAGTTACATATGTATACATGTGCCATGNTGGTG…
HHV 4 - HKNPC60	MH590571.1	5.00E-116	99	36456	intergenic	intron	4	GCCGCAATAAACATACGTGTGCATGTGTCTTTATA…
HHV 4 - Namalwa	AH002364.2	4.00E-51	91	412	repeat	intron	5	CCTGTAGTCCCAGCTACTCAGGAGGCTGAGGCAG…
HHV 4 - HKHD130	MH590499.1	7.00E-16	87	37475	intergenic	intron	6	AATAGGCTGNGTGCGGTGGCTCACACCTGTAATN…
HHV 2 - 2006-16150CAM	MH790658.1	1.00E-07	92	11357	intergenic	intron	7	ACATTAGGTATATCTCCTAATGCTATCCCTCCCCC…
HHV 4 - HKHD73	MH590442.1	3.00E-07	83	95948	intergenic	intron	8	GCACTCCAGCCTGGGCAACAGAGTGAGACCCTAAC…
HHV 4 - HKHD7	MH590376.1	1.00E-06	67	36674	intergenic	intron	9	AATTTTGTTGATCTTTTCAAAAAACCAGCTCCTGG…
HHV 4 - H002213	KP968264.1	2.00E-04	97	159585	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - VGO	KP968260.1	2.00E-04	97	159392	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - SCL	KP968259.1	2.00E-04	97	159572	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - CCH	KP968257.1	2.00E-04	97	159504	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - VA	KT001102.1	2.00E-04	97	159547	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - FNR	KR063345.1	2.00E-04	97	159600	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - H03753A	KR063342.1	2.00E-04	97	159580	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - K4123-MiEBV	KC440852.1	2.00E-04	97	159589	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - K4123-Mi	KC440851.1	2.00E-04	97	159567	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGACCCTG
HHV 4 - B95-8	AJ507799	3.00E-04	94	148162	A73 intron	intron	10	CTGCAGTCCTGCCTGGCGCAACAGAGCGAGACCCTG
HHV 4 - RPF	KR063344.1	6.00E-04	100	159601	A73 intron	intron	11	GTCTCGCTCTGTTGCCCAGGCTGGACTGCAG
HHV 4 - HKHD95	MH590464.1	8.00E-03	94	36494	intergenic	intron	12	GGCTAATTTTTTTGTATTTTTAGTAGAGATGGGG
HHV 4 - HKHD141	MH590510.1	1.30E-02	94	96579	intergenic	intron	13	TTCTCCTGCCTCAGCCTCCNGAGTAGCTGGGATT
HHV 4 - HKHD27	MH590396.1	2.60E-02	97	27439	intergenic	intron	14	CCATCTTGGCTCACTGCAACCTCCACCTCCC

A BLAST for similar sequences between APP and Herpesviridae family DNA identified the same similarity sequence as between SORL1 and EBV (Table 2). Some EBV isolates contained long sequences of APP intron alignment. For instance, isolates HKNPC60 and HKHD40 contain 2,486 and 2,357 nucleotides, respectively, aligned with 97% ungapped similarity to APP intron 13.

Table 2. A BLAST for APP and Herpesviridae family DNA identified the same similarity sequence as between SORL1 and EBV

HHV type - isolate	HHV isolate Accession	E value	Percent identity	Virus Position	Viral region	APP region	SEQ ID No.	Similarity Sequence
HHV 4 - HKNPC60	MH590571.1	1.00E-148	97	36456	intergenic	intron	4	GCCGCAATAAACATACGTGTGCATGTGTCTTT…
HHV 4 - HKHD40	MH590409.1	2.00E-132	97	158638	intergenic	intron	15	CAGGTTAGTTACATATGTATACATGTGCCATG…
HHV 4 - HKHD7	MH590376.1	5.00E-123	98	36670	intergenic	intron	16	TATCAATTTTGTTGATCTTTTCAAAAAACCA…
HHV 4 - Namalwa	AH002364.2	6.00E-51	89	412	repeat	intron	5	CCTGTAGTCCCAGCTACTCAGGAGGCTGAG…
HHV 4 - HKHD130	MH590499.1	5.00E-14	83	37423	intergenic	intron	17	GCGGTGGCTCACGCCTGTAATCCCAGCACTT…
HHV 4 - HKHD73	MH590442.1	6.00E-13	83	96041	EBNA-1	intron	18	...GTCTCACTCTGTTGCCCAGGCTGGAGTGC
HHV 2 - 2006-16150CAMe	MH790658.1	2.00E-07	92	11358	intergenic	intron	19	CATTAGGTATATCTCCTAATGCTATCCCTCC…
HHV 6 - HP36C6	KY315533.2	8.00E-05	84	82980	intergenic	intron	20	TTGTTTTAAGCCAATCTGTTTGTGATACTTTG…
HHV 4 - HKHD141	MH590510.1	1.00E-04	95	96579	intergenic	intron	21	TTCTCCTGCCTCAGCCTCCNGAGTAGCTGGGA..
HHV 4 - HKHD141	MH590510.1	1.00E-04	97	96546	intergenic	intron	22	AATCCCAGCTACTCNGGAGGCTGAGGCAGGA…
HHV 4 - HKHD95	MH590464.1	0.001	97	36461	intergenic	intron	23	GGCTAATTTTTTTGTATTTTTAGTAGAGATGGG..
HHV 4 - H002213	KP968264.1	0.001	94	159586	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - VGO	KP968260.1	0.001	94	159362	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - SCL	KP968259.1	0.001	94	159573	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - CCH	KP968257.1	0.001	94	159535	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - VA	KT001102.1	0.001	94	159548	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - FNR	KR063345.1	0.001	94	159601	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - H03753A	KR063342.1	0.001	94	159581	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - K4123-MiEBV	KC440852.1	0.001	94	159590	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - K4123-Mi	KC440851.1	0.001	94	159568	A73 intron	intron	10	CTGCACTCCAGCCTGGGCAACAGAGCGAGAC
HHV 4 - RPF	KR063344.1	0.003	94	159631	A73 intron	intron	10	CTGCAGTCCAGCCTGGGCAACAGAGCGAGAC..
HHV 4 - HKHD27	MH590396.1	0.006	100	27469	intergenic	intron	24	GGGAGGTGGAGGTTGCAGTGAGCCAAGAT

The 35-nucleotide sequence matching APP and SORL1 is from the same EBV location in intron 3 of the EBV gene A73. In SORL1 and APP, the sequence targets exclusively introns on both strands. In SORL1, the antisense sequence targets intron 3 (nucleotide position-39672) and intron 23 (126323), while a sense alignment is in intron 32 (150234) (Fig. 5A). In APP, the EBV similarity sequence maps to intron 6 (159501) and intron 8 (183750) (Fig. 5B). The EBV ncRNA contains two transcription start sites (TATA) that are located 644 and 613 nucleotides from the 5' complement site (Fig. 6). EBV may also transcribe a ncRNA complement from 3' to 5' that aligns with the intron 32 sequence and uses the TATT at 160077 or 160518. A BLAST for the EBV 35-nucleotide similarity sequence against Homo sapiens genomic and RNA sequences identified the Zinc Finger protein 677 mRNA and TMEM241 exon1 ncRNA in sense alignment.

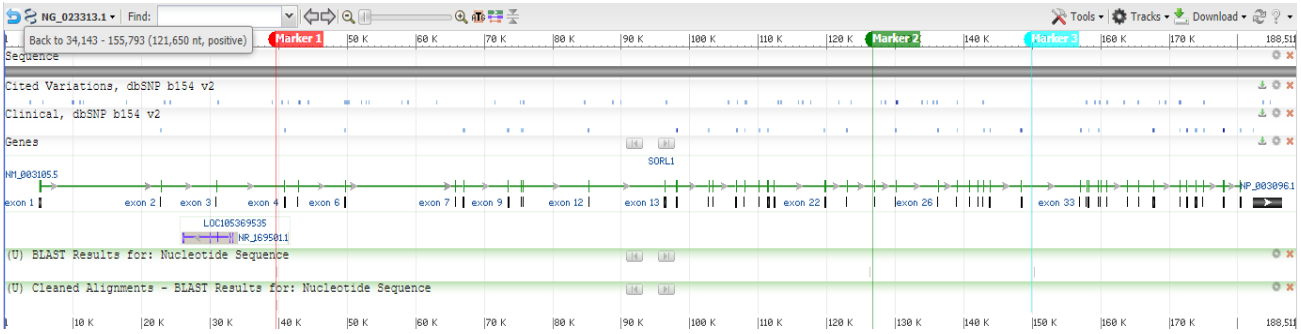

Fig. 5A. SORL1 locations with EBV sequence similarity are in intron 3 (39672) and intron 23 (126323) and sense 32 (150234).

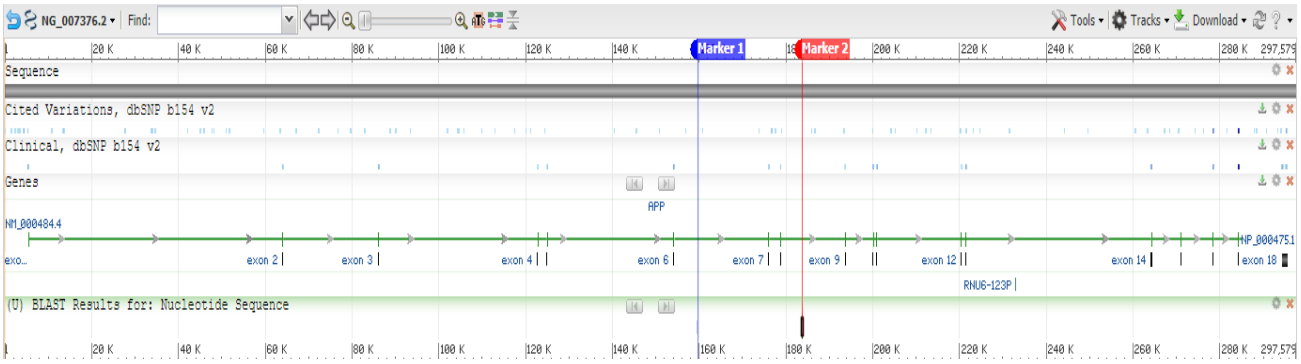

Fig. 5B. APP locations with the EBV similarity sequence are in intron 6 (159501) and intron 8 (183750).

```
158641 cgtgtgattc tcccgcgtgc caaacgaggg acactggatg tccgaggaga agcggaacag
158701 gtcgccgtgg ctggagagct cgcagactcg gaaaggaaag ctggtttgct gacgcgtggc
158761 ggtaggctgc accgtggtgg cgggggggtgc gggctgctct ggggtctgcg caccgaggcg
158821 gcacgccagg gcggctagca gcacgaccac gcttagcacc ctacgccgag tcatctctca
158881 tttggaggtg caggtagaga agggcatata gatccttaaa tacccacccc ctgccctTAT
158941 acagaagaat taggggccgg tcagagtcgt acgtgaggta aagcccatcc gggggcaggg
159001 cctggccggg gctgaccgcg tccgcccggc gcaggatcaa ggaccgcccc caggtcttgt
159061 tgtagaggga cacggttagg acggcctcgc gcagcgcccg gcacagaatt tgctggctag
159121 atgccagtga gcccccgggt acgctgtaga agctgttgaa ggaggtctct atccagtcgc
159181 tcggctcgat gcctggccat atcagggaag tcaggaacgc cttctggtga ggcagcgtac
159241 ctgcggcgtc acagcagcga gccagggcca cgttgctggg tgggggaaag agcccgctct
159301 cctccgccag gggccccgtg atgaaggtgt acaggctgtg cgtcagcgcg tgcaggtgct
159361 ccgagctcag ggtctgggta aacaggtgtg ttttgatgta cttggaattc tcaaaggcgg
159421 caccctcgcc ggcgcgcctg tcctcccagg gacccgagac gaaggcccgt ctgtagagga
159481 agtggttgcg catgcgggcc agctcccagt agaccacgtc cccccagacg cgcaggcaca
159541 gggtctcggt

 39706    SORL1    gttccagagc gagacaacgg gtccgacctc acgtc        39672
159541    HHV4    caaggtctcg ctctgttgcc caggctggag tgcagcttgg ccagaccctc
159601 ggtggccacc tggcgcaggt actgctcctt gcgcttgagc gcgtccgaga gggcgccgga
159661 cgggccgggc tctcgtgccc cagccggccg gggcacctcc gggctctccc gggacgcctc
159721 ctcctcgcct cggcccaacc gctgcatggc tcggttgagc cgcgtgtaga gctcgttcct
159781 cttttgcagg atggcccggt actgggggtg cgccgtgaag gcggcggcgc agtccgcctt
159841 cagcgcctcc accgcgtcgc ccgaggagct gtagaccccg ccgcagaaga gccgctctgt
159901 ggccccggga gccacggcat caaacaggtg agtcagcctt gcccccgcca gcgcctcctc
159961 gcaggcccgc cgcaccaggg ccaggcgacg ctcccgggca aacagggcag agaggcggga
160021 atggccgcca ccctcccccc gccccgttgc accgatagca tggccgccag agttccaata
160081 gaggagctcc gagagttccg ccacctccgg gggcactgtc gagaagacgt tgtaggtgtc
160141 cagcgctctg gtcgcccccc ctgcctccgg ccgccccggg cccgggaccg cgccctcctc
160201 tgggccgccc ggcctcgcct tctcctcagc ctccaacagg tgcccgagcc cagcctgccg
160261 gacttcattc tcaaacagtc ccgagaccgg ctccggattc accggcaccg ccaggtggtt
160321 acaggagacg tgggtccccc ctgccgtgga agggttgccg tggttgggca gaaccatcag
160381 ctcgcccaca cagcgccagc agggcacaga ggtgatgtag aggcgcgggt ctgggatggg
160441 acttacgccc cgaaagcggc ccagcagatc cagggcccgt tccaggctct ccagccccat
160501 ggtgtgagac atgcaataaa acacgctatt gattctcttc attaaaatct ctatgtcatt
```

Fig. 6. EBV ncRNA contains two transcription start sites (TATA) that are located 644 and 613 nucleotides 5' from the complement site. EBV may also transcribe the complement ncRNA from 3' to 5', which would be antisense to the sense SORL1 intron 32 sequence using TATT (AATA) at 160077 or 160518.

The Virus Pathogen Database Analysis Resource (ViPR) contains 1837 complete Herpesviridae genomes. A BLAST using this database returned essentially the same EBV sequences similarity as the GenBank BLAST. A restrictive BLAST with only EBV strains identified a 21-nucleotide sequence (aggccaggagaggcagcccca) with similarity to SORL1 that maps to intron 32 in SORL1 with an E-value of $4x10^{-07}$ (Fig. 7).

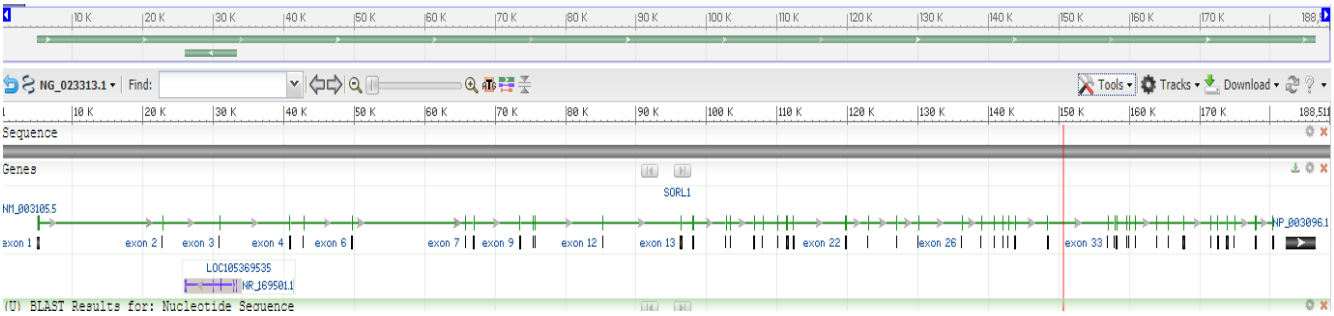

Fig. 7. EBV contains a 21-nucleotide sequence with similarity to SORL1 that maps to intron 32 in SORL1.

In the EBV isolate AJ507799.2, the 21-nucleotide sequence locates to multiple EBNA-LP introns, 3' of BWRF1 (Fig. 8). The 21-nucleotide sequence has no significant alignment to APP. A BLAST for the EBV 21-nucleotide similarity sequence against Homo sapiens genomic and RNA sequences identified an antisense alignment with the gene POU class 2 homeobox 3 gene (POU2F3). Interestingly, a KEGG pathway map shows that POU2f3 is functionally equivalent to Oct-1. Oct-1 regulates HSV-1 immediate early gene transcription. In EBV, Oct-1 enhances BRLF1-mediated regulation of active replication.[123,124]Thus, the 21-nucleotide sequence may also regulate viral transcription.

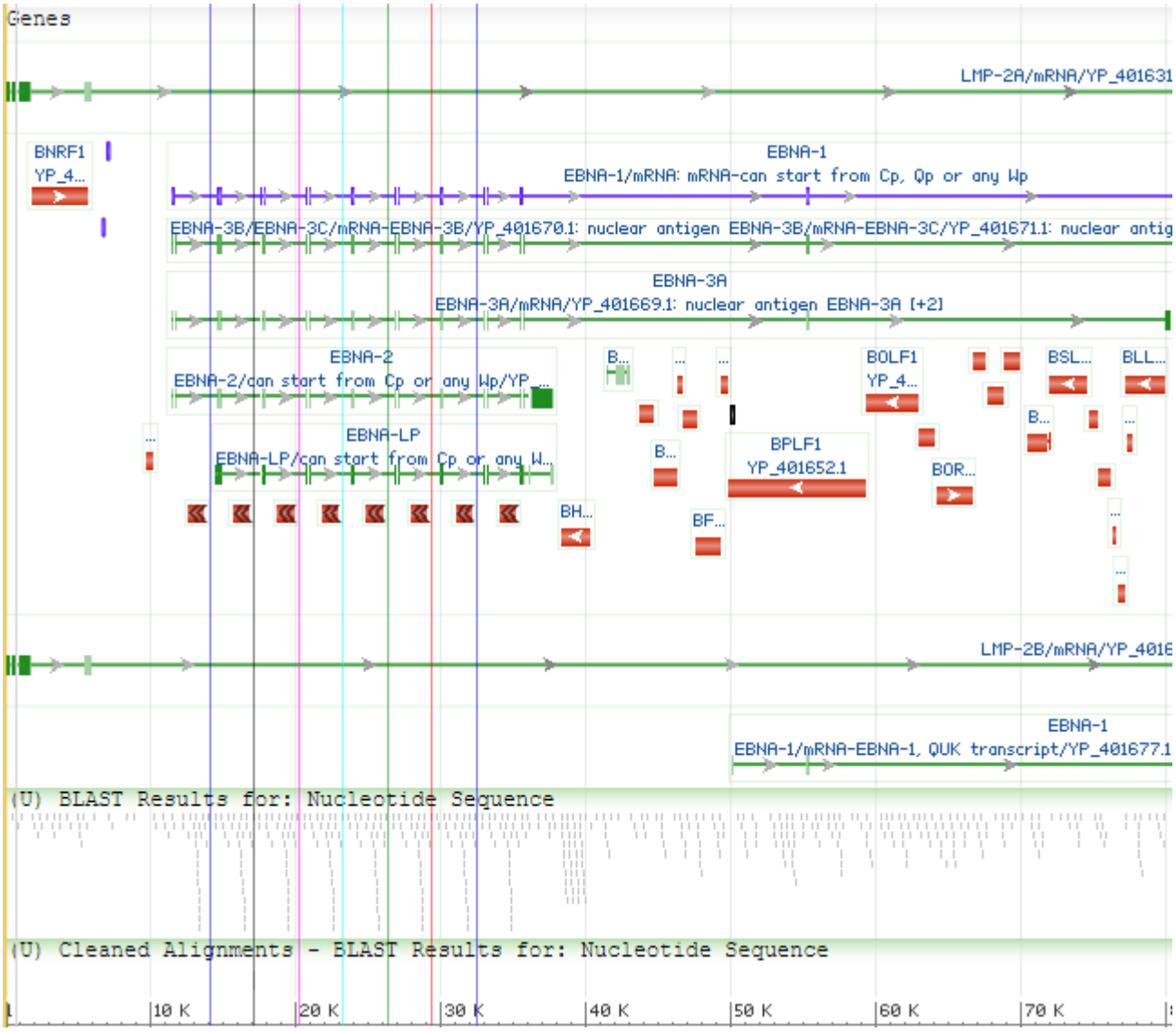

Fig. 8. DNA sequence from EBV isolate AJ507799.2, showing 7 locations of the 21-nucleotide sequence in EBNA-LP introns.

An estimated 13% of herpesvirus proteins show sequence similarity to human homologs, and about 54% of these sequences interact functionally with the host.[125] Thus, it is possible that the EBV sequence similarities do not affect human SORL1 or APP expression. However, it is curious that only EBV, except for one alignment in HSV2, identified the same 31/35 nucleotide sequence similarity in both SORL1 and APP. The EBV ncRNAs could target SORL1 and APP functional splicing elements to produce exon skipping or premature termination.[126] The EBV protein gp350 may also alter APP function. Gp350 has sequence alignment to HCMV pp150 where pp150 binds BicD1 (Fig. 3D.).[86] Thus, EBV gp350 may also divert Rab6 endosomes containing APP to the VAC. It is unknown whether gp350 is secreted like HCMV pp150.

Neurons from AD brains show abnormal expression of cell-cycle entry proteins cyclins-D and -B and increased hyperploidy.[127,128] Cell cycle entry is abnormal for postmitotic neurons and is linked to synaptic dysfunction and neuron death.[129,130] EBV expresses high levels of two short

ncRNA molecules, EBER1 and EBER2, during latency and replication. EBER expression induces IL-6 mediated activation of signal transducers and activators of transcription 3 (STAT3). STAT3 activation decreases the expression of cyclin dependent kinase inhibitor p21 and p27, which releases cyclin dependent kinases 2 and 4 inhibition, allowing cyclins to promote G1/S transition.[131,132] Cyclin-dependent kinase 4 activation induces cell death by hyperphosphorylation of the pRb family member p130. Phosphorylated p130 binds chromatin modifiers Suv39H1 and HDAC1, releasing the transcription factor E2F4, which binds transcription factors B and C-Myb to initiate transcription of the proapoptotic BH3-containing BIM. BIM activates BAX/BAK, which forms multimeric pores in the mitochondrial membrane to release cytochrome c. Cytochrome c binds the protein 14-3-3ε to release its inhibition over the apoptotic protease activating factor-1, allowing apoptosome formation and apoptosis-inducing caspase 9/3 activation.[133] Accordingly, EBER1 could be responsible for the abnormal expression of cell cycle entry proteins and increased BIM expression in the AD brain.[134]

EBER1 is secreted bound to the lupus La protein.[135] EBER1 was also found bound to the L22 ribosomal protein in uninfected cells.[136] EBER1 can bind to TLR3 on neurons to cause irreversible growth cone collapse and inhibit neurite outgrowth.[135,137] Inside the cell, EBER1 binds the RNA-dependent protein kinase (PKR) to inhibit viral RNA PKR activation and the subsequent phosphorylation of eukaryotic initiation factor 2α (EIF2α), preventing protein translation.[138] Exosomes containing EBERs are released from nasopharyngeal tumors and delivered to adjacent endothelial cells where EBERs upregulated angiogenesis.[139] Interestingly, vascular density is increased in AD hippocampus compared to controls.[140] Thus, secreted EBER could cause non-cell autonomous increase in growth cone collapse, angiogenesis, and reduced antiviral defense. Interestingly, granulomas in EBV-mediated nasopharyngeal cancers exhibit cells with intracellular amyloid deposits that do not correspond with the epithelial cells expressing EBER.[141]. However, EBV also secretes latent proteins LMP1 and LMP2A in exosomes and LMP1 is associated with CD63 in endosomes.[142,143]

In 1979, Renvoize and colleagues reported finding significantly higher EBV DNA in peripheral blood leukocytes from AD patients than age-matched control, p=0.002.[144] Acute EBV infection is associated with reduced dendritic cells.[145] and AD is associated with decreased dendritic cell number.[146] Recently, Wyss-Coray's laboratory reported finding clonally expanded T cells that target EBV antigens in brain lesions from AD patients.[147] Moreover, B cells isolated from AD patients are infected with EBV and transformed into indefinitely proliferating cell lines significantly more efficiently than B cells from healthy controls.[148] This suggests that AD risk is related to EBV entry receptor allotypes that increase EBV infection vulnerability. Consistent with a role for EBV in AD, CD35, and HLA-DRB1 gene variants are significantly associated with AD risk.[149,150] Notably, individuals with HLA-DRB1 antigen 13 have increased EBV seropositivity[151], while healthy women harboring a single nucleotide variant, HLA-DRB1*13:02, have stable gray matter volume with age compared to woman that do not.[152]

In summary, EBV is equipped to produce non-cell autonomous neurodegeneration consistent with AD pathology. EBV is already associated with diverse autoimmune syndromes, such as multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, systemic vasculitis, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, and celiac disease.[153–157] Diagnosis of one these diseases significantly increases dementia risk.[158–161]

A_2-adrenergic destabilization and norepinephrine

Normal cell surface density of α_2-adrenergic receptors depends on Sorla and APP. Sorla associates with APP at its C-terminal end and restricts APP transport to the Golgi or cell

membrane. Without Sorla, APP is endocytosed and merges with an early endosome where it is cleaved by β- and γ-secretase to produce Aβ. Norepinephrine-mediated activation of α_2-adrenergic receptors changes APP localization by disrupting Sorla and APP association.[162] APP associates with activated α_2-adrenergic receptors, stabilizing the α_2-adrenergic receptor at the cell surface, thereby preventing arrestin 3 internalization and inhibitory feedback desensitization.[163,164] The internalization of presynaptic inhibitory α_2-adrenergic receptors desensitizes feedback control, increasing norepinephrine release.[164] Accordingly, cell surface α_2-adrenergic receptor density is significantly decreased in AD brain compared to age-matched control.[165]

One-way adrenergic neurons modulate activity is by regulating vascular tone. Norepinephrine binds α-adrenergic receptors on smooth muscle cells and pericytes to induce vasoconstriction and binds β-adrenergic receptors to induce vasorelaxation. The internalization of norepinephrine-mediated postsynaptic α_2-adrenergic receptor signaling in pericytes could result in chronic vasodilation, which would explain the depressed baroreflex in AD patients.[166,167] On the hand, persistent norepinephrine-mediated postsynaptic α_1-adrenergic receptor signaling can cause smooth persistent muscle cell vasoconstriction.

Aβ deposits are a defining pathological feature in AD brains. Norepinephrine activated β_2-adrenergic receptor induces cAMP-mediated APP gene expression and amyloidogenic processing in astrocytes.[168–170] Increased norepinephrine mediated α_1-adrenergic receptor signaling stimulates glutamate and ATP release from astrocytes. Activation of α_2-adrenergic receptors upregulates calcium oscillations in astrocytes, which increases the release of the inhibitory neurotransmitter GABA. GABA in turn reduces calcium oscillation in neurons.[171] However, APP deficiency would decrease α_2-adrenergic receptor stabilization at the astrocyte cell-surface resulting in reduced norepinephrine-mediated GABA release. Accordingly, mice expressing a homozygous loss-of-function APP mutation show electrophysiology consistent with GABAergic deficit.[172] Epidemiology studies that show an association between benzodiazepine use and dementia may actually result from selection bias, wherein patients use benzodiazepine because it provides relief from GABA deficiency. GABA is also involved in regulating adult neurogenesis and neuritogenesis.[173,174] Thus, loss of tonic GABA release from astrocytes would also explain reduced neurogenesis in the AD dentate gyrus.[175]

Neurons and astrocytes express the ionotropic NMDA receptor (NMDAR). Aβ can bind to NMDARs and activate calcium influx.[176] In neurons, sustained intracellular calcium can induce long term depression, leading to neurite loss.[177] A higher intracellular calcium concentration increases intracellular chloride. Increased intracellular chloride causes chloride efflux in GABA-activated chloride channels, resulting in depolarization. This situation resembles development, wherein GABA from inhibitory interneurons acts as an excitatory neurotransmitter, stimulating neurite outgrowth.[178] This could explain the observance of abnormal neurite outgrowth surrounding Aβ deposits in AD brain. The activation of neuronal α_1- and α_2-adenergic receptors reduces the NMDAR-mediated excitatory post-synaptic potential (EPSP) amplitude but not the paired pulse response.[179,180] In neurons, Aβ oligomers may bind allosterically to α_2-adenergic receptors to enhance norepinephrine-mediated G-protein activation.[181] Decreased expression of regulator of G-protein signaling 2 (RGS2) in AD brain would potentiate decreased NMDAR EPSP.[182] Thus, increased norepinephrine and Aβ-mediated α_1- and α_2-adrenergic receptor activation could reduce neuronal EPSP amplitudes, which could affect synaptic maintenance.

Norepinephrine is a chemokine for macrophages and switches cells to the anti-inflammatory M2 phenotype. Norepinephrine can also suppress NK cell activity and dendritic cell maturation. Moreover, norepinephrine activates corticotropin-releasing hormone cells to increase

HPA mediated cortisol release.[183] Thus, herpesviruses make the host more hospitable by upregulating norepinephrine.[184] In summary, deficient APP-mediated α_2-adrenergic receptor stabilization causes (1) increased norepinephrine, (2) reduced astrocytic GABA, (3) reduced neurogenesis, (4) reduced EPSP amplitudes, and (5) immunosuppression.

Immune dysfunction and inflammation

AD is associated with biomarkers of early immune dysfunction.[185] Latent and active herpesvirus-infected cells secrete viral factors that impair intrinsic and extrinsic immunity. HCMV secretes 14 viral proteins, many of which function to alter immunity. For example, the cmvIL-10 protein is a homolog of human interleukin-10 (hIL-10) expressed during replication.[186] Human interleukin-10 signaling, through its receptor IL10R1 on leukocytes, reduces antigen presentation, immune stimulation, and T cell and NK cell response. A truncated version, LAvIL-10, is expressed during latency.[187] Both LAvIL-10 and vIL-10 function to suppress the immune response and are detectable in serum from healthy HCMV seropositive donors using sandwich ELISA.[188] EBV also secretes a homolog of hIL-10 during active infection, ebvIL-10. EbvIL-10 shares 70% amino acid sequence with hIL-10 but is less efficient at downregulating anti-inflammatory gene expression. EBV also secretes EBER1 and EBER2 during latency and replication which increases hIL-10 expression and thus immunosuppression.[189]

Herpesviruses express proteins that mimic human chemokine receptors. For example, HCMV secretes the soluble protein pUL21.5 that functions as a decoy receptor for the broad chemoattractant RANTES; blocking RANTES from binding to its cellular receptor and inducing chemotaxis.[190] HCMV also encodes additional chemokine receptors, including UL33, UL78, US27, and US28. HHV-6 expresses the transmembrane receptors U12 and U51 that bind multiple cytokines, reducing immune infiltration, and in the case of U51, downregulating RANTES expression.[191] Inhibiting chemokine signaling thwarts an antiviral immune response.

In addition to expressed factors, herpesviruses also release capsids types A and B, as well as dense bodies, without viral DNA. The capsids and dense bodies are full of viral proteins and RNA that induce immunosuppression. For instance, HCMV dense bodies consist of 95% pp65 protein, which can block the activation of interferon response factor 3, to preemptively evade antiviral control.[192,193] Controlling herpesvirus infections takes its toll on the immune system. For instance, HCMV seropositive adults have significantly fewer naïve T cells and a significantly higher frequency of terminally differentiated effector memory (EMRA) CD8+ and CD4+ T cells than age-matched seronegative adults. Furthermore, advanced age is associated with reduced naïve T cells and more EMRA in HCMV seronegative adults.[194] The EMRA phenotype is associated with immunosenescence and inflamm-aging.[195] In summary, herpesvirus infections undoubtedly compromise immunity in aged subjects, increasing susceptibility to new opportunistic microbes and reactivation of dormant microbes.[196,197] Accordingly, AD is associated with numerous microbial infections, including human papillomavirus, C. pneumonia, T. pallidum, and P. gingivalis.[198–201]

Insulin deficiency

AD is associated with reduced CNS glucose utilization and advance glycation end-products (AGE).[202,203] Optimal glucose utilization in the brain requires insulin.[204–206] Insulin is thought to be an important neurotrophic factor and is involved in regulating metabolism, learning, and memory.[207] CNS insulin is obtained from local (astrocytes) and the peripheral (pancreas) sources. Norepinephrine increases adrenergic signaling which alleviates hypoglycemia by increasing glucose production and preventing local insulin release in the CNS.[208,209] Norepinephrine also inhibits insulin release from the pancreatic β cells.[210] Insulin

secretion from β cells is also decreased with advancing age.[211] Thus, increased norepinephrine in AD could reduce systemic and cerebral insulin release, resulting in hyperglycemia.[61] Hyperglycemia increases AGE formation. AGE binds the receptor for advanced glycation end-products (RAGE) and induces oxidative stress and inflammation.[212,213] RAGE signaling also induces GSK3-mediated tau phosphorylation.[214] Prolonged hyperglycemia could produce a state of persistent insulin resistance through epigenetic or post-translational modification.[215] In summary, insulin deficiency and impaired glucose utilization can cause cognitive impairment, and RAGE activation maintains injurious proinflammatory signaling that is active in AD.

Vascular pathology and hypoxia

All Alzheimer's disease brains have vascular pathology.[140,216] In 1997, Miyakawa reported electron microscopy findings on vascular pathology in AD brains and emphasized the "disturbance of microvessels".[217] Increased amyloidogenesis in microvessel walls increases inflammatory cytokines, which leads to prothrombotic protein expression, such as increased platelet tissue factor and decreased thrombomodulin.[49] Approximately 80% of AD patients have some degree of cerebral amyloid angiography (CAA) characterized by Aβ deposits in small arterials.[218] CAA narrows the vascular lumen and is associated with hemorrhagic bleeds, microinfarcts, and white matter pathology. Aβ aggregates are also found among degenerating microvessels.[217] At the same time, angiogenesis is upregulated in the hippocampus and proangiogenic factors are increased.[140,219]

Alphaherpesvirus infected adrenergic neurons release Aβ and virions at hippocampal vessels and cells causing inflammation and infection, respectively. Inflammatory signaling can increase vasoconstriction by activating the renin angiotensin system.[220] Congruently, HSV2 IgG seropositivity is significantly associated with hypertension risk.[221] Age-related loss of estrogen reduces β_1- and β_3-adrenergic receptor expression leading to decreased vasodilation.[222] Consequently, menopause is associated with a two-fold increase in hypertension risk.[223] Likewise, vasoconstriction increased by two-fold in ovariectomized rats.[224]

AD patients have significantly higher activated factor VII, von Willebrand factor, fibrinogen, and fibrin levels in their blood compared to age-matched controls.[118–121] In addition, herpesvirus antigens and Aβ can activate the complement system, explaining the presence of C3a and C5a in AD blood.[225] Upregulation of cell adhesion molecules in activated endothelial cells increases neutrophil attachment and aggregation that can occlude microvessels.[226] Occluded microvessels collapse and degenerate, resulting in chronic local hypoxia. Microvessel thrombosis can cause silent microinfarcts. The number of microinfarcts in AD brains could be in the hundreds to thousands and are currently only visible using high-field-strength MRI.[227] Accordingly, reduced cerebral blood perfusion is an early sign of AD.[228] Similarly, herpesvirus-infected endothelial cells in the glymphatic and meningeal lymphatic system can become occluded, preventing metabolic waste and Aβ disposal.[229]

Hypoxia can cause tau hyperphosphorylation and increase Aβ deposits.[114,230,231] Additionally, entorhinal cortical cells are highly sensitive to hypoxia.[232] A mitigating multigenic response to hypoxia is mediated by the hypoxia inducible factor alpha 1 (HIF-1) system. However, the HIF-1 response may be dysfunctional in the elderly since HIF-1 hypoxia-response element binding is deficient in senescent mice.[233] In summary, herpesvirus-mediated vascular pathology can result in ischemic brain injury, leading to cognitive impairment.

APP and Sorla

Herpesvirus-mediated disruption of APP and Sorla function can have severe consequences. APP regulates the localization of nerve growth factor (NGF) receptor, tropomyosin receptor kinase A (TrkA).[234] APP deficiency reduced TrkA at the cell surface. Moreover, misprocessed APP prevents retrograde NGF endosomal trafficking. Reduced NGF signaling causes neurite outgrowth collapse and cholinergic neuron degeneration. APP also binds and regulates activity of the high-affinity choline transporter (CHT). The CHT recycles choline back into the presynaptic terminal and is the rate-limiting step for cholinergic neurotransmission.[235] APP also stabilizes the iron efflux pore, ferroportin, at the cell surface.[236] Reduced cell-surface ferroportin increases intracellular iron stores and cell death by ferroptosis.[238] Reduced ferroportin in macrophages can cause the iron-deficiency anemia associated with AD[237].

Sorla regulates the trafficking of brain derived neurotrophic factor (BDNF) receptor (TrkB).[239] And BDNF signaling regulates tau phosphorylation and localization in neurites.[240] Reduced hippocampal volume and memory impairment is associated with lower BDNF levels with age.[241] Also, herpesvirus-mediated reduction in the neurotrophin receptors, TrkA and TrkB, is compounded by the age-related decline in their ligands.[242] In summary, substantial AD neuropathology can result from herpesvirus-mediated disruption of APP and Sorla function.[243]

Summary

Occult neurotropic alphaherpesvirus infection of adrenergic neurons in the LC could be the gateway infection to herpesvirus-mediated AD pathogenesis. Multiple herpesvirus types encode factors that induce endocytic dysfunction by interacting with endosomal proteins like BicD1 or APP processing factors, or downregulating Sorla or APP expression. The absence of APP in α_2-adrenergic neurons increases norepinephrine release and decreases astrocytic GABA release. Increased norepinephrine causes vasoconstriction, decreased ESPS amplitude, immunosuppression, and decreased insulin signaling and glucose utilization. Inflammatory cytokines, viral mimics, and norepinephrine can target the reactivation of multiple herpesvirus to areas of adrenergic innervation. APP and Sorla deficiency reduce NGF and BNDF neurotrophic signaling, respectively, resulting in reduced plasticity and survival. APP deficiency also decreases high-affinity choline transporter and ferroportin function, resulting in reduced acetylcholine availability and iron dyshomeostasis, respectively. Furthermore, herpesvirus-mediated systemic immunosuppression increases vulnerability to opportunistic infections. Aging is the largest AD risk factor. Aging increases AD risk by increasing herpesvirus reactivity, vasoconstriction, insulin resistance, increased cholesterol, and immunosenescence. An age-related increased in FOXO3a transcription factor binds the FOXO binding sites iP1 and iP2 in the major immediate early promoter to reactivate the virus. Notably, it appears that AD can be mediated by a relatively small number of herpesvirus-infected cells that secrete viral proteins to produce non-cell autonomous neurodegeneration.[244] Thus, an absence of herpesvirus DNA in AD brain could result because the reverse transcriptase reaction may be beyond detection limits in the presence of significant background RNA.[245] In conclusion, alphaherpesvirus, in combination with a betaherpesvirus (HCMV and HHV-6/HHV-7) and the Epstein-Barr virus, are likely to play a major role as the non-genetic driver of AD.