Understanding Your Risk for Late-Onset Alzheimer's Disease:

The relationship between the SHARPIN R274W Variant and APOE e4

Thais Nisenbaum[1* †], Rachel Battle[1†], Sarah Macon[1†]

Department of Biotechnology, Advanced Academic Programs, John Hopkins University

Baltimore, Maryland, USA

*Corresponding author. E-mail: tgallon1@jhu.edu ORCID: 0009-0007-2850-0313

Contributing Authors: rbatte1@jhu.edu ORCID: 0009-0009-1788-7609; smacon3@jhu.edu

† These authors contributed equally to this manuscript

Acknowledgments:

We would like to thank Sijung Yun, Ph.D. (syun16@jhu.edu) and Sajung Yun, Ph.D. (syun15@jhu.edu) for guidance in completing this paper.

Abstract

The SHARPIN (SHANK-associated RH domain interactor) Arg274Trp variant, which has been associated with late-onset Alzheimer's Disease (LOAD) in the Japanese population, plays a crucial role in mediating inflammatory responses through the regulation of the nuclear factor-κB (NF-κB) pathway. Alterations in gene expression have been associated with the pathogenesis of various diseases, including Alzheimer's disease (AD). SHARPIN has been linked to AD through the regulation of amyloid-β (Aβ) phagocytosis and NLRP3 expression, resulting in the accumulation of Aβ and neuron death. In this study, we analyzed the clinical, functional, and biochemical consequences of the SHARPIN R274W variant in the ubiquitin-like domain (UBL) and explored the regulatory regions of the gene. We also examined whether individuals carrying the SHARPIN R274W variant also had the APOE Cys112Arg variant, which is the strongest risk factor for the development of LOAD. Our preliminary analysis revealed that the SHARPIN Arg274Trp mutation may play a role in Alzheimer's Disease independently of APOE e4. Further analysis of LOAD patients across different populations may help to clarify the relationship between these two variants in Alzheimer's Disease. Overall, our findings contribute to a better understanding of the genetic risk factors for LOAD and may have important implications for the development of future therapies.

Keywords: Alzheimer's, SHARPIN, LOAD, ApoE4, NF-κB signaling pathway, ubiquitination, LUBAC

Introduction

Alzheimer's Disease

Alzheimer's disease (AD) is the most common type of dementia that affects a person's memory, thinking, and behavior. There are two types of AD: early onset, where symptoms begin before the age of 65, and late onset, where symptoms start after age 65. Late-onset Alzheimer's Disease (LOAD) is the most common form of AD. The most common symptom is memory loss. As things progress, individuals can develop symptoms such as having challenges with daily tasks, solving problems, and even speaking or writing. They can also face difficulties with understanding time or space and even suffer from changes in mood and behavior, leading to withdrawal from activities and work [1, 16]. When the disease has progressed significantly, individuals will show the most symptoms. These symptoms arise because neurons and cells in the brain are being destroyed. This occurs when there is a buildup of beta-amyloid 42, a protein found between neurons in the brain. The beta-amyloid 42 form is particularly toxic, and in individuals with Alzheimer's disease, it can be produced in abnormal levels that clump together and form plaques between neurons. The buildup of these plaques disrupts cell function. In addition to the beta-amyloid 42 plaques, neurofibrillary tangles, and protein tau clumps form inside the neurons that block the neuron's transport system [23]. When the neurons in the brain can no longer communicate with each other, the symptoms of Alzheimer's become

present. As the destruction of neurons progresses, they begin to destroy the rest of the brain, and the symptoms of Alzheimer's get worse.

ApoE4

The apolipoprotein E type 4 (ApoE4) allele is one of three major apolipoprotein E types [21] ApoE2 and ApoE4 have been associated with LOAD, while ApoE3 remains neutral [21] ApoE2 has been shown to be protective, while ApoE4 has been shown to increase the risk of developing LOAD [20,21]. The difference between the three variants falls on two amino acids. ApoE2 has the mutation Arg158Cys, ApoE3 has Cys112 and Arg158 and ApoE4 has the mutation Cys112Arg [20,21,26]. This amino acid change has been linked to "two key properties, domain interaction and reduced protein stability that likely contribute to ApoE4-associated neuropathology" [20]. When the protein folds, the Arg-112 in the N-terminal domain ApoE4 causes the side chain Arg-61 in the same domain to bend away from the helix and interact with Glu-255 of the C-terminal domain. This doesn't occur in ApoE2 or 3. This domain interaction has been known to mediate the effects of ApoE4, such as increasing amyloid-beta production, amyloid-beta-induced lysosomal leakage, and enhancing proteolytic cleavage in neurons, all of which have been associated with LOAD [20,23]. Another study determined that individuals with ApoE4 had reduced glucose metabolism in the same areas of the brain as individuals with probable LOAD [27,30]. The full mechanism in which ApoE4 acts is not yet fully understood, but it is apparent that having the ApoE4 allele increases one's risk of developing LOAD.

SHARPIN

The SHANK-associated RH domain interactor (SHARPIN) was initially found as a post-synaptic protein that co-localizes with Shank family proteins (SHANK1) at excitatory synapses in mature neurons [2,33]. The gene itself contains nine coding regions (Figure 1), while the C-terminus of the Sharpin protein interacts with Shank, and the N-terminus mediates homomultimerization [1]. Further information about the locations of the exons and introns of SHARPIN can be found in *Supplemental Table 3*. The SHANK-associated RH domain interactor gene is part of the LUBAC (linear ubiquitin chain assembly complex) along with RBCK1 (HOIL-1L) and RNF31 (HOIP) [2,33].

Figure 1 The SHANK-associated RH domain interactor (SHARPIN) gene, located on the complementary strand of chromosome 8 (see arrow), contains 9 exons with 8 coding exons. mRNA transcript shows the boundaries of the 5' and 3' UTRs (indicated by the gray color) and the coding region within the gene spanning 8 of the 9 exons

LUBAC is an E3 ubiquitin ligase responsible for targeting proteins for proteasomal degradation. The C-terminal of SHARPIN contains the Ubiquitin-like domain (UBL) and the Npl4-zinc finger domain (NZF). The UBL transfers linear ubiquitin chains to proteins and targets them for proteasomal degradation, while the Npl4-zinc finger domain (NZF) interacts with LUBAC subunits [35]. As part of LUBAC, SHARPIN is involved in protein polyubiquitination and regulation of the nuclear factor-κB (NF-κB) pathway [33,38]. The polyubiquitination of NF-κB Essential Modulator (NEMO/IKBKG) and RIPK1 activates signal transduction of the NF-κB pathway and the JNK signaling pathway [33]. NF-κB is a transcription factor that regulates the activation and differentiation of immune cells and plays a role in cellular growth and apoptosis. The polyubiquitination of NEMO allows NF-κB to translocate to the nucleus through the canonical NF-κB pathway [12]. The ubiquitination mediated by the LUBAC complex interferes with TNF-induced cell death reducing inflammatory response [33] The JNK pathway is involved

in cellular growth and apoptosis and is associated with neurodegeneration and tumor progression [4]. SHARPIN is often upregulated in several cancer types and is associated with melanoma progression by regulating Ras-associated protein-1(Rap1), which mediates the JNK signaling pathway [41].

SHARPIN's primary role is associated with LUBAC, but it also functions independently of the complex [17]. The pleckstrin homology domain (PH), located at the N-terminal, is associated with the SHARPIN's functions independent of LUBAC. SHARPIN is thought to be associated with lymphocyte migration by binding to lymphocyte-function-associated antigen-1 (LFA-1), resulting in the inhibition of intercellular adhesion molecule-1 (ICAM-1)-mediated lymphocyte adhesion. Additionally, SHARPIN is associated with platelet function by binding to integrin αIIbβ3 and preventing the formation of blood clots [1].

SHARPIN is highly conserved across species and is crucial in mediating inflammatory responses by interacting with different proteins and regulating signaling pathways. All variants identified by NCBI and Ensembl can be seen in *Supplemental Table 2*. Alterations in SHARPIN expression are associated with the pathogenesis of various diseases. One alteration is dbSNP rs77359862.

This SNP has been previously shown to increase the risk of LOAD, just as ApoE4 has been shown to do [2]. Currently, there are two published studies based on an Asian population associating the SNP of interest with LOAD and AD [2,29]. While studies have not explicitly looked at whether this SHARPIN variant works with ApoE4, one study found that another SHARPIN variant, specifically dbSNP rs572750141, works independently of ApoE4 [3]. We believe that if one SHARPIN variant works independently of ApoE4, it is highly like that most, if not all, including our variant, work independently of ApoE4.

The newly discovered SHARPIN variant dbSNP rs77359862 and the ApoE4 variant both have been shown to increase a person's risk of getting LOAD, however, we believe they work independently from each other. As such, we assume an individual with both mutations would have a greater risk of developing LOAD.

Materials & Methods

Biomart

A Biomart query for the SHARPIN gene (gene stable ID: ENSG00000179526) using the Ensembl Variation 109 database and Human Short Variants (SNPs and indels excluding flagged variants) (GRCh38.p13), filtered to the dbSNP database as a source, resulted in 2,339 results, 43 of which are benign and the remaining 2,296 have no data. 2,292 SNPs had no associated phenotype, and 11 were "ClinVar: phenotype not specified." *Supplemental Table 1* includes a list of SNPs identified to be associated with a phenotype, which consists of 36 results for 3 SNPs (rs12550729 (2), rs34173062 (32), rs77359862 (2)).

ENSEMBL

The population allele frequencies associated with dbSNP rs77359862 were determined according to the population genetics data available in Ensembl [7].

A total of 42 individuals containing the SHARPIN R274W mutation from the 1000 Genomes Project Phase 3 were identified across various populations. Individuals were then cross-referenced with the population genetics data for dbSNP rs429358 [6].

ENCODE

The Encyclopedia of DNA Elements (ENCODE) Project combines bioinformatic and laboratory methods to provide information on DNA elements. Histone modifications, or histone marks, are identified by chromatin immunoprecipitation followed by sequencing (ChIP-seq). The ENCODE project includes data from various regulatory elements such as H3K4Me1, H3K4Me3, and H3K27Ac. H3K4Me1 refers to the mono-methylation of lysine 4 of H3 histone, H3K4Me3 refers to the tri-methylation of lysine 4 of H3 histone, and H3K27Ac refers to the acetylation of lysine 27 of the H3 histone protein [9].

NGS Analysis in Galaxy

Eight individuals were selected for sequencing analysis in Galaxy. Alignment files from low coverage whole genome sequencing (WGS) data, sequenced as part of the 1000 Genomes on GRCh38, were obtained from the International Genome Sample Resource (IGSR) [154and used as input for the analysis. FreeBayes Bayesian, genetic variant detector, was used for calling the variants, and the results were limited to the SNP positions (rs77359862 – chr8: 144099379, rs429358 – chr19: 44908684); see Figure 2 for workflow [11,36,37].

Overview of Galaxy Workflow

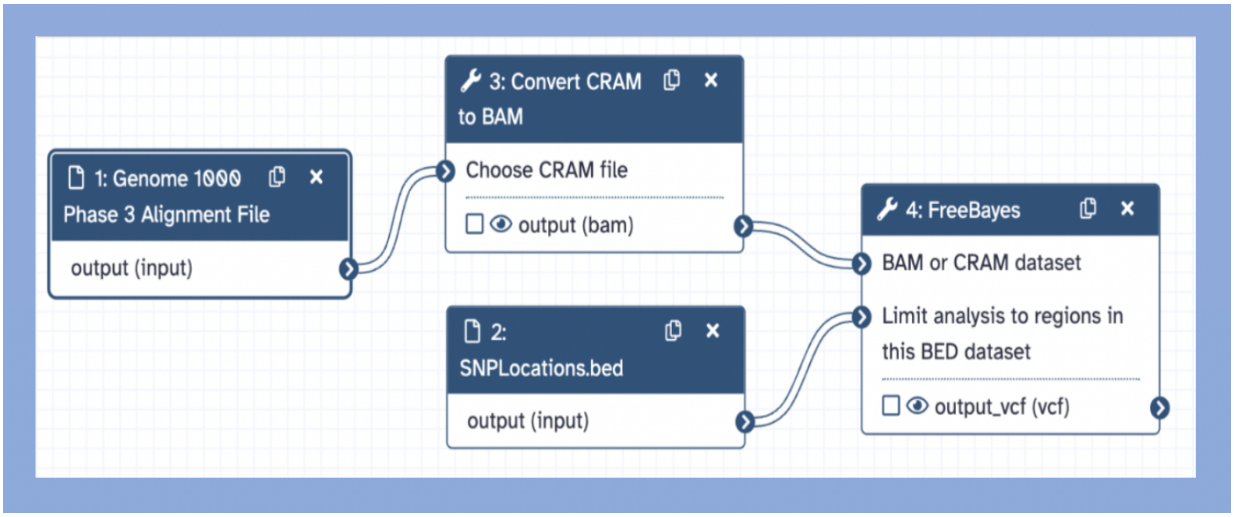


Figure 2 Overview of Galaxy workflow starting with the input data, conversion of CRAM to BAM file, and FreeBayes analysis

Data Visualization

UCSC Genome Browser

Layered H3K4Me1, H3K4Me3, and H3K27Ac data for 7 different cell lines are available in the UCSC Genome Browser for visualization (Figure 7). The different cell lines include GM12878 (lymphoblastoid cell line), H1-hESC (The human Embryonic Stem Cell (hESC) line H1), HSMM (Human Skeletal Muscle Myoblasts), HUVEC (Human umbilical vein endothelial cells (HUVEC), K562 (K-562 are lymphoblast cells isolated from the bone marrow), NHEK (Normal Human Epidermal Keratinocytes), and NHLF (Human Lung Fibroblasts) [9].

Integrative Genomics Viewer (IGV)

The H3K4Me1, H3K4Me3, and H3K27Ac data for HSMM, NHLF, NHEK, K562, H1-hESC, GM12878, and HUVEC cells were exported from the UCSC Genome Browser, using the "Table Browser" tool, and visualized in IGV (GRCh38) using annotations from RefSeq genes (Figure 7).

The BAM files generated in Galaxy, using the "Convert CRAM to BAM" tool, were exported and visualized in IGV (GRCh38) using annotations from RefSeq genes and All SNPs 1.4.2 (Figures 9A and 9B).

Results

SNP Geographical and Ethnic Expression

Overall, dbSNP rs77359862 has a MAF value of 0.01 [7]. According to almost all published studies, the SHARPIN variant, dbSNP rs77359862, is primarily found in East Asia/Asia/Asian with the highest alternate allele (G>A) frequency being 0.0492 [13,31]. Most studies separated East Asia/Asian, but even then, typically, East Asia/Asian had the highest frequency. Across Asia, the population in Kihn in Ho Chi Minh, Vietnam, has the highest allele frequency with a MAF of 0.0056. Only one study, the PAGE Study, showed another geographic area/ethnicity having a

higher frequency: Native Hawaiian, with a frequency of 0.0618 [31]. Given that East Asians/Asians have the highest frequency of the missense variant, next-generation sequencing data of the population allow researchers to further analyze the complications associated with dbSNP rs77359862.

GWAS Studies Associated with SHARPIN R274W

GWAS studies, GCST90095658 and GCST90095659, involving 2,643 Korean ancestry individuals, associate dbSNP rs77359862 with decreases in hippocampal volume and entorhinal cortical thickness, respectively [24].

Clinical Consequences of SHARPIN R274W

SHARPIN is thought to play a role in Alzheimer's disease (AD) pathogenesis, regulating amyloid-β (Aβ) phagocytosis and NLRP3 expression, which forms inflammasomes and activates AD [16]. AD is associated with the accumulation of Aβ and activation of microglia and macrophages as a response to inflammation. Macrophage polarization to an M1 phenotype is characteristic of AD, which occurs in response to inflammation and is a process mediated by NLRP3. Elevated Aβ is associated with a higher expression of SHARPIN, which polarizes macrophages, activates an inflammatory response, and ultimately results in neuron death [18]. However, low expression of SHARPIN leads to defects in the (Aβ) phagocytosis, which also leads to the accumulation of Aβ and causes neuron death [17].

Looking more specifically as to what within the SHARPIN gene leads to these defects, recent studies have found several SNPs within the SHARPIN gene that have been linked to AD; one is dbSNP rs77359862. Asanomi et al. (2019) reported the novel SHARPIN variant, dbSNP rs77359862, and compared it to other SHARPIN variants, such as dbSNP rs572750141. The novel SHARPIN variant (dbSNP: rs77359862) is a G > A single nucleotide variation, resulting in the amino acid substitution Arg274Trp (Figure 3). The arginine residue is in SHARPIN's Ubiquitin-like domain (UBL), which interacts with HOIP, a component of LUBAC. The UBL is responsible for transferring ubiquitin linear chains to proteins and targeting them for degradation [2]. In normal conditions, SHARPIN's polyubiquitination function is associated with activating the nuclear factor-κB (NF-κB) pathway [38]. As such, the missense variant Arg274Trp is associated with reduced activation of the NF-κB pathway and, therefore, changes in immune responses. Additionally, under normal conditions, SHARPIN is associated with the degradation of mutant Huntingtin, Ataxin-3, SOD1, and TDP-43, all of which are associated with neurodegenerative disease [16]. Furthermore, The Arg274Trp causes SHARPIN to localize in uneven granule clumps when compared to an even distribution associated with the wild type [2]. As described by Asanomi et al. (2022), the changes in immune responses due to this mutation are associated with an increased risk of late-onset Alzheimer's disease (LOAD). In a separate study by Park et al. (2021), the SHARPIN rs77359862 variant has been associated with entorhinal cortical thickness, hippocampal volume, and accumulation of amyloid-β, contributing to brain damage associated with Alzheimer's disease (AD) [29].

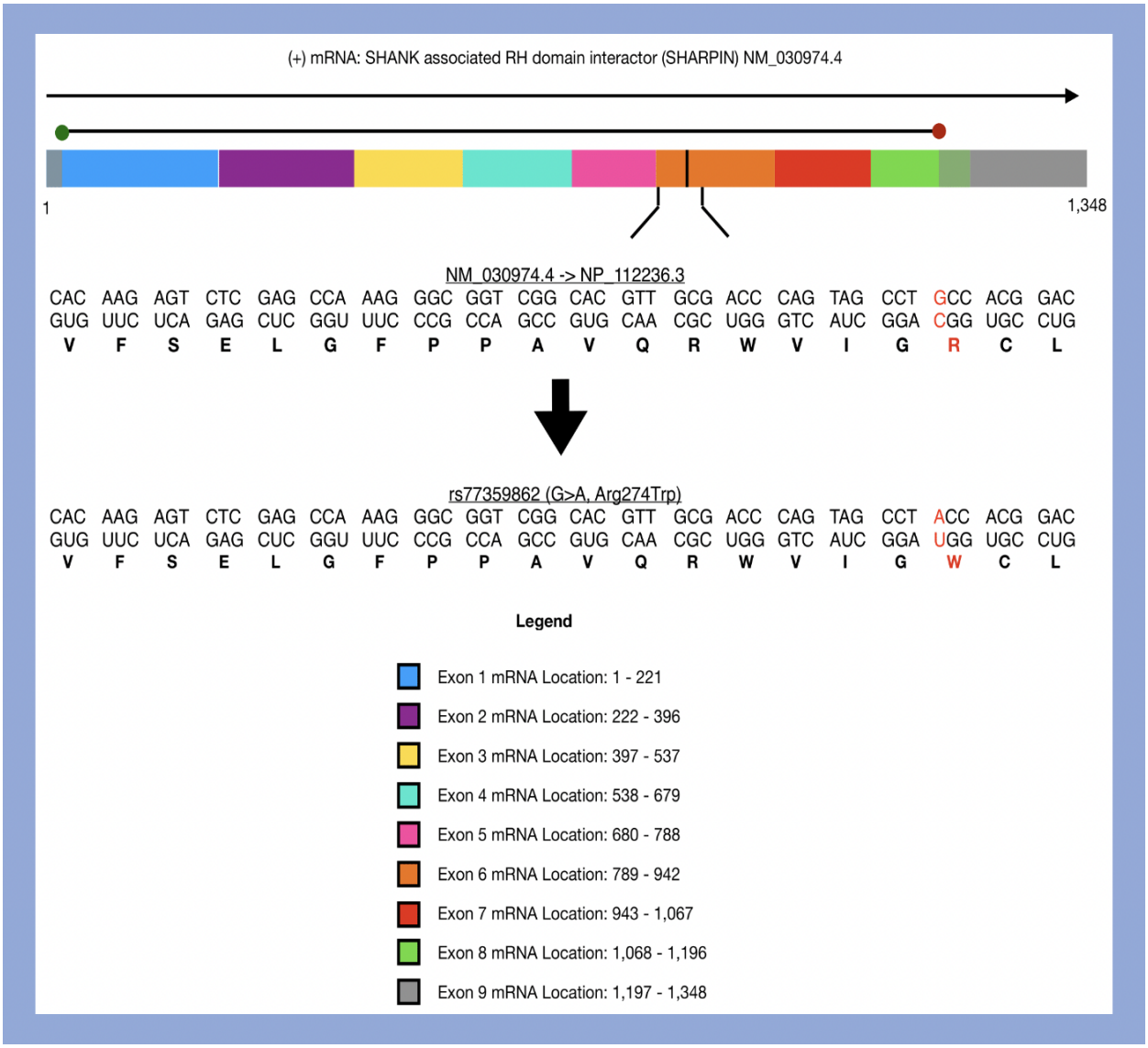


Figure 3 The SHANK-associated RH domain interactor (SHARPIN) mRNA transcript shows the boundaries of the 5' and 3' UTRs (indicated by the gray color) and the coding region within the gene spanning 8 of the 9 exons. The single nucleotide variation is located on the sixth exon at nucleotide 144,099,379 and amino acid 274. The figure shows the approximate location of the mutation, indicated by a black line. The mutation causes a substitution from Arg > Trp, which has been classified as a non-synonymous missense mutation. Comparisons between the DNA, mRNA, and protein sequences for the reference sequences and SNP can be seen, with the mutation location highlighted in red

Asanomi et al. (2019), the authors who reported the novel SHARPIN variant, compare dbSNP rs77359862 to dbSNP rs572750141, a previously reported SHARPIN variation also associated with an increased risk of LOAD. The rs572750141 variant results in the amino acid substitution Gly186Arg. The glycine residue at position 186 is highly conserved and is located near SHARPIN's UBL. Similar to Arg274Trp, the Gly186Arg mutation leads to reduced activation of the NF- κ B pathway and uneven granule clumps in the cytosol [3].

An additional SHARPIN variant (dbSNP: rs34173062) causing G/A/C allele changes has been reported in several studies. Soheili-Nezhad et al. (2020) associates this variant with bilateral entorhinal cortical thickness and heritability of AD. The mutation involves an amino acid change

from serine to phenylalanine in the protein's N-terminal (Ser17Phe) and affects the β1-integrin pathway [34]. The rs34173062 variant has also been studied by Olafsdottir et al. (2020), who report it to be associated with an increased risk of asthma and higher eosinophil counts [25]. Furthermore, a study conducted by Seymore et al. (2007) associates SHARPIN homozygous loss of function mutation with multiorgan inflammation, dysregulation of the immune system, and dermatitis in mice [32].

Functional Consequences of SHARPIN R274W

SHARPIN Domains

SHARPIN contains three domains that are crucial in understanding its implications in disease (Figure 4). At the N-terminal is the PH domain that is involved in integrin and tumor regulation but is not implicated with the dbSNP rs77359862. At the C-terminal is the NZF domain which can bind ubiquitin, but its structures are not yet understood. In the center is the UBL domain, which is key to understanding the functional consequences of dbSNP rs77359862. Located from 219-288, the UBL domain is involved in the formation of the LUBAC and can recognize the UBA domain of HOIP of LUBAC.

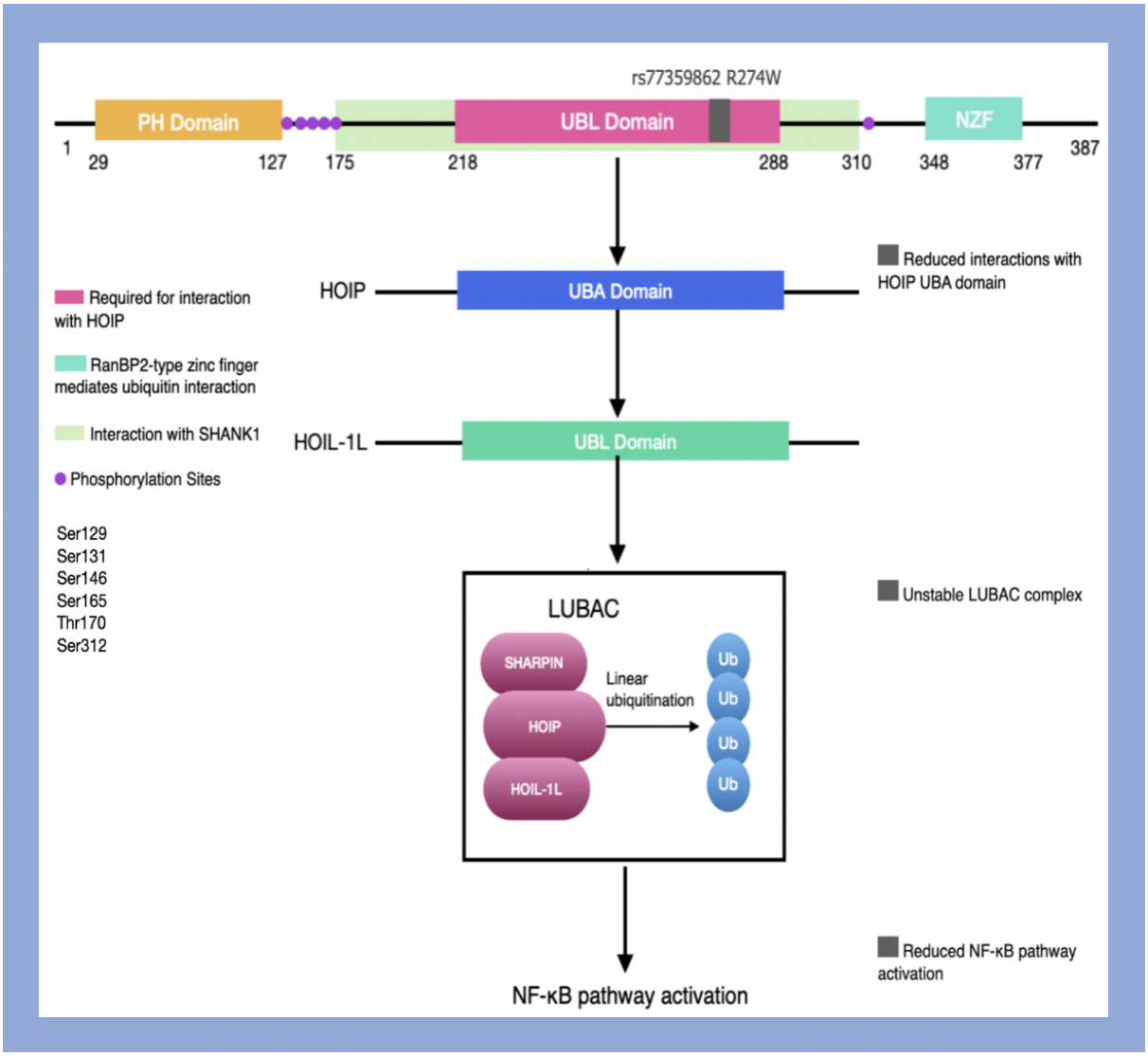


Figure 4 Interaction between the UBL domain of SHARPIN, UBA domain of HOIP, and UBL domain of HOIL-1 for the formation of the LUBAC complex. The effects resulting from the R274W variant located in the UBL domain of SHARPIN are demonstrated in grey on the right of the figure

Reduced interactions with the HOIP UBA domain

The reduction in interactions with the HOIL-1 interacting protein (HOIP) UBA domain has been determined to be a resulting factor of the amino acid change within the dbSNP rs77359862. This variant is located within the SHARPIN UBL domain, which mediates the binding of SHARPIN to HOIP. When the polar amino acid arginine is substituted with a hydrophobic tryptophan, the chemical properties of the change in amino acid affect the binding capability of the SHARPIN UBL domain. Park et al. (2021) determined that the interaction in the wild-type HOIPUBA-SHARPINUBL complex was made from ten hydrogen bonds and seven salt bridges. When they looked at the interaction in the mutant HOIPUBA-SHARPINUBL complex, they saw a reduction to four hydrogen bonds and zero salt bridges [29]. The introduction of a hydrophobic amino acid leads to a reduction in hydrogen bonds and salt bridges, ultimately leading to a weaker interaction. In addition to reducing interactions with the HOIP UBA domain, the dbSNP

rs77359862 could potentially impact the activation of HOIP. When alone, HOIP stays in an autoinhibited state (not activated). It isn't until the LUBAC complex is formed between HOIL-1L, SHARPIN, and HOIP that the autoinhibited state is released (becomes activated) [40]. The variant is in the UBL domain, which is required for SHARPIN's interaction with HOIP, and, therefore, could affect the release of HOIP's autoinhibition [40].

Unstable LUBAC complex

The stability of the linear ubiquitin chain assembly complex (LUBAC) depends on the interaction of SHARPIN, HOIP, and HOIL-1L. The interaction of the SHARPIN UBL domain, HOIP UBA domain, and HOIL-1L UBL domain form the ternary complex structure of LUBAC. Studies of this ternary complex structure found LUBAC-tethering motifs (LTMs) present between the HOIL-1L and SHARPIN subunits. These LTMs, when heterodimerized, cause the HOIL-1L and SHARPIN to form a spherical structure that helps maintain the stability of the HOIL-1L UBL and SHARPIN UBL domains, which in turn helps maintain the stability of the HOIP UBA domain. Maintaining the stability of all three domains individually helps maintain the stability of the ternary LUBAC structure and, thus, the LUBAC as a whole. One of the domains is altered, and that alteration affects the stability of that domain, the stability of the LUBAC is diminished [3].

Predicted Biochemical Consequences of the Arg to Trp Mutation

Our mutation of interest has been classified as an Arginine to Tryptophan transition on the 274 amino acid. Arginine (R) is an essential polar amino acid with a positively charged guanidino group. Tryptophan (W) is a non-polar amino acid with a large aromatic side chain. The difference between the two amino acid natures could explain the results seen in the paper by Asanomi et al. (2022), with the mutant protein organizing into uneven granule clumps in the cell's cytosol [2]. The mutant nature of clumping may be due to the difference in the hydrophobic-hydrophilic forces of the amino acids on the protein chain. In biological proteins, the hydrophobic effect has the greatest influence on protein stability and folding patterns in the cell. The hydrophobic effect, which causes non-polar substances to minimize their contact with water, is the major determinant of the native protein structure. The aggregation of non-polar side chains into the interior of a protein is favored because of the entropy of the water molecules to organize bonds around non-polar side chains. The hydrophobic residues tend to sequester together into the protein's interior during protein folding because this is the lowest energy state, thus, very stable. Arginine is a hydrophilic, polar side chain, and its nature as a hydrophilic amino acid affects protein folding, so it can interact with the cytosol. In contrast, tryptophan has a hydrophobic nature and tends to be on the inside of the protein. This hydrophobic interaction in the improper position may lead to the association and granule formation Asamomi et al. (2022) saw in the mutant protein [2]. Protein clumping and aggregation may be due to the tryptophan amino acids associated with each other forming a quaternary structure that acts to lower the energy state of the protein and imitate the interior protein environment for the tryptophan.

Arginine is also well-designed to bind the phosphate anion and is often found in the active centers of proteins that bind phosphorylated substrates. As a cation, arginine also could play a role in maintaining the overall charge balance of the protein. The activity of the arginine within the ubiquitin-like domain of the protein may stabilize the interaction with the LUBAC complex. Besides the possible change in the folding activity of the mutant protein, tryptophan could also be too large with its non-polar aromatic side chain to allow for proper interaction with the complex.

Reduced activation of the NF-κB pathway

The activation of the nuclear factor (NF)-κB pathway depends on linear ubiquitin chain conjugation. Reduction in the activation of the NF-κB pathway occurs as a direct consequence of substrate deficiency. SHARPIN is not able to produce linearized ubiquitin modifications, but it can activate LUBAC by binding to HOIP. The LUBAC complex plays a key role in conjugating linear ubiquitin chains to substrates. Deficiency in the substrates leads to a reduction of LUBAC in the NF-κB pathway. This prevents SHARPIN-mediated linear ubiquitination. Similarly, deficiency of SHARPIN can lead to inhibition of LUBAC-mediated linear polyubiquitination of substrates [3].

Regulation Regions of SHARPIN

The SHANK-associated RH domain interactor (SHARPIN) gene, located on the complementary strand of chromosome 8, has multiple regulatory regions identified by Ensembl (Figure 5A and *Supplementary Figure 1*). First, Ensembl identified a large promoter region with flanking promoter regions. The entire region is 6,597 bps long and located from CDS location 144,106,595 – 144,099,998. The identified promoter also has the transcription start site within its region located at 144,103,753 – 144,103,755. There are two enhancer regions identified between the 4th and 7th exon of the SHARPIN gene, each being about 200 bps long. The last regulatory region is the CTCF binding located downstream of SHARPIN, encompassing the 8th and 9th exons. The regulatory regions in the SHARPIN SNP rs77359882 can be seen in Figure B, which shows the expanded exon 5 through exon 7 region with the approximate location of the R274W mutation on exon 6. Below the gene is the enhancer and miRNA identified by Ensembl with the approximate location in association with the SHARPIN gene. The first is an enhancer located at CDS location 144,099,201 – 144,099,400, located within the site of rs77359862 mutation. The second is miRNA-3661 or has-miR-3661, located at the end of exon 6 at CDS location 144,099,273 – 144,099,298.

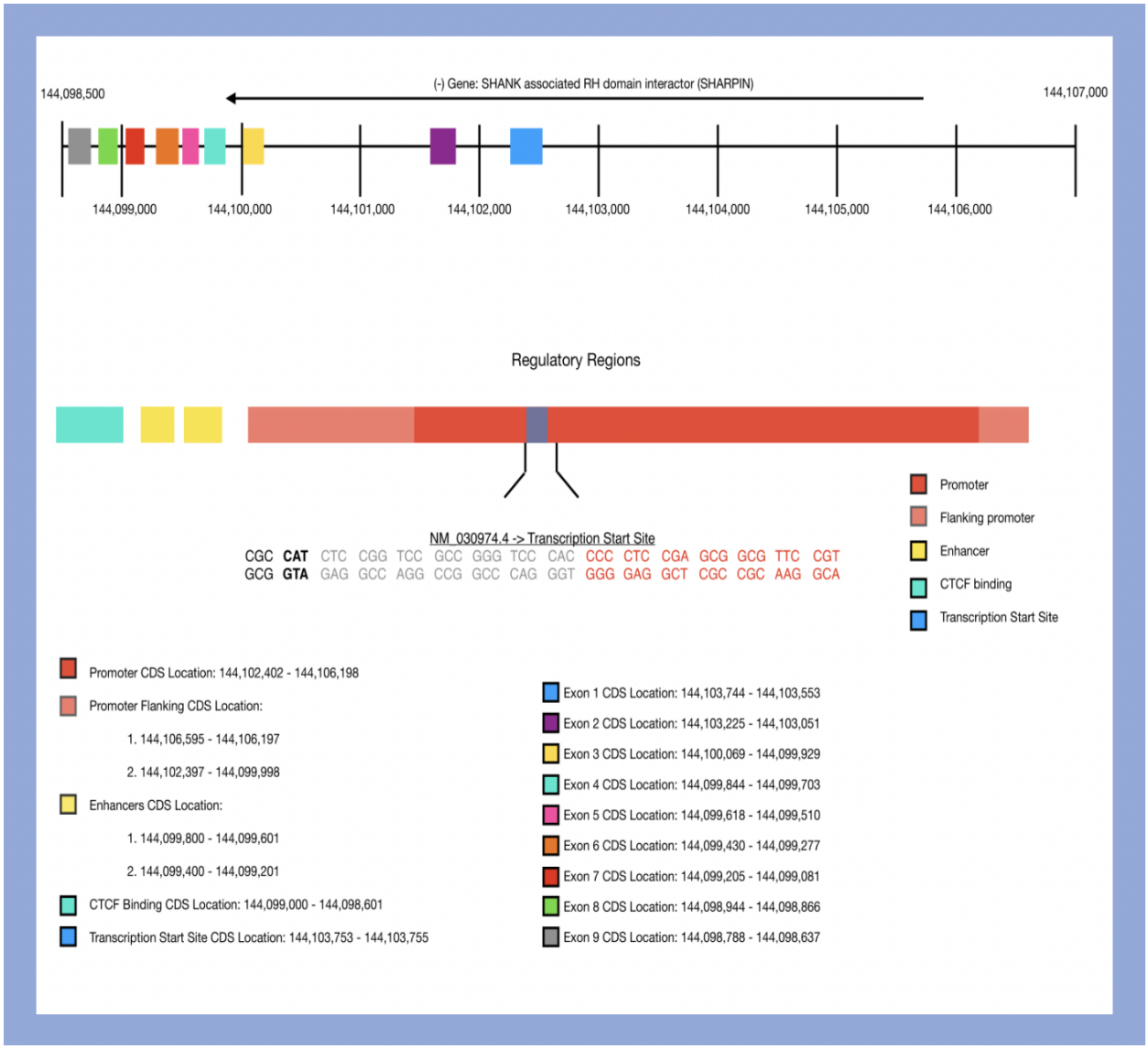


Figure 5a Graphical representation of the regulatory regions within the SHARPIN gene from ENSEMBL (ENSG00000179526) [5]

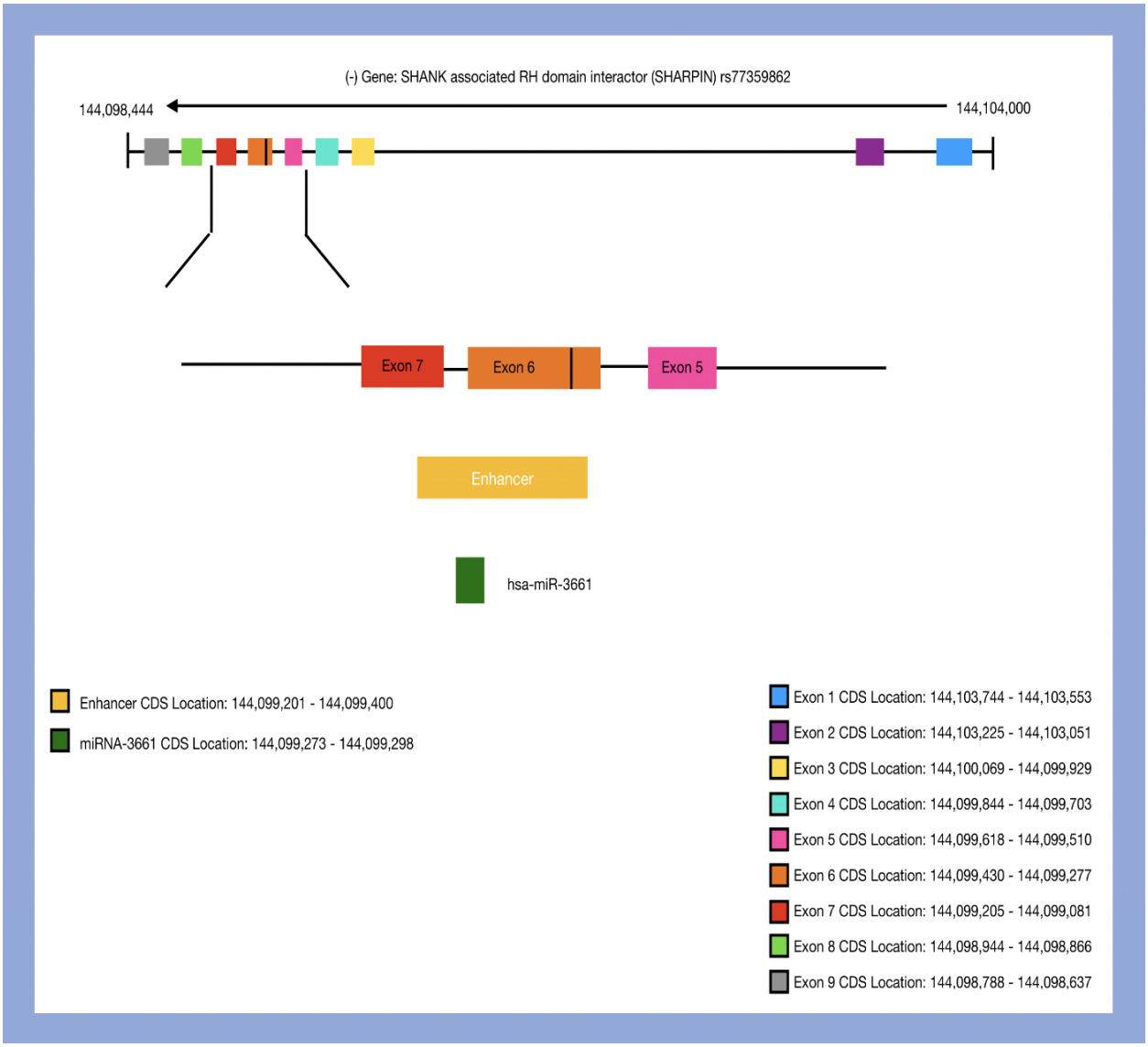


Figure 5b Graphical representation of the regulatory regions within the SHARPIN rs77359862 from ENSEMBL [7]

Histone Acetylation and Methylation in SHARPIN

Chemical modifications to histones, such as acetylation and methylation, regulate gene expression by opening and closing chromatin. Histone acetylation modulators include histone acetylases (HATs), which add acetyl groups to lysine residues in histone tails, and histone deacetylases (HDACs), which remove acetyl groups. The addition of an acetyl group to lysine neutralizes the positively charged lysine, and as DNA is negatively charged, this results in the opening of chromatin and activates transcription [14] Histone methylation adds one, two, or three methyl groups to lysine or arginine amino acid residues in histones. In general, histone methylation leads to transcription suppression but can result in transcription activation depending on which lysine or arginine gets methylated [39].

The layered H3K4Me1, H3K4Me3, and H3K27Ac tracks for GM12878, H1-hESC, HSMM, HUVEC, K562, NHEK, and NHLF associated with histone modifications in SHARPIN are seen in Figure 6.

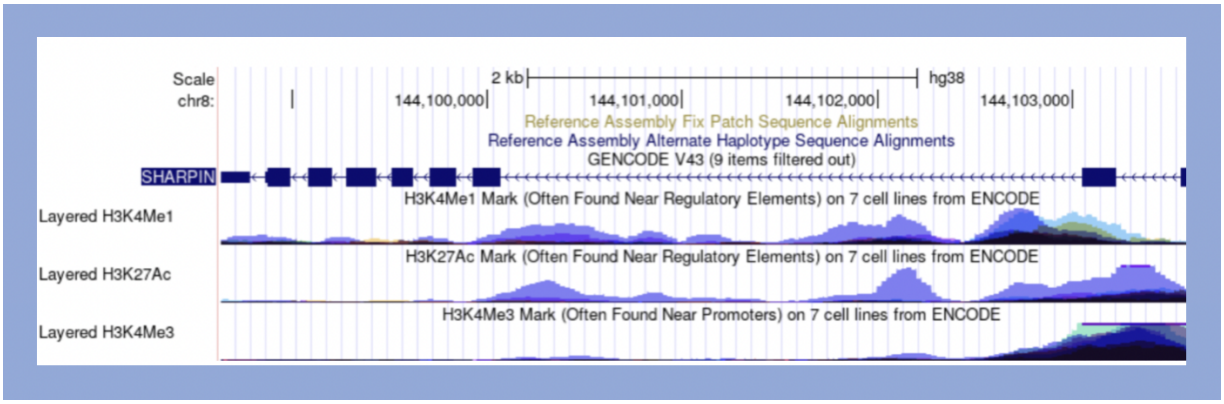


Figure 6 Layered histone modification data in seven cell lines from ENCODE tracks displayed in the UCSC Genome Browser

The H3K4Me1, H3K4Me3, and H3K27Ac tracks demonstrate areas associated with promoters/transcription start sites (TSS) and regulatory elements, such as enhancers. The TSS regions are denoted by enrichment in H3K27Ac and H3K4Me3, and regulatory regions are denoted by enrichment in H3K27Ac and H3K4Me1 (Figure 7) [8]. Regulatory regions around exon 6, where dbSNP rs77359862, are seen in H1-hESC cells.

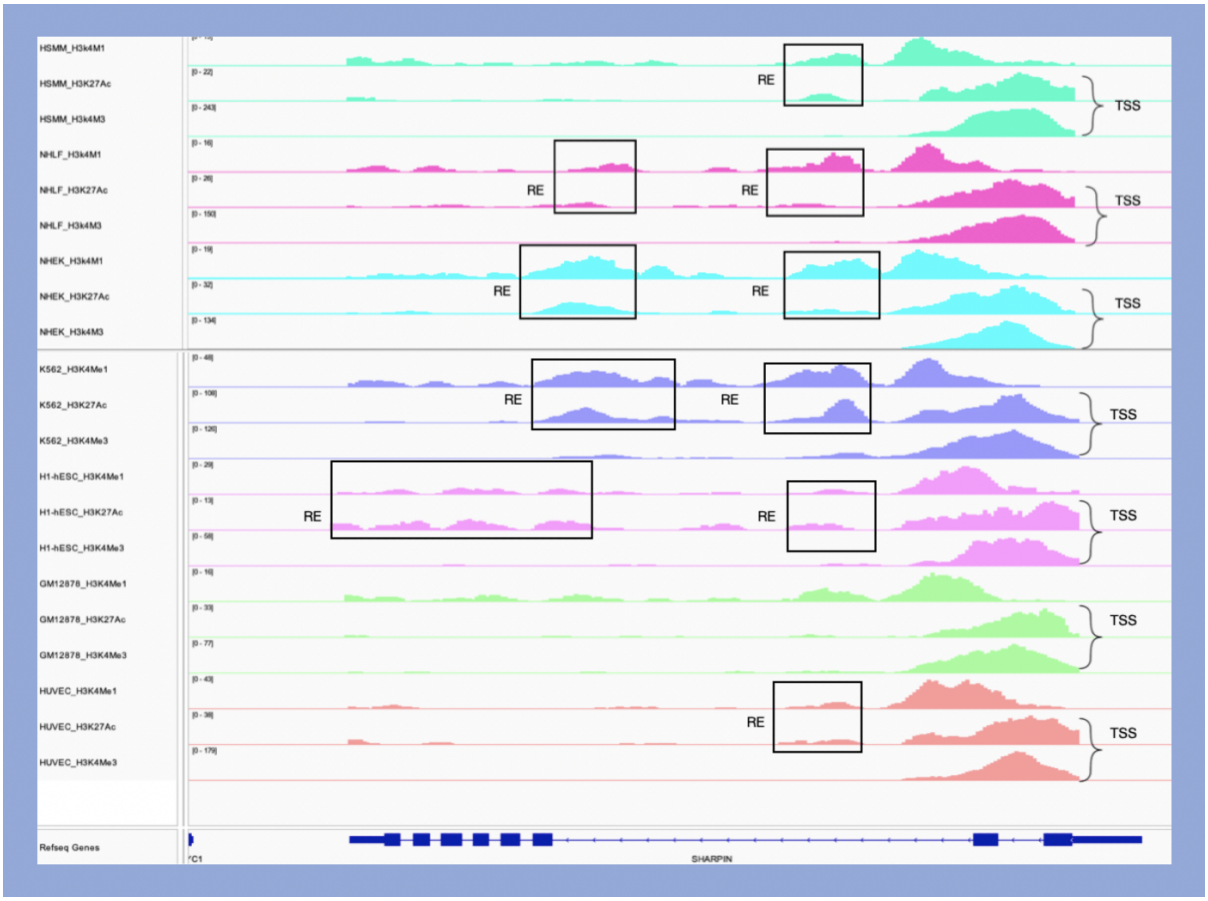


Figure 7 SHARPIN TSS and regulatory regions in HSMM, NHLF, NHEK, K562, H1-hESC, GM12878, and HUVEC cells displayed in IGV. RE = Regulatory element

Individuals containing only the SHARPIN Arg274Trp (rs77359862) mutation or both SHARPIN Arg274Trp (rs77359862) and APOE Cys112Arg(rs429358) mutations

A total of 42 individuals from the 1000 Genomes Projects Phase 3 contain the G>A mutation in SHARPIN, 40 of which are of East Asian ancestry, 1 of European ancestry, and 1 of American ancestry. Of the 42 individuals, 8 also contain the T>C mutation in APOE. A summary of these results is graphically represented in Figure 8. Figure 9 contains the list of individuals and their genotypes at positions chr8:144099379 and chr19:44908684.

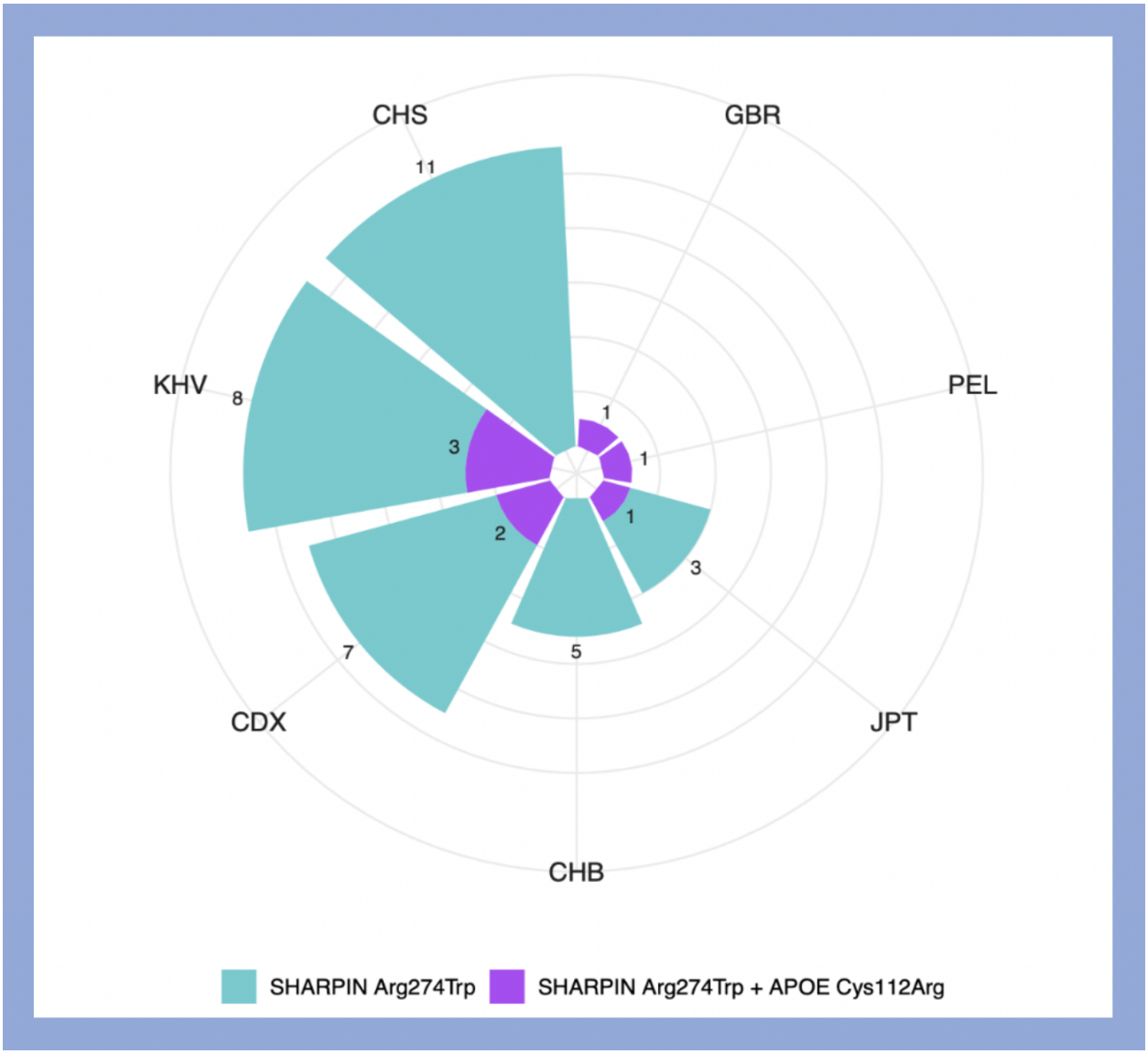


Figure 8 Summary of the number of individuals containing only the SHARPIN Arg274Trp (rs77359862) mutation or both SHARPIN Arg274Trp (rs77359862) and APOE Cys112Arg(rs429358) mutations separated by population

a. Han Chinese South, East Asian Ancestry (CHS)

Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype
HG00437 (F)	A|G	T|T
HG00464 (F)	A|G	T|T
HG00536 (M)	A|G	T|T
HG00557 (F)	G|A	T|T
HG00589 (M)	A|G	T|T
HG00657 (F)	A|G	T|T
HG00663 (F)	A|G	T|T
HG00671 (M)	G|A	T|T
HG00684 (F)	A|G	T|T
HG00693 (F)	A|G	T|T
HG00717 (F)	A|G	T|T

b. Han Chinese in Beijing, China, East Asian Ancestry (CHB)

Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype
NA18546 (M)	G|A	T|T
NA18547 (F)	G|A	T|T
NA18557 (M)	G|A	T|T
NA18627 (F)	A|G	T|T
NA18629 (M)	G|A	T|T

c. Chinese Dai in Xishuangbanna, China, East Asian (CDX)

Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype
HG01031 (M)	G|A	T|T
HG01046 (F)	G|A	T|T
HG01804 (F)	A|G	T|T
HG01812 (F)	G|A	T|T
HG01815 (F)	A|G	T|C
HG01817 (F)	G|A	T|T
HG02164 (F)	G|A	C|T
HG02187 (F)	A|G	T|T
HG02395 (M)	G|A	T|T

d. Japanese in Tokyo, Japan, East Asian Ancestry (JPT)

Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype
NA18939 (F)	A|G	T|T
NA18943 (M)	A|G	T|T
NA18944 (M)	G|A	T|C
NA18997 (F)	G|A	T|T

e. Kinh in Ho Chi Minh City, Vietnam, East Asian Ancestry (KHV)

Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype
HG01595 (F)	A|G	T|T
HG01848 (F)	G|A	T|C
HG01852 (M)	A|G	C|T
HG01863 (F)	G|A	T|C
HG01874 (F)	A|G	T|T
HG02028 (F)	G|A	T|T
HG02049 (F)	G|A	T|T
HG02067 (M)	G|A	T|T
HG02076 (M)	A|G	T|T
HG02113 (F)	G|A	T|T
HG02121 (F)	A|G	T|T

f. British in England and Scotland, European Ancestry (GBR)

Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype
HG00123 (F)	G|A	C|T

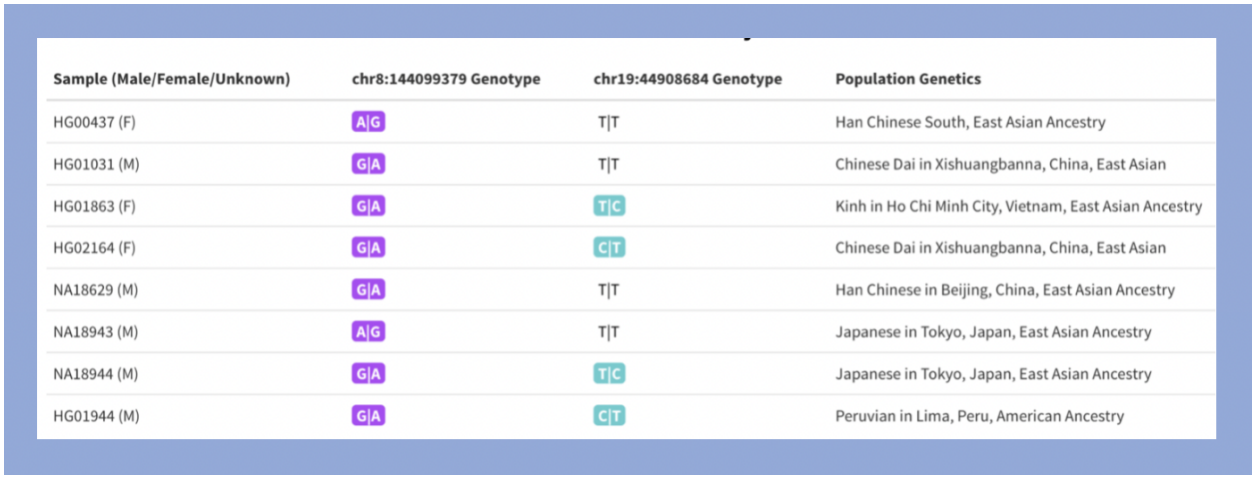
g. Peruvian in Lima, Peru, American Ancestry (PEL)

Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype
HG01944 (M)	G|A	C|T

Figure 9 List of individuals and their corresponding genotype at positions chr8:144099379 and chr19:44908684 separated by population. All selected individuals have the SHARPIN Arg274Trp variation. An allele change of T > C at chr19:44908684 is indicative of the APOE Cys112Arg mutation

NGS Analysis

Figure 10 contains the list of individuals, and their genotypes, selected for further NGS analysis in Galaxy. BAM files were uploaded into IGV for visualization. Figures 11A and 11B demonstrate position 144099379 on chromosome 8 and position 44908684 on chromosome 19, respectively, for the eight individuals in Figure 10. Allele changes were as expected.



Sample (Male/Female/Unknown)	chr8:144099379 Genotype	chr19:44908684 Genotype	Population Genetics
HG00437 (F)	A|G	T|T	Han Chinese South, East Asian Ancestry
HG01031 (M)	G|A	T|T	Chinese Dai in Xishuangbanna, China, East Asian
HG01863 (F)	G|A	T|C	Kinh in Ho Chi Minh City, Vietnam, East Asian Ancestry
HG02164 (F)	G|A	C|T	Chinese Dai in Xishuangbanna, China, East Asian
NA18629 (M)	G|A	T|T	Han Chinese in Beijing, China, East Asian Ancestry
NA18943 (M)	A|G	T|T	Japanese in Tokyo, Japan, East Asian Ancestry
NA18944 (M)	G|A	T|C	Japanese in Tokyo, Japan, East Asian Ancestry
HG01944 (M)	G|A	C|T	Peruvian in Lima, Peru, American Ancestry

Figure 10 List of 8 individuals selected for NGS analysis. Four individuals contain only the SHARPIN Arg274Trp mutation, and four individuals contain both the SHARPIN Arg274Trp and the APOE Cys112Arg mutations

BAM File Visualization in IGV – chr8: 144099379

Figure 11a Visualization of BAM files in IGV demonstrating position 144099379 on chromosome 8 (rs77359862)

BAM File Visualization in IGV – chr19:44908684

Figure 11b Visualization of BAM files in IGV demonstrating position 44908684 on chromosome 19 (rs429358)

The FreeBayes Bayesian genetic variant detector allows for the detection of haplotypes from short DNA sequences. FreeBayes identified variants in each sample that were supported by at least 2 observations by at least 20% of the reads [11]. Table 1 demonstrates the results using the BAM files above as input, the reference genome GRCh38, and the SNP positions (rs77359862 – chr8: 144099379, rs429358 – chr19: 44908684) filter. As expected, the variants detected were supported by the low-coverage WGS data from the 1000 Genomes on GRCh38. The sample's allele frequencies are represented by the column AF, which demonstrates heterozygosity with a value of 0.5. The total read depth for each individual is represented by the column DP. The Phred quality scores for the reference and alternate alleles are shown in columns QR and QA, respectively. The mean mapping quality for the reference and alternate alleles, denoted by columns MQM and MQMR, is 60 for all individuals. The mapping quality

distribution is calculated using Q=-log10(P), where Q represents the Phred score, and P represents the probability that the read is in the incorrect location. A score of 60 is associated with high mapping quality [22].

Table 1 Summary of VCF file outputs from FreeBayes Bayesian genetic variant detector was used for calling the variants filtered to positions chr8: 144099379 and chr19: 44908684

SAMPLE	CHRM	POS	REF	ALT	QUAL	AF	DP	QR	QA	MQM	MQMR
HG00437	chr8	144099379	G	A	16.7047	0.5	17	368	136	60	60
HG01031	chr8	144099379	G	A	23.8308	0.5	6	42	78	60	60
HG01863	chr8	144099379	G	A	135.713	0.5	19	312	264	60	60
	chr19	44908684	T	C	12.4782	0.5	8	121	93	60	60
HG02164	chr8	144099379	G	A	17.1513	0.5	7	92	82	60	60
	chr19	44908684	T	C	22.5523	0.5	7	126	93	60	60
NA18629	chr8	144099379	G	A	101.008	0.5	10	114	196	60	60
NA18943	chr8	144099379	G	A	53.7698	0.5	7	82	126	60	60
NA18944	chr8	144099379	G	A	10.3443	0.5	10	171	88	60	60
	chr19	44908684	T	C	13.8267	0.5	5	66	76	60	60
HG01944	chr8	144099379	G	A	125.985	0.5	13	136	246	60	60
	chr19	44908684	T	C	18.8615	0.5	10	120	98	60	60

Discussion

Our investigations suggest that the SHARPIN Arg274Trp variant functions independently of APOE4 in association with the development of LOAD. The activation of immune cells, such as microglia and macrophages, in response to inflammatory responses, along with the accumulation of Aβ, are characteristic of Alzheimer's disease. SHARPIN plays a role in mediating inflammatory responses through the regulation of the NF-κB pathway as part of the LUBAC. The changes in immune response, as a result of the mutation, have been attributed to the development of LOAD by Asanomi et al. (2019). Furthermore, the regulation of Aβ phagocytosis is thought to be associated with varying levels of SHARPIN expression. According to observations by Park et al. (2021), brain damage associated with AD, including cortical thickness, hippocampal volume, and accumulation of amyloid-β, has been attributed to the mutation.

Investigations of the functional consequences of the SHARPIN R274W variant demonstrate the mutation leads to LUBAC instability and reduced activation of the NF-κB pathway. The SHARPIN

R274W variant is in the UBL domain, which is required for the interaction with the HOIP UBA domain for the formation of LUBAC. Additionally, the Arginine residue in the UBL domain may play a role in the overall charge balance and stabilization of the complex.

Among the forty-two individuals with the SHARPIN Arg274Trp from the 1000 Genomes Project, forty are from East Asia, the continental region with the highest prevalence of this SNP. Only eight of these individuals have the ApoE4 Cys112Arg, suggesting that there is a high percentage of people who are at risk of developing LOAD solely because of the SHARPIN Arg274Trp mutation. The two individuals who are not of East Asian descent that have the SHARPIN Arg274Trp variant also have the ApoE4 Cys112Arg mutation. However, because there are so few individuals from the 1000 Genomes Project who are not of East Asian descent, it is challenging to determine the distribution of the SHARPIN Arg274Trp variant alone versus with ApoE4 Cys112Arg within ethnic groups. Obtaining more genomes of individuals who are not of East Asian descent but have the SHARPIN variant would allow for a better understanding of the prevalence of the variant as well as the prevalence of the variant when ApoE4 is also present, ultimately providing a clearer picture of the relationship between the two variants as it pertains to ethnicity.

Further analysis of LOAD patients across different populations may elucidate the relationship between these two variants in Alzheimer's Disease. Understanding who develops LOAD, at what age, and how fast the progression is could help to unravel the relationship between the most common indicator of increased risk (APOE4) and the novel SHARPIN Arg274Trp SNP. Additionally, it's important to remember that the presence of a genetic variant associated with a disease does not necessarily mean that the individual will develop the disease. Many factors, including environmental and lifestyle factors, can also play a role in disease development. Given the limited data, we do not know whether the individuals analyzed had developed LOAD, and we acknowledge the limitations of our analysis. In the future, it would be useful to conduct a longitudinal study of individuals with the SHARPIN R274W variant alone and of individuals with both the SHARPIN R274W variant and the ApoE4 Cys112Arg variant to investigate whether they have an increased risk of developing LOAD.

We also investigated the regulatory regions of SHARPIN and found enrichment in H3K27Ac and H3K4Me1 associated with the SNP position in H1-hESC cells. Embryonic stem cells are capable of producing all types of cells in the body. The use of these pluripotent cells could be combined with organ-on-a-chip (OoC) technology to create disease models and understand the pathophysiology of LOAD based on a patient's genetic background. This approach could be further enhanced by using induced pluripotent stem cells (iPSC) in combination with CRISPR to study specific genes or variations. [10,28].

Data Availability

1. Sequencing data used in this study are available at the following URL: https://www.internationalgenome.org/
2. All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Statements and Declarations

The authors have no competing interests to declare that are relevant to the content of this article.

References

1. Alzheimer's Association (no date) Alzheimer's Disease and Dementia. Alzheimer's Association. Available at: https://www.alz.org/
2. Asanomi, Y., Shigemizu, D., Akiyama, S., Miyashita, A., Mitsumori, R., Hara, N., Ikeuchi, T., Niida, S., & Ozaki, K. (2022). A functional variant of SHARPIN confers increased risk of late-onset Alzheimer's disease. Journal of human genetics, 67(4), 203–208. https://doi.org/10.1038/s10038-021-00987-x
3. Asanomi, Y., Shigemizu, D., Miyashita, A., Mitsumori, R., Mori, T., Hara, N., Ito, K., Niida, S., Ikeuchi, T., & Ozaki, K. (2019). A rare functional variant of SHARPIN attenuates the inflammatory response and associates with increased risk of late-onset Alzheimer's disease. Molecular medicine (Cambridge, Mass.), 25(1), 20. https://doi.org/10.1186/s10020-019-0090-5
4. de Los Reyes Corrales, T., Losada-Pérez, M., & Casas-Tintó, S. (2021). JNK Pathway in CNS Pathologies. International journal of molecular sciences, 22(8), 3883. https://doi.org/10.3390/ijms22083883
5. EMBL's European Bioinformatics Institute. "Human (GRCh38.p13): SHARPIN." Ensembl, Mar. 2023, useast.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000179526;r=8:144098633-144108124
6. EMBL's European Bioinformatics Institute. "Human (GRCh38.p13): Rs429358 SNP." Ensembl, Feb. 2023. https://www.ensembl.org/Homo_sapiens/Variation/Explore?r=19:44908184-44909184;v=rs429358;vdb=variation;vf=201931519
7. EMBL's European Bioinformatics Institute. "Human (GRCh38.p13): Rs77359862 SNP." Ensembl, Feb. 2023,

useast.ensembl.org/Homo_sapiens/Variation/Population?r=8:144098879-144099879;v=rs77359862;vdb=variation;vf=172599439

8. ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. Nature, 489(7414), 57–74. https://doi.org/10.1038/nature11247
9. ENCODE regulation tracks. (n.d.). Retrieved from https://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=1591108903_6kEcD9Jdmgof363zhIaNnbYGSuy1&db=hg19&c=chr8&g=wgEncodeReg
10. Fanizza, F., Campanile, M., Forloni, G., Giordano, C., & Albani, D. (2022). Induced pluripotent stem cell-based organ-on-a-chip as personalized drug screening tools: A focus on neurodegenerative disorders. Journal of tissue engineering, 13, 20417314221095339. https://doi.org/10.1177/20417314221095339
11. Garrison, E. (2012). Haplotype-based variant detection from short-read sequencing. http://arxiv.org/abs/1207.3907
12. Gilmore T. D. (2006). Introduction to NF-kappaB: players, pathways, perspectives. Oncogene, 25(51), 6680–6684. https://doi.org/10.1038/sj.onc.1209954
13. gnomAD. gnomad.broadinstitute.org/variant/8-144099379-G-A?dataset=gnomad_r3.
14. Gujral, P., Mahajan, V., Lissaman, A. C., & Ponnampalam, A. P. (2020). Histone acetylation and the role of histone deacetylases in normal cyclic endometrium. Reproductive biology and endocrinology : RB&E, 18(1), 84. https://doi.org/10.1186/s12958-020-00637-5
15. *IGSR: The International Genome Sample Resource*. 1000 Genomes | A Deep Catalog of Human Genetic Variation. (2008). https://www.internationalgenome.org/
16. Isik, A. T. (2010). Late-onset Alzheimer's disease in older people. Clinical Interventions in Aging, 5, 307-311. https://doi.org/10.2147/CIA.S11718
17. Krishnan, D., Menon, R. N., & Gopala, S. (2022). SHARPIN: Role in Finding NEMO and in Amyloid-Beta Clearance and Degradation (ABCD) Pathway in Alzheimer's Disease?. Cellular and molecular neurobiology, 42(5), 1267–1281. https://doi.org/10.1007/s10571-020-01023-w
18. Krishnan, D., Menon, R. N., Mathuranath, P. S., & Gopala, S. (2020). A novel role for SHARPIN in amyloid-β phagocytosis and inflammation by peripheral blood-derived macrophages in Alzheimer's disease. Neurobiology of aging, 93, 131–141. https://doi.org/10.1016/j.neurobiolaging.2020.02.001
19. Lim, S., Sala, C., Yoon, J., Park, S., Kuroda, S., Sheng, M., & Kim, E. (2001). Sharpin, a novel post-synaptic density protein that directly interacts with the shank family of

proteins. Molecular and Cellular Neuroscience, 17(2), 385–397. https://doi.org/10.1006/mcne.2000.0940

20. Mahley, R. W., Weisgraber, K. H., and Huang, Y. (2006). Apolipoprotein E4: A casuative factor and therapeutic target in neuropathology, including Alzheimer's disease. PNAS, 103(15), 5644-5651. www.pnas.orgcgidoi10.1073pnas.0600549103

21. Mahley, R. W., Weisgraber, K. H., & Huang, Y. (2009). Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. The Journal of Lipid Research, 50 Supplement, S183-S188. https://doi.org/10.1194/jlr.R800069-JLR200

22. Miller, C. A., Qiao, Y., DiSera, T., D'Astous, B., & Marth, G. T. (2014). bam.iobio: a web-based, real-time, sequence alignment file inspector. Nature methods, 11(12), 1189. https://doi.org/10.1038/nmeth.3174

23. N/A. (2017). What Happens to the Brain in Alzheimer's Disease?. National Institute on Aging, U.S. Department of Health and Human Services. https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease#:~:text=In%20Alzheimer's%20disease%2C%20as%20neurons,significant%20loss%20of%20brain%20volume

24. Nhgri, Tony Burdett Emma Hastings, Dani Welter, Spot, Embl-Ebi,. GWAS Catalog. www.ebi.ac.uk/gwas/variants/rs77359862.

25. Olafsdottir, T. A., Theodors, F., Bjarnadottir, K., Bjornsdottir, U. S., Agustsdottir, A. B., Stefansson, O. A., Ivarsdottir, E. V., Sigurdsson, J. K., Benonisdottir, S., Eyjolfsson, G. I., Gislason, D., Gislason, T., Guðmundsdóttir, S., Gylfason, A., Halldorsson, B. V., Halldorsson, G. H., Juliusdottir, T., Kristinsdottir, A. M., Ludviksdottir, D., Ludviksson, B. R., … Stefansson, K. (2020). Eighty-eight variants highlight the role of T cell regulation and airway remodeling in asthma pathogenesis. Nature communications, 11(1), 393. https://doi.org/10.1038/s41467-019-14144-8

26. Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: 107741: 06/27/2022. World Wide Web URL: https://omim.org/

27. Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University, Baltimore, MD. MIM Number: 104310: 11/03/2020. World Wide Web URL: https://omim.org/

28. Palasantzas, V. E. J. M., Tamargo-Rubio, I., Le, K., Slager, J., Wijmenga, C., Jonkers, I. H., Kumar, V., Fu, J., & Withoff, S. (2023). iPSC-derived organ-on-a-chip models for personalized human genetics and pharmacogenomics studies. Trends in genetics : TIG, 39(4), 268–284. https://doi.org/10.1016/j.tig.2023.01.002

29. Park, J. Y., Lee, D., Lee, J. J., Gim, J., Gunasekaran, T. I., Choi, K. Y., Kang, S., Do, A. R., Jo, J., Park, J., Park, K., Li, D., Lee, S., Kim, H., Dhanasingh, I., Ghosh, S., Keum, S., Choi, J. H., Song, G. J., Sael, L., … Alzheimer's Disease Neuroimaging Initiative (2021). A missense

variant in SHARPIN mediates Alzheimer's disease-specific brain damages. Translational psychiatry, 11(1), 590. https://doi.org/10.1038/s41398-021-01680-5

30. Reiman, E. M., Caselli, R. J., Yun, L. S., Kewei Chen, M. S., Bandy, D., Minoshima, S., Thibodeau, S. N., and Osborne, D. (1996). Preclinical Evidence of Alzheimer's Disease in Persons Homozygous for the e4 allele for Apolipoprotein E. New England Journal of Medicine, 334, 752-758. https://doi.org/10.1056/NEJM199603213341202

31. "Rs77359862 RefSNP Report - dbSNP - NCBI." dbSNP, 21 Sept. 2022, www.ncbi.nlm.nih.gov/snp/rs77359862#frequency_tab.

32. Seymour, R. E., Hasham, M. G., Cox, G. A., Shultz, L. D., Hogenesch, H., Roopenian, D. C., & Sundberg, J. P. (2007). Spontaneous mutations in the mouse Sharpin gene result in multiorgan inflammation, immune system dysregulation and dermatitis. Genes and immunity, 8(5), 416–421. https://doi.org/10.1038/sj.gene.6364403

33. SHARPIN: Q9H0F6. UniProt. (n.d.). Retrieved February 24, 2023, from https://www.uniprot.org/uniprotkb/Q9H0F6/entry

34. Soheili-Nezhad, S., Jahanshad, N., Guelfi, S., Khosrowabadi, R., Saykin, A. J., Thompson, P. M., Beckmann, C. F., Sprooten, E., Zarei, M., & Alzheimer's Disease Neuroimaging (2020). Imaging genomics discovery of a new risk variant for Alzheimer's disease in the post-synaptic SHARPIN gene. Human brain mapping, 41(13), 3737–3748. https://doi.org/10.1002/hbm.25083

35. Stieglitz, B., Haire, L. F., Dikic, I., & Rittinger, K. (2012). Structural analysis of SHARPIN, a subunit of a large multi-protein E3 ubiquitin ligase, reveals a novel dimerization function for the pleckstrin homology superfold. The Journal of biological chemistry, 287(25), 20823–20829. https://doi.org/10.1074/jbc.M112.359547

36. Tange, O. (2011). GNU Parallel - The Command-Line Power Tool. ;;Login: The USENIX Magazine, 36(1), 42–47. http://www.gnu.org/s/parallel

37. The Galaxy Community. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update, Nucleic Acids Research, Volume 50, Issue W1, 5 July 2022, Pages W345–W351, doi:10.1093/nar/gkac247

38. Tokunaga, F., & Iwai, K. (2012). LUBAC, a novel ubiquitin ligase for linear ubiquitination, is crucial for inflammation and immune responses. Microbes and infection, 14(7-8), 563–572. https://doi.org/10.1016/j.micinf.2012.01.011

39. Whetstine, J. R. (2010). Chapter 287 - histone methylation: Chemically inert but chromatin dynamic. In R. A. Bradshaw, & E. A. Dennis (Eds.), Handbook of cell signaling (second edition) (pp. 2389-2397). Academic Press. https://10.1016/B978-0-12-374145-5.00287-4

40. Yu, Beiming, et al. "Advances in the Structural and Physiological Functions of SHARPIN." Frontiers in Immunology, vol. 13, Frontiers Media, Apr. 2022, https://doi.org/10.3389/fimmu.2022.858505.

1. Zhou, S., Liang, Y., Zhang, X., Liao, L., Yang, Y., Ouyang, W., & Xu, H. (2020). SHARPIN Promotes Melanoma Progression via Rap1 Signaling Pathway. The Journal of investigative dermatology, 140(2), 395–403.e6. https://doi.org/10.1016/j.jid.2019.07.696