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Next Generation Precision Medicine: CRISPR-mediated Genome Editing for the Treatment of Neurodegenerative Disorders

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Abstract

Despite significant advancements in the field of molecular neurobiology especially neuroinflammation and neurodegeneration, the highly complex molecular mechanisms underlying neurodegenerative diseases remain elusive. As a result, the development of the next generation neurotherapeutics has experienced a considerable lag phase. Recent advancements in the field of genome editing offer a new template for dissecting the precise molecular pathways underlying the complex neurodegenerative disorders. We believe that the innovative genome and transcriptome editing strategies offer an excellent opportunity to decipher novel therapeutic targets and develop patient-specific precision-targeted personalized therapies to effectively treat neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, frontotemporal dementia etc. However, despite significant advancements, we would caution the researchers that since the CRISPR field is still evolving, currently we do not know the full spectrum of CRISPR-mediated side effects. In the wake of the recent news regarding CRISPR-edited human babies being born in China, we urge the scientific community to maintain high scientific and ethical standards and utilize CRISPR for developing *in vitro* disease in a dish model, *in vivo* testing in nonhuman primates and lower vertebrates and for the development of neurotherapeutics for the currently incurable diseases. Here, we review the latest developments in the field of CRISPR-mediated genome editing and provide unbiased futuristic insights regarding its translational potential to improve the treatment outcomes and minimize financial burden.

Graphical Abstract

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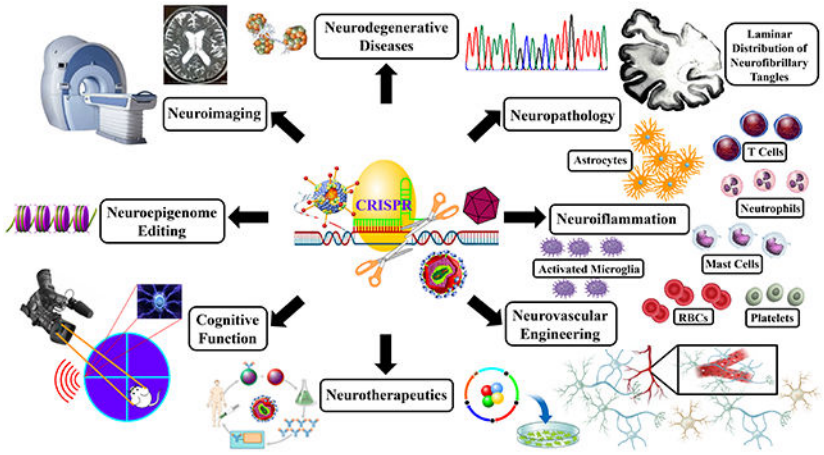

Keywords

Alzheimer's disease; Amyotrophic lateral sclerosis; CRISPR; Frontotemporal dementia; genome editing; Huntington's disease; neuroinflammation; neurodegeneration; Parkinson's disease

Introduction

Neurodegenerative diseases are becoming increasingly common in the ageing population and are a significant cause of socio-economic burden worldwide. Due to the current knowledge gap, the precise and highly complex molecular and cellular mechanisms underlying neurodegenerative diseases are not very well understood. However, intervention at the earliest stages of neurodegenerative disorders holds tremendous promise for the prevention as well as the treatment of various neurodegenerative disorders and the recent trend indicates a paradigm shift from single therapeutic target to a multi-target approach (Cao et al., 2018). In this regard, CRISPR/Cas9 (Clustered Regularly interspaced short palindromic repeats/CRISPR-associated 9)-mediated genome editing offers a novel approach to either halt or delay the progression of neuroinflammation as well as neurodegeneration. Neuroinflammation, which is a hallmark of various neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and multiple sclerosis (MS), plays a crucial role in the development and progression of these neurodegenerative diseases (Chitnis and Weiner, 2017; Lall and Baloh, 2017). The elements of the innate immune response within the CNS including microglia and astrocytes are the key cellular mediators of neuroinflammation. We believe that precision-targeted genome editing of the key signaling molecular mechanisms underlying neuroinflammation offers a novel therapeutic approach to effectively treat neurodegenerative disorders and significantly reduce the economic burden. Although there are several excellent review articles on various genome editing approaches utilizing zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALEN), meganucleases and targeterons, our discussion will be specifically focused upon CRISPR-mediated genome editing in the central nervous system. For the basic understanding of genome editing in mammalian brain there are several excellent reviews available (Nishiyama, 2018; Vesikansa, 2018). Here, we describe the status of genome

editing especially in relation to neuroinflammation and neurodegeneration. To demonstrate the significant potential of CRISPR-mediated genome editing we have made sincere efforts to highlight the progress in various neurodegenerative disorders especially AD, PD, ALS, FTD and HD.

CRISPR/Cas9: A Novel Gene Editing Tool

Initial discovery of a Type II CRISPR system in *Streptococcus pyogenes* wherein 4 genes including Cas9, Cas1, Cas2, Csn1 and two non-coding RNAs (pre-crRNA and tracrRNA) act simultaneously to target and degrade foreign DNA in a sequence specific manner has ushered in a new era in the field of genome editing (Garneau et al., 2010; Horvath and Barrangou, 2010; Jinek et al., 2012; Wiedenheft et al., 2012). In the type II CRISPR/Cas system that is very widely used, Cas9 is complexed with two small RNAs termed as CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) to form a sequence-specific RNA-guided endonuclease (RGEN). In this system the tracrRNA:crRNA-guided Cas9 protein utilizes distinct endonuclease domains (HNH and RuvC-like domains) to cleave the two strands in the target DNA. The CRISPR/Cas9 system was subsequently exploited to allow double strand breaks in the mammalian genome at specified locations that could be repaired by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR) (Cong et al., 2013; Mali et al., 2013a; Mali et al., 2013b; Ran et al., 2013a; Sander and Joung, 2014).

Most recently, a set of CRISPR-Cas systems from uncultivated archaea that contain exceptionally compact Cas14 proteins capable of targeted single-stranded DNA cleavage without restrictive sequence requirements have been reported (Harrington et al., 2018). Hiroshi et al have engineered CRISPR-Cas9 nuclease with an expanded targeting space (Nishimasu et al., 2018). Unlike the wild type SpCas9 that requires an NGG protospacer adjacent motif (PAM), the rationally engineered SpCas9-NG can recognize relaxed NG PAMs. SpCas9-NG induces indels at endogenous target sites bearing NG PAMs in human cells. Additionally the fusion of SpCas9-NG and the activation-induced cytidine deaminase (AID) mediates the C-to-T conversion at target sites with NG PAMs in human cells. In comparison to xCas9 (SpCas9 variant with A262T/R324L/S409I/E480K/E534D/M694I/E1219V mutations) the cleavage kinetics of SpCas9-NG are superior. Further, the nuclease-inactive version of SpCas9-NG-AID in which SpCas9-NG D10A nickase is fused to AID (nSpCas9-NG-AID referred to as Target-AID-NG mediates C-to-T conversion at 32 endogenous target sites with NG PAMs in human cells. A new study has explored the potential utility of CRISPR-C to generate functional extrachromosomal circular DNA (eccDNA) which will be very useful for studying the cellular impact, persistence and function of eccDNAs (Moller et al., 2018). This technology allows the generation of eccDNA from intergenic and genic loci in human cells with a size ranging from a few hundred base pairs up to 47.4 megabase-sized ring chromosomes. However, it remains to be explored whether eccDNAs may play a role in neurodegenerative disorders. The list of various CRISPR systems and their respective PAM recognition sequences are provided in Table 1.

While there are several studies that have utilized either lentiviral or AAV vectors for successful genome editing *in vivo* especially in the brain, there are potential concerns regarding off target effects, random integration of viral vectors and immune response. To overcome these potential limitations, recent studies have demonstrated promising genome editing results in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes (Staahl et al., 2017). The engineered variants of Cas9 with multiple SV40 nuclear localization sequences revealed a tenfold increase in the efficiency of neuronal genome editing *in vivo*. However, astrocytes did not undergo editing using this approach. Hence, it would be important to devise appropriate genome editing strategies depending upon the cell type.

Multiple studies have successfully utilized constitutively active form of CRISPR/Cas9. However, it would be desirable to achieve regulated expression of CRISPR/Cas9 to maximize gene editing efficiency while simultaneously reducing the off target side effects. In this direction, an interesting study has described the development of a doxycycline inducible dual AAV based CRISPR/cas9 system (de Solis et al., 2016). Their system utilizes an inducible gRNA AAV vector designed to express the gRNA from a H1/TO promoter and the Tet repressor to regulate the expression of the gRNAi in a doxycycline dependent manner. Their initial results utilizing systemic injection of AAV2/DJ-P_{Tight}-Cas9 and AAV2/DJ-$gRNA_{Tet2}$/rtTA-GFP in to the basal and lateral amygdala and feeding the animals with or without doxycycline led to gene editing that was not doxycycline dependent due to leaky Cas9 expression. Subsequently, by multiple rounds of genetic engineering they developed AAV2/DJ8-P_{Mecp2}-Cas9 and AAV2/DJ8-$gRNAi_{Tet2}$ vectors which upon systemic infusion in to the basal and lateral amygdala were able achieve genome editing in doxycycline dependent manner in as little as 24 hours. These research findings are exciting because regulated *in vivo* gRNA expression will significantly reduce off targeted effects while achieving precise gene editing in a very short time. To further improve upon these results, they have very recently developed a Cre-loxP system to conditionally regulate the expression and gene editing of *Streptococcus aureus* Cas9 (SaCas9) via the expression of Cre-*recombinase in vitro* and *in vivo* (Kumar et al., 2018). In their refined approach, they investigated AAV-mediated floxed SaCas9/CRISPR for achieving Cre-dependent gene editing *in vivo*. However, as compared to the earlier system, the Cre-dependent system appeared to edit at a much lower efficiency. Therefore, there is significant scope to further refine *in vivo* gene editing efficiency by developing better inducible expression vectors. Additionally, it would be worthwhile to explore various other AAV serotypes for achieving targeted gene editing in the neurons, microglia as well as astrocytes.

Although CRISPR has proven to be extremely versatile tool for genome editing in a wide variety of applications, a major caveat is the potential off target effects (Cho et al., 2014; Lin et al., 2014). Hence, there is a significant concern that such off target effects may negatively impact experimental results thereby limiting the potential utility of the CRISPR/Cas9 system especially in the clinical studies. Recent studies have evaluated genome-wide target specificities of CRISPR RNA-guided programmable deaminases by utilizing modified Digenome-seq (Kim et al., 2015). There are several sophisticated technologies available for the detection of off-target effects including GUIDE-seq, BLESS, HTGTS, IDLV capture, SITE-seq, CIRCLE-seq and BLISS (Frock et al., 2015; Tsai et al., 2015; Bolukbasi et al.,

2016; Tsai and Joung, 2016; Tycko et al., 2016; Cameron et al., 2017; Tsai et al., 2017; Yan et al., 2017). A wide variety of approaches have been developed to minimize the off target effects including rationally engineered Cas9, evolved Cas9 variants, Sniper-Cas9, optimized base editors, modified or shortened guide RNAs, FokI-Cas9 fusion nucleases, purified Cas9 ribonucleoproteins, VSV-G-enveloped vesicles carrying CRISPR-SpCas9 ribonucleoprotein complexes VEsiCas, paired catalytic mutant Cas9 nickases, $Cas9^{D10A}$ nickase and HiFi Cas9 and combining ribonucleoprotein delivery with AAV donor vectors for homology directed genome-editing (Ran et al., 2013b; Fu et al., 2014; Guilinger et al., 2014; Kim et al., 2014; Shen et al., 2014; Tsai et al., 2014; Wyvekens et al., 2015; Chiang et al., 2016; Slaymaker et al., 2016; Gaj et al., 2017b; Gopalappa et al., 2018; Hu et al., 2018a; Lazzarotto et al., 2018; Lee et al., 2018a; Montagna et al., 2018; Vakulskas et al., 2018; Zafra et al., 2018).

Recently, various technological advances have made it possible to determine genome wide activity of CRISPR/Cas9 nucleases (Bae et al., 2014; Tsai et al., 2015; Tsai et al., 2017; Lazzarotto et al., 2018). New studies have analyzed the influence of flanking DNA sequence on the repair outcome by measuring the edits generated by >40,000 guide RNAs in synthetic constructs (Allen et al., 2018). Their comprehensive studies suggest that the majority of reproducible mutations are in fact insertions of a single base, short deletions or longer microhomology-mediated deletions depending on the cell line.

Immune response to CRISPR therapeutics and the presence of pre-existing antibodies to Cas9 proteins could prove to be a significant hurdle especially for *in vivo* gene editing (Cromer et al., 2018; Simhadri et al., 2018). Development of codon optimized CRISPR may hold the key to overcome this hurdle. However, the emergence of novel delivery systems especially ribonucleoprotein complexes could potentially overcome the issues associated with the immune response (Staahl et al., 2017).

Gene Editing for Neurodegenerative Diseases

Within the central nervous system, microglial-astrocyte-monocyte-neuronal cross talk plays a crucial role in maintaining homeostasis during normal brain development, function, regeneration, repair as well as recovery. Neuroinflammation plays a very crucial role in the etiopathogenesis of various neurodegenerative diseases (Ransohoff, 2016; Becher et al., 2017). Neuroinflammation is a highly complex and orchestrated biological process within the central nervous system that is tightly regulated by the pro as well as anti-inflammatory mediators and cell types. Normally acute neuroinflammation is often beneficial and is neuroprotective as it induces an adaptive response that enables the host to defend against the invading pathogens. However, on the other end of the spectrum, chronic neuroinflammation is indeed deleterious and results in neuronal dysfunction, which ultimately leads to the development of various neurodegenerative diseases. Currently, despite significant scientific advancements, there is no effective cure for the neurodegenerative diseases. Hence, there is an urgent need to develop novel approaches to successfully treat various neurodegenerative diseases. We believe that CRISPR-mediated gene editing offers a novel approach to develop precision-targeted therapies against various neurodegenerative disorders (Figure 1). Here we

provide a glimpse of the latest CRISPR-mediated gene editing approaches that have been used to target various neurodegenerative diseases Table 2.

Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes an irreversible cognitive decline in an estimated 5.5 million Americans with $259 billion in healthcare costs in 2017 (Alzheimer's Association). If the present trend continues, by 2050 the number of AD patients will surpass 16 million with ~$1.1 trillion in healthcare costs thereby necessitating the development of novel therapeutic strategies to effectively treat AD. The pathological hallmarks of AD is abnormal accumulation of amyloid beta (Aβ) and neurofibrillary tangles due to aggregates of hyperphosphorylated tau protein, pathological events including oxidative stress, reactive glial and microglial changes and genetic mutations in APP, PSEN1, PSEN2 and higher association with APOE4 allele (Wang et al., 2017a). Recent studies have provided novel insights regarding neurogenetic contributions to Aβ and tau spreading in the human cortex (Sepulcre et al., 2018). Impaired processing and elimination of these proteins leads to their abnormal buildup and spread (Menzies et al., 2017; Boland et al., 2018). Here, we present the recent developments in the CRISPR-mediated genome editing of various AD-linked genes.

APP, PSEN1, PSEN2 and BACE1

Successful rescue of the PSEN1 mutations by CRISPR-knockout of APP in human stem-cell-derived cortical neurons has been recently achieved (Hung and Livesey, 2018). Their studies reveal that mutations in APP and PSEN1 leads to major defects in lysosome function and autophagy in iPS cell-derived human neurons. Deletion of *APP* in *PSEN1* Y115C neurons reduced LAMP1 protein and increased the axonal transport of lysosomes when compared with isogenic *PSEN1* Y115C neurons. Further, APP deletion also rescued autophagy defects. Overall, their results suggest that either reducing input of APP to the lysosomal-autophagy system, enhancing axonal transport or augmenting lysosome function at the early stages could be exploited to develop potential therapeutic strategies to attenuate autophagic defects in AD. Further, it would be most appropriate to compare the results of CRISPR-knockout iPS cell-derived neurons with corresponding AD patient specific iPS cell line derived neurons (Li et al., 2018). In a recent study, it was demonstrated that CRISPR/cas9-mediated correction of the PSEN2 point mutation abolished the electrophysiological deficit thereby restoring the maximal number of spikes and spike height as compared to the levels observed in the controls. Moreover, increased Aβ42/40 was also normalized post CRISPR/Cas9-mediated correction of the $PSEN2^{N141I}$ mutation (Ortiz-Virumbrales et al., 2017).

Generation of two iPS cell lines from the skin biopsy obtained from a healthy male individual of African descent and generated either a heterozygous V717I (London) or a heterozygous KM670/671NL (Swedish) mutation in the APP gene by CRISPR-mediated gene knock-in have been successfully achieved (Frederiksen et al., 2018). Recent trend indicates that ethnicity may play a role in AD pathogenesis. Keeping in view this paradigm, these iPS cell lines will be useful to study AD pathogenesis in comparison to the iPS cell

lines derived from other ethnic backgrounds. We believe that more research is needed to decipher the role of ethnic background in determining the risk as well as the severity of AD pathogenesis.

In AD APP cleavage by the enzyme β-secretase BACE1 is the rate-limiting step in the amyloidogenic pathway. By exploiting the lentiviral and AAV9 based CRISPR/Cas-9-mediated gene editing, Sun et al have developed a strategy by which they were able to reciprocally manipulate the amyloid pathway by simultaneously attenuating APP-β-cleavage and Aβ production while up-regulating neuroprotective APP-α cleavage (Sun et al., 2019). For their studies, they identified three PAM sites at the APP C-terminus that were conserved in both human and mouse and synthesized sgRNAs targeting these regions. Their *in vitro* studies revealed that APP-sgRNA predicted to cut human APP at the 659 aa position was most efficient both in editing APP as well as in attenuating Aβ. Co-injection of AAV9-APP-sgRNA-GFP and AAV9-HA-Cas9 into mouse hippocampus led to efficient transduction of both sgRNA as well as Cas9 causing endogenous APP *in vivo* editing. Overall, their data suggest that the gene-editing approach does not have a major effect on post-Golgi trafficking of APP but attenuates APP endocytosis from the cell surface and consequently its interaction with BACE-1 in endosomes. A potential caveat of these studies is that they have used 8 weeks old C57BL/6 mice to validate their results. A better approach would be to test the APP *in vivo* editing in AD mouse models and perform neurocognitive functional analysis. A combinatorial study utilizing CRISPR/Cas9 and relevant APP knock in mouse model has revealed deletion mutations that are protective against AD-like pathology (Nagata et al., 2018).

The *KM670/671NL* APP Swedish mutation, which is located on the β-secretase site results in increased enzymatic cleavage by β-secretase of APP thereby causing increased Aβ levels. Selective disruption of the human mutant APP^{SW} allele using CRISPR/Cas9 has been achieved (Gyorgy et al., 2018). Utilizing two separate AAVs expressing APP^{SW}-specific guide RNA and Cas9, they were able to achieve site-specific indel formation in the primary neurons isolated from APP^{SW} transgenic (Tg2576) mouse embryos as well as following intra-hippocampal co-injections. However, a drawback of their study is that they were able to detect an approximate 2% indel formation in the mutant alleles within the injected area. These extremely low levels of indel formation may not be sufficient to correct the disease phenotype. A potential solution would be to use a single AAV coexpressing SaCas9 as well as the sgRNA as we have recently demonstrated (Raikwar et al., 2018).

Perusal of current literature suggests a strong linkage between T2D and AD (Craft and Watson, 2004; Akter et al., 2011; Stanley et al., 2016; Kandimalla et al., 2017; Moreno-Gonzalez et al., 2017; Pugazhenthi et al., 2017; Shinohara and Sato, 2017). Although, the current literature suggests that there is a strong linkage between T2D and AD (Moreno-Gonzalez et al., 2017; Pugazhenthi et al., 2017; Shinohara and Sato, 2017), there is a significant knowledge gap regarding the precise molecular mechanism/s underlying the linkage and interaction between T2D and AD. In an interesting study, Moreno et al have investigated the role of insulin signaling in AD pathophysiology (Moreno et al., 2018). It has previously been demonstrated that γ-secretase hypofunction underlies insulin resistance in adipocytes (Sparling et al., 2016). Furthermore, mutations in PSEN1 or PSEN2 are

associated with hypofunction of the γ-secretase thereby leading to an increase in the Aβ 42:40 ratio and Aβ42. Based upon this report, Moreno et al analyzed the effect of insulin signaling on Aβ speciation in iPS cell-derived wild type and FAD mutant $PSEN2^{N141I}$ neurons as well as their CRISPR/Cas9-corrected counterparts.

An interesting study has identified that in the neurons in the human brain, *APP* mRNA undergoes reverse transcription to produce cDNA which then reintegrates as genomic complementary DNA (gencDNA) and accumulation of mutations leads to a range of gencDNA *APP* variants that lack one or more exons (Lee et al., 2018b). Consequently, some gencDNAs yield toxic proteins causing cell death and might contribute to sporadic AD. However, the molecular mechanism underlying the generation of mutant gencDNA are presently unknown. Potential CRISPR-mediated targeting of gencDNAs may yield unique clues underlying novel molecular mechanisms underlying AD pathogenesis.

Glia Maturation Factor

Glia maturation factor (GMF), a proinflammatory molecule discovered in our laboratory is significantly upregulated in various neurodegenerative diseases (Lim et al., 1989; Lim et al., 1990; Lim and Zaheer, 1991; Zaheer et al., 2001; Zaheer et al., 2002; Zaheer et al., 2007a; Zaheer et al., 2007b; Zaheer et al., 2008; Zaheer et al., 2011; Thangavel et al., 2012; Kempuraj et al., 2013; Thangavel et al., 2013; Zaheer et al., 2013; Khan et al., 2014a; Khan et al., 2014b; Kempuraj et al., 2015; Khan et al., 2015; Ahmed et al., 2017; Thangavel et al., 2017; Kempuraj et al., 2018b; Kempuraj et al., 2018a; Kempuraj et al., 2018c; Selvakumar et al., 2018b; Thangavel et al., 2018). Therefore, we believe that GMF represents a novel and an attractive therapeutic target to disrupt glia-neuron interactions and possibly delay and or halt the progression of multiple neurodegenerative diseases (Figure 2). Capitalizing on the success of CRISPR/Cas9, we have generated and successfully tested a recombinant neurotropic adeno-associated virus (AAV-B1-CRISPR-SaCas9-GMF-sgRNA) simultaneously coexpressing CRISPR-SaCas9 and GMF sgRNA for GMF gene editing in murine neuronal and microglial cell lines. We have also recently demonstrated that dual lentiviral-mediated CRISPR based GMF gene editing in BV2 microglial cells leads to suppression of p38 MAPK phosphorylation, which is crucial for reducing neuroinflammation (Raikwar et al., 2018). Our *in vitro* results suggest that GMF gene editing leads to indels in exons 2 and 3 which in turn leads to reduced microglial activation due to reduction in p38 MAPK phosphorylation following LPS challenge. These exciting results suggest that targeted GMF gene editing offers a novel approach to develop the next generation of precision guided personalized therapy for the treatment of AD. Our ongoing studies will provide novel insights into CRISPR-mediated GMF gene editing *in vivo* in the murine models of various neurodegenerative diseases.

TREM2

Microglia dysfunction plays a crucial role in the pathogenesis of neurodegenerative disorders. To investigate the role of microglia and monocyte derived TREM2 in phagocytosis, generation of isogenic $TREM2^{+/R47H}$, $TREM2^{+/-}$ and $TREM2^{-/-}$ human pluripotent stem cells using CRISPR/Cas9 has now been reported (Claes et al., 2018).

Differentiation of these isogenic lines into monocytes and microglia-like cells and their subsequent functional analysis revealed that the phagocytosis of *E. coli* fragments and human amyloid plaques was not affected by $TREM2^{+/R47H}$ mutation but was significantly impaired in $TREM2^{+/-}$ and $TREM2^{-/-}$ progeny. It has been established that R47H variant of TREM2 confers greatly increased risk for AD. Therefore, deciphering the role of R47H variant could provide valuable insights underlying molecular mechanisms operational in AD pathogenesis and progression. In this regard, CRISPR/Cas9 to generate a mouse model of AD harboring one copy of the single nucleotide polymorphism encoding the R47H variant in murine TREM2 is indeed very interesting (Cheng-Hathaway et al., 2018). Their results suggest that R47H variant increases the risk of AD by conferring a loss of TREM2 function and enhancing neuritic dystrophy around plaques. Similarly, development of TREM2 R47H mice using CRISPR/cas9-assisted gene targeting strategy has been achieved (Xiang et al., 2018). Their results suggest that R47H variant activates a cryptic splice site that generates miss-spliced transcripts leading to TREM2 haploinsufficiency. However, caution needs to be exercised and humanized TREM2 R47H knock-in mice need to be generated to investigate cellular consequences caused by the human TREM2 R47H.

RANK

RANK signaling plays a crucial role in regulating the activation of the microglia. In an interesting study has reported on the development of a BV2 cell line lacking RANK receptor ($RANK^{-/-}$ BV2) by utilizing CRSIPR-mediated gene editing (Kichev et al., 2017). Their data suggest that knocking out RANK receptor abolishes the anti-inflammatory effect but does not alter the inhibition of TLR3 expression caused by RANKL pretreatment. Further, $RANK^{-/-}$ BV2 cells are resistant to the reduction in expression of the adaptor proteins MyD88 and TRIF following RANKL pretreatment. Moreover, reduced BV2 microglial activation led to reduced expression of inflammatory markers iNOS and COX2.

APOE4

APOE4 is a major genetic risk factor for sporadic AD but the underlying molecular and cellular mechanisms are currently unknown. CRISPR/Cas9 was utilized to create isogenic iPS cell lines harboring homozygous APOE4 alleles to examine APOE4 effects on human brain cell types (Lin et al., 2018). Their studies revealed that APOE4 iPS cell-derived neurons, astrocytes and microglia-like cells recapitulate phenotypes associated with AD at multiple levels. Further, transcriptional profiling identified hundreds of differentially expressed genes in each cell type with the most affected involving synaptic function (neurons), lipid metabolism (astrocytes) and immune response (microglia-like cells). In comparison to isogenic APOE3 neurons, the APOE4 neurons exhibited increased synapse number and elevated $A\beta_{42}$ secretion. Additionally, APOE4 astrocytes displayed impaired Aβ uptake and cholesterol accumulation and APOE4 microglia-like cells exhibited altered morphologies, which correlated with Aβ phagocytosis. Remarkably, six months old APOE4 organoids exhibited increased Aβ accumulation and tau phosphorylation as compared with APOE3 organoids. In the next series of experiments, they utilized CRISPR/Cas9 approach to create isogenic lines homozygous for APOE3 from sAD iPS cells that were homozygous for APOE4. Differentiation of the APOE3 iPS cells into functional neurons, glia and organoids

revealed that most of the AD-related phenotypes observed in APOE4 iPS cell-derived brain cell types and organoids could be reversed by CRISPR/Cas9-mediated editing of APOE4 to APOE3. Given the fact that APOE2, APOE3 and APOE4 differentially activate APP transcription, studies using AAVs expressing dCas9 and gRNA targeting the AP-1 binding sequence of the APP promoter have revealed that AP-1-dependent regulation of APP expression operates physiologically *in vivo* (Huang et al., 2017). Conversion of APOE4 to APOE3 by gene editing was able to rescue GABAergic neurons and reduce tau phosphorylation (Wang et al., 2018a). Overall, these elegant studies highlight the potential of CRISPR-mediated gene editing to decipher novel molecular mechanisms underlying AD pathology.

STIM1

In familial AD patients STIM1 cleavage by the presenilin-1-associated γ-secretase leads to dysregulation of calcium homeostasis. Recent studies have investigated the expression levels of STIM1 in the medium frontal gyrus of pathologically confirmed AD patients (Pascual-Caro et al., 2018). Their results suggest that STIM1 protein expression level decreased with the progression of neurodegeneration. Therefore, to investigate the role of STIM1 in neurodegeneration, they performed CRISPR/Cas9-nickase-mediated STIM1 gene editing in the SH-SY5Y neuroblastoma cell line. Their *in vitro* data suggests that STIM1 is not required for the differentiation but is essential for the cell survival in differentiating cells. Additionally STIM1 loss triggers mitochondrial depolarization and senescence because of calcium dysregulation. Taken together these results suggest that STIM1 is an attractive therapeutic target for AD drug discovery. Development of an *in vitro* model system based on CRISPR transcriptional activation analysis of APP and/or BACE1 revealed increased Aβ in skin fibroblasts as well as γ-secretase processivity defects in FAD fibroblasts (Inoue et al., 2017). This activated CRISPR skin fibroblast model will prove beneficial to probe the role of various genetic modifiers of sporadic AD.

Clusterin

Clusterin (CLU) protein plays a crucial role in amyloid β processing and mutations in the CLU gene are a major risk factor for AD (May et al., 1990; Harold et al., 2009; Lambert et al., 2009; Thambisetty et al., 2010). CLU binds to TREM2 and therefore may play a crucial role in the activation of microglia (Yeh et al., 2016). A novel CLU-knockout iPS cell line by CRISPR/Cas9-mediated gene editing was developed to investigate Aβ-mediated neurodegeneration in cortical neurons differentiated from wild type and CLU knockout iPS cells (Robbins et al., 2018). They utilized a targeting construct containing a 2A GFP floxed PGK neo to integrate into the exon 3 of CLU gene by CRISPR/cas9-mediated homologous recombination. Their studies demonstrated that the neurons lacking CLU did not show neurodegeneration in response to 1 μM $A\beta_{1-42}$ treatment, unlike CLU wild type neurons. They further performed a transcriptome wide expression to map the effects of CLU on pathways that are active in human neurons and demonstrated dysregulation of CCND1, KLF10, FOS, EGR1 and NAB2 in CLU knockout neurons. Based on their data, lack of neurodegeneration in the CLU knockout neurons in response to Aβ as compared with the wild type neurons supports the role of CLU in Aβ-mediated AD pathogenesis.

ANK3

Ankyrin G, which is encoded by ANK3 gene, plays a crucial role in neuronal development and signaling. New studies have examined the role of ANK3 a well-established risk gene for psychiatric illness by utilizing CRISPR-dCas9-KRAB transcriptional repressor in mouse neuro-2a cells (Garza et al., 2018). ANK3 gene editing led to increased EB3 expression, decreased tubulin acetylation and increased soluble:polymerized tubulin ratio indicating enhanced microtubule dynamics. Further, Ank3 repression in neuro-2a cells increased GSK3 activity (reduced inhibitory phosphorylation) and elevated collapsing response mediator protein 2 (CRMP2) phosphorylation. Their data suggest that ANK3 functions in neuronal microtubule dynamics through GSK3 and its downstream substrate CRMP2. These findings highlight the molecular and cellular mechanisms underlying brain-specific ANK3 disruption, which might play a potential role in psychiatric illness as well as AD.

TAU/MAPT

TAU aggregation is vital for the progression of AD. In order to develop an *in vitro* model of tauopathy, which is suitable for the drug, discovery screenings, new studies have described the development a footprint free triple MAPT-mutant human iPS cell line (Garcia-Leon et al., 2018). They introduced three mutations (N279K, P301L and E10+6) in and next to exon 10 of the MAPT gene in the wild type healthy-derived human iPS cells. This was achieved by utilizing CRISPR-FokI nuclease to minimize off-target effects and homology-directed recombination with the piggyback transposase-mediated excision of a selectable cassette, introduced during the homology-directed recombination. The resulting cell line resulted in an altered 3R/4R-TAU isoform expression with high levels of 4R-TAU and displayed considerably increased levels of oxidative stress, ER/UPR stress and activation of inflammatory-related response marker genes. Overall, this cell line reproduces multiple neurodegenerative phenotypes associated with tauopathies including altered TAU expression including phosphorylated isoforms, TAU aggregation, defective neurite conformation, altered neuronal maturation and enhanced electrophysiological excitability. This cell line will prove valuable for in vitro drug discovery screens and identification of novel therapeutic targets for AD.

In order to unravel the function of a rare Tau variant A152T which has been shown to play a role in neuroinflammation and pose a significantly increased risk for FTD and other neurodegenerative diseases (Kara et al., 2012; Labbe et al., 2015; Decker et al., 2016; Sydow et al., 2016), new studies have reported on the generation of human iPS cells from A152T carriers and derivation of neuronal progenitor cells and differentiated neuronal cells (Silva et al., 2016). Utilizing various biochemical and cellular assays their studies revealed that as compared to the control neurons, A152T neurons exhibit accumulation, redistribution and decreased solubility of TAU. Further, TAU upregulation was coupled to enhanced stress-inducible markers and cell vulnerability to proteotoxic, excitotoxic and mitochondrial stressors and CRISPR/Cas9-mediated targeting of TAU was sufficient to rescue viability. These remarkable findings highlight the potential of iPS-derived neuronal model and CRISPR/Cas9-mediated gene editing for developing the next generation of precision targeted gene and stem cell-based therapies to treat various neurodegenerative disorders.

TAU and α-synuclein aggregates, which are the hallmarks of tauopathies and α-synucleinopathies, require transcellular propagation, which is mediated by binding to heparan sulfate proteoglycans on the cell surface and their subsequent cellular uptake and intracellular seeding and fibrillization (Goedert et al., 1996; Holmes et al., 2013). Recent studies have determined the genes required for the aggregate uptake by inducing CRISPR-mediated knockout of the major genes of the heparan sulfate proteoglycans synthesis pathway (Stopschinski et al., 2018). Their studies revealed that the CRISPR-mediated knockouts of the extension enzymes exostosin 1 (*EXT1*), exostosin 2 (*EXT2*) and exostosin-like 3 (*EXTL3*) as well as N-sulfotransferase (*NDST1*) or 6-O-sulfotransferase (*HS6ST2*) significantly reduced TAU uptake while *EXT1, EXT2, EXTL3* or *NDST1* but not *HS6ST2* reduced α-synuclein uptake. These studies provide a novel platform for the development of mechanism based therapies to block transcellular propagation of TAU, α-synuclein and amyloid protein-based pathologies.

Fibrinogen

Fibrinogen deposition is observed in a wide variety of CNS diseases with blood brain barrier disruption. By directly affecting the neurons, microglia and the astrocytes, fibrinogen promotes neuroinflammation as well as glial scar formation. CRISPR/Cas9 was utilized to investigate the role of fibrinogen in oligodendrocyte progenitor cells differentiation and myelination (Petersen et al., 2017). CRISPR/Cas9-mediated knockout of the activing A receptor type 1 (ACVR1) in the primary oligodendritic progenitor cells reduced fibrinogen-induced nuclear accumulation of phosphorylated Smad1/5 and Id1 expression and enhanced the formation of mature MBP+ oligodendrocytes post fibrinogen treatment. Overall, their results suggest that therapeutic depletion of fibrinogen decreases BMP signaling and enhances remyelination *in vivo*.

Base Editing

Base editing is a novel genome editing approach that allows the programmable conversion of one base pair into another without double-stranded DNA cleavage, homology-directed repair or excess stochastic deletions or insertions. Base editing is achieved by utilizing a base editor which is either a fusion protein between SpCas9 and rat APOBEC1 (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 1) (Komor et al., 2016) or Target-Aid (activation-induced cytidine deaminase) which is composed of SpCas9 and sea lamprey PmCDA1 (*Petromyzon marinus* cytosine deaminase) (Nishida et al., 2016). New studies have introduced pathogenic mutations into the mouse *Psen1* gene by base editor and target-AID and generated multiple mouse lines harboring point mutations (Sasaguri et al., 2018). Their studies reveal that base editor has consistently higher base-editing efficiency (10.0-62.8%) as compared to that of Target-AID (3.4-29.8%). Most importantly, their *in vivo* studies revealed that Psen1-436S and Psen1-P117L mice have alteration of their Aβ profile similar to human fAD patients. Additionally, they also identified Psen1-P436L as a potential novel pathogenic mutation that has not been previously reported. These exciting studies suggest that base editing could prove beneficial for the identification of unknown pathogenic mutations as well as developing novel disease models.

Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by locomotor dysfunction with rigidity, bradykinesia and resting tremor. PD is the second most common neurodegenerative disease after AD with an overall incidence of around 15 per 100,000 people. Genetic studies have revealed the involvement of various genes and signaling pathways involved in PD pathogenesis. Familial PD can result due to mutations in the LRRK2, PARK7, PINK1, PRKN or SNCA genes.

SNCA

Human clinical trials in PD patients utilizing human fetal mesencephalic cells have shown that the transplanted grafts are susceptible to Lewy body formation. To overcome this potential challenge, engineered synucleinopathy-resistant human dopaminergic neurons by CRISPR-mediated deletion of the SNCA gene in human ES cells have been reported (Chen et al., 2018). Their studies utilizing $SNCA^{+/-}$ and $SNCA^{-/-}$ neurons suggest that these neurons were resistant to the formation of protein aggregates following treatment with recombinant α-synuclein pre-formed fibrils. In a different study, an all in one lentiviral vector has achieved downregulation of SNCA expression by targeted editing of DNA methylation (Kantor et al., 2018).

PD modeling using iPS cells has proven to be very valuable (Chung et al., 2013). To further refine the approach, an efficient method to derive biallelic genomic-edited populations using CRISPR-Cas9 has now been developed (Arias-Fuenzalida et al., 2017). Their approach known as FACS-assisted CRISPR-Cas9 editing (FACE) allows the derivation of correctly edited polyclones carrying a positive selection fluorescent module and the exclusion of non-edited, random integrations and on-target allele NHEJ-containing cells. They generated an isogenic set of human SNCA mutants for PD-specific cellular modeling. These isogenic lines contain disease-associated mutations p.A30P or p.A53T in the SNCA gene.

NLRP3

NLRP3 plays a crucial role in various inflammatory diseases including neuroinflammation especially in AD and PD. In an interesting study, a CRISPR activation approach was used to systematically identify regulators of neuronal fate specification (Liu et al., 2018). An optimized cationic lipid-assisted nanoparticle (CLAN) to deliver Cas9 mRNA and guide RNA targeting NLRP3 ($CLAN_{mCas9/gNLRP3}$) into macrophages has been reported (Xu et al., 2018). This strategy offers a promising new avenue for treating NLRP3-dependent inflammatory diseases. Interestingly, deubiquitinases USP7 and USP47, which play a crucial role in inflammasome activation in macrophages, have been subjected to doxycycline-inducible CRISPR-mediated knockdown causing alterations in NLRP3 ubiquitination levels (Palazon-Riquelme et al., 2018). These studies suggest that NLRP3, USP7 and USP47 could be attractive therapeutic targets for treating neuroinflammation.

GBA

Pathogenic aggregation and misfolding of α-synuclein are implicated in the pathogenesis of familial and sporadic PD as well as other α-synucleinopathies. However, the precise molecular mechanisms underlying the regulation of α-synuclein tetramer formation are currently unknown. Systematic studies involving CRISPR-GBA1 knockout have demonstrated that mutations in glucocerebrosidase 1 (GBA1) and depletion-induced GBA1 deficiency leading to the accumulation of glycosphingolipids are sufficient to cause destabilization of α-synuclein tetramers as well as increase the susceptibility of human dopaminergic neurons to cytotoxicity due to the exposure to pathologic α-synuclein fibrils (Kim et al., 2018). Their results suggest that prevention of lipid dyshomeostasis could offer novel therapeutic strategies to treat PD.

TFAM

Chronic manganese exposure leads to neurotoxicity and leads to the development of PD. Lentiviral CRISPR/Cas9-mediated gene editing of TFAM transcription factor was used to generate a mitochondrially defective dopaminergic cell model by (Langley et al., 2017). Using MitoPark mice and TFAM knockout, they systematically characterized neurobehavioral, neurochemical and biochemical changes contributing to nigral dopaminergic neurodegeneration. Their studies revealed that low dose manganese toxicity significantly accelerates and exacerbates the motor deficits, striatal dopamine depletion and tyrosine hydroxylase neuronal loss in MitoPark mice. Additionally, knocking out TFAM induces mitochondrial deficits by impairing both basal and ATP-linked respiration capacity in a dopaminergic neuronal cell model.

P13

Mitochondrial dysfunction in the nigrostriatal dopaminergic neurons is crucial to the development of PD. Mitochondrial genome editing has the potential to revolutionize the development of novel mitochondria-targeted therapies. A proof of concept studies has demonstrated the development of MitoCas9 for the mitochondria-targeted genome editing (Jo et al., 2015). However, caution needs to be exercised as no mitochondrial DNA sequencing data was provided to conclusively validate their data. To investigate the function of the mitochondria-localized protein P13 in PD, new studies have described the generation of P13 knockout mice using CRISPR/Cas9 (Inoue et al., 2018). Their results suggest that heterozygous p13 knockout prevents mitochondrial dysfunction, toxin-induced motor deficits and the loss of dopaminergic neurons in the substantia nigra.

CHCHD2

Coiled-coil-helix-coiled-coil-helix domain containing protein 2 (CHCHD2 plays a role in AD, PD and FTD. Generation of CRISPR-based isogenic human ES cell lines harboring different PD-linked CHCHD2 mutations has led to the identification of new pathogenic mechanisms (Zhou et al., 2018c). The differentiated neural progenitor cells harboring CHCHD2 R145Q or Q126 mutation revealed impaired mitochondrial function. Further, PD-

linked CHCHD2 mutations lost their interactions with CHCHD10. Their studies suggest that CHCHD2-CHCHD10 complex may be a novel therapeutic target for PD and related neurodegenerative disorders.

LRRK2

By exploiting CRISPR/Cas9-mediated gene editing of PD patient-specific iPS cells researchers have generated isogenic PD astrocytes and ventral midbrain dopaminergic neurons lacking the *LRRK2* G2019S mutation (di Domenico et al., 2019). Their coculture studies of control ventral midbrain dopaminergic neurons on top of PD astrocytes revealed morphological signs of neurodegeneration and abnormal astrocyte-derived α-synuclein accumulation due to inhibition of α-synuclein lysosomal degradation. In contrast, coculture of PD ventral midbrain dopaminergic neurons on top of control astrocytes prevented the appearance of disease-related phenotypes. Further, they observed that macroautophagy was also markedly impaired in these cells. Their findings represent a direct indication that dysfunctional astrocytes play a crucial role during PD pathogenesis. Transcriptional repression of SNCA, MAPT, HTT and APP via targeting to the transcriptional start sites using dCas9, dCas9-KRAB and dCas9-VPR effector domains and the use of double-nicking CRISPR/Cas9 to exert precise alterations in SNCA will enable to dissect molecular as well as temporal course of pathogenic events underlying PD and other neurodegenerative diseases (Heman-Ackah et al., 2016; Heman-Ackah et al., 2017).

PRKN

Parkin gene PRKN encodes E3 ubiquitin ligase, which plays a crucial role in mitochondrial quality control and turnover (Arkinson and Walden, 2018). Various PRKN mutations are related to autosomal recessive PD. PRKN Genome-wide CRISPR screen for PARKIN regulators has revealed THAP11 as a negative regulator in multiple cell types (Potting et al., 2018). Human iPS cell-derived inducible Neurogenin 2 (iNGN2) neurons in which THAP11 was targeted by CRISPR/Cas9 revealed de-repression of PARK2 transcription and enhanced phosphoS65-ubiquitin accumulation thereby demonstrating the impact of PARKIN-level regulation.

Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

Amyotrophic lateral sclerosis (ALS) also known as Lou Gehrig’s disease is a neurodegenerative disorder that is caused by gradual deterioration and death of motor neurons. In ALS, both the upper motor neurons as well as the lower motor neurons undergo degeneration thereby leading to muscle weakness, stiffness, fasciculations and atrophy. ALS is caused by the mutations in SOD1 and C9ORF72. Frontotemporal dementia (FTD) is a group of neurodegenerative disease conditions resulting from the progressive degeneration of the temporal and frontal lobes of the brain. FTD is the second most common cause of dementia following AD and is caused by the mutations in MAPT, GRN, C9ORF72, VPC, CHMP2B, TARDP, FUS, ITM2B, TBK1 and TBP genes.

C9ORF72

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are characterized by hexanucleotide repeat (GGGGCC) expansions in the C9ORF72 gene. Unfortunately, these hexanucleotide repeats produce sense and antisense RNA foci and are translated into aggregation prone neurotoxic DPR proteins by RAN translation. CRISPR-mediated genome-wide gene-knockout screens for suppressors and enhancers of C9ORF7 DPR toxicity have been established recently (Kramer et al., 2018). These studies have led to the identification of multiple DPR toxicity modifiers, which will be very useful not only to study the molecular mechanisms underlying neurodegeneration but also to develop novel therapeutic strategies. Previously, a deactivated form of Cas9 was used to impede transcription of microsatellite expansion (Pinto et al., 2017). These studies revealed a repeat length-, PAM-, and strand-dependent-reduction of repeat-containing RNAs following direct dCas9 targeting to the repeat sequences. Further, they demonstrated that aberrant splicing patterns were rescued in DM1 cells and production of RAN peptides characteristic of DM1, DM2 and C9ORF72-ALS/FTD cells was drastically decreased. Additionally, systemic delivery of dCas9/gRNA by AAV led to reductions in pathological RNA foci, rescue of chloride channel 1 protein expression and decreased myotonia.

Most recently, the role of C9ORF72 in ALS/FTD has been investigated (Shi et al., 2018b). Using human induced motor neurons their studies found that repeat-expanded C9ORF2 was haplsoinsufficient in ALS and it interacted with endosomes and was required for normal vesicle trafficking and lysosomal biogenesis in motor neurons. Further, the repeat expansion reduced C9ORF72 expression thereby causing neurodegeneration via dual mechanisms including the accumulation of glutamate receptors leading to excitotoxicity and impaired clearance of neurotoxic dipeptide repeat proteins derived from the repeat expansion. To confirm that reduced C9ORF72 protein levels were sufficient to cause neurodegeneration, they harnessed CRISPR/Cas9-mediated genome editing to introduce a frameshift mutation into either one or both C9ORF72 alleles in the control iPS cells. Their data suggest that targeting one allele reduced C9ORF72 transcript levels, which were more severely reduced in homozygous mutant cells. Moreover, eliminating C9ORF72 protein expression from one or both alleles reduced the survival of the induced motor neurons that was comparable to those of patient iPS cell-derived induced motor neurons. Restoring the expression of C9ORF72 in $C9ORF72^{+/-}$ and $C9ORF72^{-/-}$ induced motor neurons rescued the survival of the induced motor neurons. Overall, these results suggest that depletion of C9ORF2 by CRISPR/Cas9-mediated genome editing led to the observed neurodegeneration. In a separate study utilizing iPS cell-derived motor neurons from ALS patients carrying C9ORF72 mutation, studies have interrogated the consequences of G4C2 expansion in human motor neurons and found that increased expression of GluA1 AMPA receptor (AMPAR) subunit occurs in the motor neurons with C9ORF72 mutations leads to increased CA^{2+} permeable AMPAR expression and results in enhanced selective vulnerability to excitotoxicity (Selvaraj et al., 2018). However, this vulnerability was abolished by CRISPR/Cas9-mediated correction of the C9ORF72 repeat expansion in the motor neurons.

TREM2

How homozygous TREM2 mutations lead to FTD is not very well understood. Recent studies have utilized CRISPR-Cas9 to generate a Trem2p.T66M knock-in mouse model expressing the FTD-like syndrome (Kleinberger et al., 2017). These mice exhibit an intracellular accumulation of immature mutant TREM2 and reduced generation of soluble TREM2 as well as delayed resolution of inflammation upon *in vivo* lipopolysaccharide stimulation. Further, FDG-microPET imaging studies revealed a significant reduction in cerebral blood flow and brain glucose metabolism. Overall, their data suggest that a TREM2 loss of function mutation causes brain-wide metabolic alterations possibly through microglia-regulated brain glucose metabolism.

CHMP2

iPS cell-derived neurons from two symptomatic FTD3 patients for modeling FTD with mutation in CHMP2B gene has recently been reported (Zhang et al., 2017). The FTD3 causing mutation 31449G>C was repaired via CRISPR/Cas9 in the FTD3 isogenic human iPS cells. Functional studies using the FTD3 iPS cell-differentiated neurons revealed significant upregulation of LRRK2, down regulation of APOE, endosomal dysregulation as well as abnormal mitochondrial ultrastructure and function including increased oxidative stress. APOE plays a vital role in neuronal lipid transport, brain injury repair, facilitates the clearance of soluble amyloid beta and suppresses microglia activation. Therefore, downregulation of APOE in FTD3 neurons may contribute to microglia activation and trigger neuroinflammation. RNA sequencing revealed that the CHMP2B mutation manifests in dysregulated expression of the key genes associated with PD and AD due to perturbed iron homeostasis.

MAPT

Neuronal models of FTLD-Tau by Neurogenin2-induced direct neuronal differentiation from FTLD-Tau patient iPS cells have been created (Imamura et al., 2016). The studies reported that FTLD-Tau neurons either with an intronic MAPT mutation or with an exonic MAPT mutation developed accumulation and extracellular release of misfolded tau followed by neuronal death. The investigators generated isogenic control iPS cells by targeting of the MAPT gene by CRISPR/Cas9 in combination with the *piggyBac* tet-on expression system to introduce Ngn2. Further, they assessed the mechanistic links between the excitation of neurons and misfolded tau by incorporating DREADDs consisting of human muscarinic acetylcholine M4 receptor bearing artificial mutations into FTLD-Tau iPS cells. Their findings suggest that neurodegeneration in FTLD-Tau involves misfolded Tau and that it is modulated by neuronal activity.

To identify the genes and pathways that are dysregulated in autosomal dominant frontotemporal lobar degeneration with tau inclusions (FTLD-tau), researchers have performed transcriptomic analyses in iPS cell-derived neurons carrying MAPT p.R406W and CRISPR/Cas9-corrected isogenic controls (Jiang et al., 2018). They found that the expression of MAPT p.R406W mutation was sufficient to create a significantly different

transcriptomic profile compared with that of the isogenic controls leading to differential expression of 328 genes, which were enriched for pathways involving gamma-aminobutyric receptors and pre-synaptic function. These genes may play a role in the pathogenesis of FTLD-tau and other primary tauopathies.

Astrocytes play a crucial role in in the normal brain including supporting neurogenesis and synaptogenesis, provide trophic support to neurons, and protect them from oxidative stress. However, whether astrocytes play a crucial role in FTD pathogenesis is not very clear. CRISPR/Cas9-mediated gene editing in human FTD patient-specific iPS cell-derived neural progenitor cells to repair the FTD-associated N279K MAPT mutation in the exon 10 has been reported recently (Hallmann, 2017). Their study demonstrates profound intrinsic pathological changes in N279K MAPT astrocytes in regards to cell morphology, TAU expression, protein ubiquitination, susceptibility to oxidative stress and whole genome expression profiles. Further, co-culture of healthy control neurons with FTD or control astrocytes revealed alterations in stress responses and changes in gene expression patterns. Specifically, they observed down regulation of DHRS2, MCAT, EMP3, RNF7, RDH12, CDH23 and CLCNKA genes. The upregulated genes included CXCR4, ESPL1, MX1, KLRG1, TDG, ANXA2, CSDE1, NHLH1, NTF3, EFS and RBPJL.

p35

Aberrant Cdk5 activation and induction of neuroinflammtion, synaptic loss, Aβ accumulation and tau hyperphosphorylation due to accumulation of p25 is observed in various neurodegenerative disorders including AD. To validate the role of p25/Cdk5 in tauopathy, new studies have utilized FTD-patient-derived iPS cells carrying the Tau P301L mutation to generate P301L:Δp35KI isogenic iPS cell lines in which p35 is replaced with noncleavable mutant Δp35 using CRISPR/Cas9 genome editing (Seo et al., 2017). Creation of cerebral organoids using these isogenic iPS cell lines revealed that blockade of p25 generation reduced the levels of phosphorylated Tau and increased the expression of synaptophysin. These studies suggest that inhibition of p35 can remedy neurodegenerative processes in the presence of pathogenic tau mutation.

FUS

Mutations in the fused in sarcoma gene are responsible for the juvenile as well as adult onset ALS. Researchers have generated and characterized iPS cells from the ALS patients with different FUS mutations as well as healthy controls (Guo et al., 2017). The differentiation of the ALS patient derived iPS cells into motor neurons revealed typical cytoplasmic FUS pathology, hyperexcitability and progressive axonal transport defects. Axonal transport defects were successfully rescued by CRISPR/Cas9-mediated genetic correction of the FUS mutation in the ALS patient derived iPS cells. Further, development of novel cell lines with the FUS gene modified by CRISPR has been reported (An et al., 2019). Specifically, to mimic genetic alterations typical observed in ALS, the investigators generated cell lines with the deletion of the genomic sequences encoding the 12 C-terminal amino acids of FUS by CRISPR/Cas9-mediated gene editing. These cell lines revealed that endogenous levels of mutant FUS cause accumulation of NEAT1 isoforms and dysfunctional paraspeckles.

Recent studies have identified defects in DNA nick ligation and oxidative damage repair in a subset of ALS patients (Wang et al., 2018b). Their studies show that the loss of nuclear FUS caused DNA nick ligation defects in motor neurons due to reduced recruitment of XRCC1/LiglII to DNA strand breaks. These DNA ligation defects in ALS patient-derived iPS cell lines carrying FUS mutations and in motor neurons generated there from are rescued by CRISPR/Cas9-mediated correction of mutation.

SOD1

Capitalizing on the role of superoxide dismutase in ALS, interesting studies have demonstrated that CRISPR/Cas9 can be harnessed to disrupt mutant SOD1 expression in the G93A-SOD1 mouse model of ALS following in vivo genome editing post AAV gene therapy (Gaj et al., 2017a). Genome editing reduced the expression of the mutant SOD1 protein by >2.5 fold in the lumbar and thoracic spinal cord thereby resulting in improved motor function and reduced muscle atrophy. Surprisingly, the ALS mice treated by CRISPR-mediated SOD1 genome editing had ~50% more neurons at end stage and displayed a ~37% delay in disease onset and a ~25% increase in survival compared to control animals.

Huntington’s Disease

Huntington’s disease (HD), a severe autosomal dominant neurodegenerative disorder is caused by an expanded polyglutamine repeat in the N-terminal of the Huntingtin gene, which leads to multiple cellular dysfunction (Bates et al., 2015; Wild and Tabrizi, 2017). Huntington’s disease is characterized by a combination of cognitive, motor and psychiatric symptoms as well as atrophy of the basal ganglia and the cerebral cortex and runs a progressive course leading to death within 5-20 years post manifestation of the symptoms.

Huntingtin

Earlier studies have shown that depletion of normal huntingtin in adult mouse brains does not affect animal survival, growth or neuronal viability (Wang et al., 2016a). Moreover, the N terminal region of Huntingtin has been shown to be non-essential for early embryonic development. Therefore, targeted genome editing of the N-terminal region of Huntingtin could offer a unique approach to treat Huntington’s disease. Permanent suppression of endogenous Huntingtin expression in the striatum of the mutant Huntingtin expressing mice (HD140Q-knockin mice) by AAV-CRISPR/Cas9-mediated genome editing effectively depleted Huntingtin aggregates and attenuated early neuropathology (Yang et al., 2017). Further, their data suggest that reducing Huntingtin expression in striatal neuronal cells does not affect the viability of the adult HD140Q-knockin mice but alleviates their motor deficits as well as neurological symptoms. CRISPR/Cas9-mediated Huntingtin gene inactivation was able to reverse the neuropathology and behavioral phenotypes even in the mice that were 9 months old, thereby suggesting that the old neuronal cells still have the ability to clear the accumulated mutant proteins and repair early injury once the expression of the mutant proteins is blocked.

Utilizing a slightly different approach, studies have demonstrated AAV-mediated targeted deletions that are sufficient to terminate Huntingtin expression (Monteys et al., 2017). They screened genomic regions adjacent to the Huntingtin exon 1 to identify SNPs that were prevalent and were within the critical position for CRISPR/cas9- or CRISPR/Cpf1-directed editing and tested their utility for allele-specific editing in HD patient cell lines and a mouse model expressing full length mutant human Huntingtin. Selective excision of ~44 kb DNA spanning promoter region, transcription start site and the CAG expansion mutation of the mutant HTT gene, results in complete inactivation of the mutant allele without impacting the normal allele (Shin et al., 2016). These investigators used personalized CRISPR/Cas9 strategy to target two patient specific PAM sites simultaneously to inactivate the mutant alleles from the source in a completely allele-specific manner. Yet another study has utilized a different approach to correct HD human iPS cells using CRISPR/Cas9 and piggyback transposon system (Xu et al., 2017). These studies revealed that both HD and corrected isogenic human iPS cells could be differentiated into excitable, synaptically active forebrain neurons. Further, the phenotypic abnormalities in HD iPS cell-derived neural cells including impaired neural rosette formation, increased susceptibility to growth factor withdrawal and deficits in mitochondrial respiration were rescued in isogenic controls.

A lentiviral based KamiCas9 self-inactivating gene editing system to achieve transient expression of the Cas9 protein and high gene editing efficiency has been reported (Merienne et al., 2017). This system utilizes an additional sgRNA under the transcriptional control of the weak 7SK promoter to target the ATG of Cas9 in order to block its translation. Using the KamiCas9 targeted genome editing of the mutant huntingtin led to efficient inactivation in the Huntington disease patient-specific iPS neuronal derivatives as well as in the mouse models of HD. Due to the transient expression of the Cas9 by self-inactivation, this system offers unique advantages including high on target gene editing, reduced off target effects as well as minimal immune or inflammatory response. As such, KamiCas9 will prove very beneficial for the development of therapeutic approaches to treat neuroinflammation and neurodegenerative disorders.

Conclusions and Future Perspectives

Targeted genome editing holds a tremendous potential to decipher the complex molecular mechanisms underlying neuroinflammation and neurodegeneration. We realize that it is simply beyond our scope to review most of the neurodegenerative disorders that can be successfully targeted by CRISPR-mediated genome editing (Figure 3). Further, in the years to come we will see a significant emphasis on CRISPR-mediated targeted genomic and epigenomic editing for the development of the next generation of neurotherapeutics. These are exciting times for harnessing the vastly unlimited and untapped potential of genome editing for the treatment of currently incurable and devastating neurodegenerative diseases. We believe that the discovery of novel CRISPR enzymes with precision-targeted genome editing with minimal off target effects will herald in a new era in the field of patient-specific precision-targeted regenerative therapies for neurological disorders. Future genome editing approaches will immensely benefit from the cutting-edge scientific advancements as described in the following sections.

CRISPR-Cas12a

Recently, a newer catalytically active CRISPR-Cas12a fused to a transcriptional-activator domain has been developed for enabling flexible switching between genome editing and transcriptional activation by altering guide length (Breinig et al., 2019). This was achieved by fusing a transcriptional activator complex VPR to catalytically active Cas12a and generating Cas12a-VPR and utilizing tetracycline-response element (TRE)-driven RFP. They were able to demonstrate AsCas12a-VPR-mediated orthogonal gene control in vivo by simultaneously targeting the TRE promoter for activation and Trp53 for indel formation in TRE-GFP reporter mice. Their research findings suggest that the ability of AsCas12a-VPR to activate genes while maintaining DNA cleavage activity, together with the simplicity of Cas12a-mediated multiplexing will be particularly valuable for in vivo applications as well as deciphering the higher-order genetic interactions that underlie the complex phenotypic traits in both healthy and diseased states.

CRISPR/Cas13

The current field of CRISPR-mediated genome editing is constantly evolving at an unprecedented pace. In addition to the most widely used Type II CRISPR systems, a new field of RNA editing using type VI CRISPR/Cas systems is gaining immense popularity (Cox et al., 2017). These investigators have profiled type IV CRISPR/Cas system and engineered a Cas13 orthologue capable of robust knockdown and demonstrated RNA editing by using catalytically inactive Cas13 (dCas13) to direct adenosine-to-inosine deaminase activity by ADAR2 (adenosine deaminase acting on RNA type 2) to transcripts in mammalian cells. Their system aptly referred to as RNA Editing for Programmable A to I Replacement (REPAIR) has no strict sequence constraints and can be successfully used to edit full-length transcripts containing pathogenic mutations. They have further refined their system by creating a high-specificity variant with a miniaturized version that can be easily packaged for efficient viral delivery. Similar studies have demonstrated that the class 2 type VI RNA-guided RNA-targeting CRISPR/Cas effector Cas13a derived from *Leptotrichia wadei* (LwaCas13a) can be utilized for the targeted knockdown of endogenous transcripts (Abudayyeh et al., 2017). LwaCas13a provides a platform for a range of transcriptomic analysis tools as well as therapeutic applications. Development of a platform termed SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) that combines isothermal preamplification with Cas13 to detect single molecules of RNA or DNA which can be used for diagnostic applications in patient liquid biopsy samples rapidly using a portable format that can be easily deployed in the field (Gootenberg et al., 2018). The Cas13-based SHERLOCK platform can detect Zika virus and dengue virus in patient samples at concentrations as low as 1 copy per microliter (Myhrvold et al., 2018). They have developed HUDSON (heating unextracted diagnostic samples to obliterate nucleases) protocol that pairs with SHERLOCK for viral detection directly from body fluids thereby enabling instrument-free detection of dengue and Zika virus directly from patient samples in less than 2 hours.

TUNR Flexible Gene Editing Technology

A simple and predictable protocol for generating hypomorphic mutations by targeting translation elongation has been described recently (Arthur et al., 2017). This system involves adding consecutive adenosine nucleotides or polyA tracks to the gene coding sequence of interest to decrease translation elongation efficiency, which in turn decreases mRNA stability and protein expression. An advantage of this system is that the protein expression is adjustable independent of promoter strength and it can be further modulated by changing the sequence features of the polyA tracks.

Neural Blastocyst Complementation and Cortical Organoids

Neural blastocyst complementation to study the development and function of specific forebrain regions using CRISPR-Cas9 will be very useful (Chang et al., 2018). Neural blastocyst complementation was achieved by the targeted ablation of host-derived dorsal telencephalic progenitors during development thereby leading to the creation of vacant forebrain niche in the host embryo resulting in agenesis of the cerebral cortex and hippocampus. Subsequent injection of doublecortin-deficient ES cells-generated by CRISPR-Cas9 produced neural blastocyst complementation chimaeras that recapitulated the phenotype of conventional, germline doublecortin-deficient mice. This new approach has significant potential to generate complex mouse models for studying forebrain functions as well as organogenesis. New studies have developed functional cortical organoids that spontaneously display glutamatergic and GABAergic signaling dependent periodic and regular oscillatory network events (Trujillo and Muotri, 2018).

Predicting Genome Editing Outcome

Most recently, studies have extensively analyzed the indel profiles at over 1000 genomic sites in human cells and uncovered general principles guiding CRISPR-mediated DNA editing (Chakrabarti et al., 2018). Their studies suggest that precise targets and editing outcome can be predicted based on simple rules that mainly depend on the fourth nucleotide upstream of the protospacer adjacent motif. If the fourth nucleotide is an A or a T, the genome editing outcome will be a very precise insertion, on the other hand a C will lead to a relatively precise deletion while a G will lead to many imprecise deletions. Further, the team also discovered that DNA sequence features including chromatin state influences indel profiles without altering dominant indels at precise sites. These interesting studies suggest that choosing the target sites with A or T nucleotide at −4 position of the target region is the most effective way to induce predictable insertions.

RNA Tracking

In an interesting study, a nuclease-inactive *S. pyogenes* CRISPR/Cas9 that can bind RNA in a nucleic-acid-programmed manner and allow endogenous RNA tracking in living cells has been developed (Nelles et al., 2016). This DNA targeted RCas9 which binds to RNA has multiple potential applications including directed cleavage, imaging, transcription modulation and targeted methylation. Optimization of libraries for CRISPR-Cas9 genetic

screens for the systemic interrogation of gene function have been described recently (Sanson et al., 2018). These studies evaluated genome-wide libraries for CRISPRko, CRISPRi and CRISPRa with *S. pyogenes* Cas9 via 14 screens across 3 cell lines. Recent scientific advancements have enabled precise conversion of single base or base pair into desired base or base pair without generating excess undesired editing by-products (Rees and Liu, 2018).

in utero CRISPR/Cas9

Multiple studies have demonstrated the potential of *in utero* CRISPR/Cas9-mediated gene editing (Cheng et al., 2016; Kalebic et al., 2016; Mikuni et al., 2016; Rannals et al., 2016b; Rannals et al., 2016a; Shinmyo et al., 2016; Tsunekawa et al., 2016; Uemura et al., 2016; Shinmyo and Kawasaki, 2017; Shinmyo et al., 2017; Hu et al., 2018b; Lu et al., 2018; Ribierre et al., 2018; Rossidis et al., 2018; Wang et al., 2018c). In an interesting study, taking advantage of a high-throughput electroporation-based gene editing in the zygotes, researchers have developed knock-in mice in which a hemagglutinin epitope was inserted in the *Nlgn1* gene (Nozawa et al., 2018). In these mice, the HA-Nlgn1 was enriched at synapses between parallel fibers and molecular layer interneurons and the glomeruli in which mossy fiber terminals synapse onto granule cell dendrites.

A pioneering approach SLENDR (single-cell labeling of endogenous proteins by CRISPR/Cas9-mediated HDR) developed recently, utilizes *in utero* electroporation to introduce an epitope tag or a fluorescent reporter in to a gene of interest by CRISPR/Cas9-mediated HDR (Mikuni et al., 2016). Using this approach allows simultaneous multiplex labeling of multiple neuronal proteins and the protein dynamics can be visualized in real-time at a very high resolution by live imaging. To further advance their technology, they have now reported the development of vSLENDR (virus-mediated single-cell labelling of endogenous proteins via HDR) by combining CRISPR/Cas9-mediated DNA cleavage and AAV-mediated delivery of donor template in the mature postmitotic neurons in the mouse brain (Nishiyama et al., 2017). They utilized a dual AAV system one expressing Cas9 and the other expressing a guide RNA and the donor DNA. In the next decade, gazing beyond the realms of the scientific boundaries we can envision CRISPR and base editors being harnessed for in utero correction of genetic mutations underlying neurodegenerative disorders in human embryos and fetus.

in vivo Bar Coding

In an exciting study, to address the complexity of mammalian development, investigators have utilized *in vivo* barcoding and engineered a mouse line via homing CRISPR carrying 60 independent loci of homing guide RNAs (Kalhor et al., 2017; Kalhor et al., 2018). Crossing the hgRNA line with a Cas9 line resulted in developmentally barcoded offspring due to the fact that hgRNAs stochastically accumulate mutations throughout the gestation period thereby generating unique mutations in each lineage without deleting the prior mutations. These barcoded offspring were used to address lineages above the first lineages in the tree with a focus on the establishment of the anterior-posterior (A-P) axis versus the lateral (L-R) axis in the brain. Their results suggest that commitment of the A-P axis is

established before commitment to the L-R axis during development of the central nervous system. These studies may be very useful for connectome mapping in the brain.

dCas9 Transgenic Mice

In a an interesting study, scientists have generated a transgenic mouse using an improved dCas9 system that enables simultaneous and precise *in vivo* transcriptional activation of multiple genes and long noncoding RNAs in the nervous system (Zhou et al., 2018a). Using these mice, they were able to achieve *in vivo* direct conversion of astrocytes into functional neurons by targeted activation of endogenous genes *Ascl1, Neurog2* and *NeuroD1*. To induce neuronal conversion of astrocytes *in vivo*, AAV-sgRNAs targeting *Ascl1, Neurog2* and *NeuroD1* was coinjected with AAV-GFAP-mCherry to label astrocytes in the midbrain. They observed a significant increase in the proportion of $mCherry^{+}NeuN^{+}$ cells one-month post AAV injection. Using further refinements, they were able to simultaneously target and control ten genes within the brain.

AD-BXD

A new emerging paradigm is that herpesviruses play a crucial role in AD pathology (Costa et al., 2017; Eimer et al., 2018; Kristen et al., 2018; Readhead et al., 2018). Furthermore, gut microbes have been demonstrated to play a crucial role in neuroinflammation, autoimmunity, neurogenesis and neurodegenerative diseases including AD and PD (Kumar et al., 2016; Mohle et al., 2016; Fung et al., 2017). Hence, there is a need to develop novel *in vitro* as well as *in vivo* models that accurately recapitulate AD pathology. In this regard, a novel AD-BXD panel that faithfully recapitulates key aspects of human AD including phenotypic variation in disease onset and severity, sensitivity to genetic variations in genes known to confer risk for human late-onset AD and a high level of concordance with transcriptional aspects of human disease has been developed (Neuner et al., 2018). Latest research findings suggest that a sushi-containing neurotransmitter receptor in the brain, $GABA_BR1a$ interacts with APP as an activity-dependent negative-feedback mechanism to preserve homeostatic control of neural circuits in the aged brain (Rice et al., 2019).

3K3A-APC

Exciting studies have shown that vasculoprotective, neuroprotective and anti-inflammatory factor 3K3A-activated protein C (3K3A-APC) blocks amyloidogenic BACE1 pathway and improves functional outcome in 5XFAD mouse model of AD (Lazic et al., 2019). These studies found that 3K3A-APC effectively inhibited parenchymal accumulation of Aβ40 and Aβ42 in the hippocampus and cortex respectively, normalized hippocampus-dependent behavioral deficits and cerebral blood flow responses, improved cerebrovascular integrity and diminished neuroinflammatory responses. Thus, it would be interesting to supplement CRISPR-mediated genome editing with 3K3A-APC treatment for maximizing the synergistic effects.

BDNF

Brain derived neurotrophic factor has been shown to be essential for establishing activity-related neural plasticity. Reducing BDNF production by CRISPR-Cas9 in the ventral hippocampal neurons impairs recall of avoidance extinction (Rosas-Vidal et al., 2018). For testing the effect of BDNF knockdown in ventral hippocampal neurons, rats were infused with a lentiviral vector expressing CRISPR/Cas9 and BDNF specific sgRNA. The treated rats revealed significant reduction of BDNF in ventral hippocampal neurons and exhibited extinction of avoidance. These findings support the growing body of knowledge implicating the role of the hippocampal-prefrontal pathway in anxiety-related disorders and extinction-based therapies.

Identification of Phagocytosis Regulators

Phagocytosis plays a crucial role in a wide variety of biological processes including development, apoptotic cell clearance, synaptic pruning and elimination of cellular debris post injury. Studies utilizing genome wide CRISPR screens have led to the identification of phagocytosis regulators (Haney et al., 2018). These remarkable studies have uncovered the role of NHLRC2 as a key player in phagocytosis by regulating RhoA-Rac1 signaling cascade. These studies have shown that a previously uncharacterized AD-associated gene TM2D3 can preferentially influence the uptake of amyloid-β aggregates. Additionally, an important finding was that the very-long-chain fatty acids are essential for efficient phagocytosis of certain substrates.

Future research will determine the molecular and cellular mechanisms and uncover novel signaling pathways that may prove very beneficial for the development of the next generation of precision-targeted personalized gene and stem cell-based therapies for the treatment of neurodegenerative disorders. We believe that CRISPR-mediated genetic engineering is a powerful new approach to develop novel and robust *in vivo* models of neurodegenerative disorders for improved rigor and reproducibility in preclinical studies.

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References

Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, Verdine V, Cox DBT, Kellner MJ, Regev A, Lander ES, Voytas DF, Ting AY, Zhang F (2017) RNA targeting with CRISPR-Cas13. Nature 550:280–284. [PubMed: 28976959]

Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DB, Shmakov S, Makarova KS, Semenova E, Minakhin L, Severinov K, Regev A, Lander ES, Koonin EV, Zhang F (2016) C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science 353:aaf5573. DOI: 10.1126/science.aaf5573. [PubMed: 27256883]

Agis-Balboa RC, Pinheiro PS, Rebola N, Kerimoglu C, Benito E, Gertig M, Bahari-Javan S, Jain G, Burkhardt S, Delalle I, Jatzko A, Dettenhofer M, Zunszain PA, Schmitt A, Falkai P, Pape JC, Binder EB, Mulle C, Fischer A, Sananbenesi F (2017) Formin 2 links neuropsychiatric phenotypes at young age to an increased risk for dementia. EMBO J 36:2815–2828. [PubMed: 28768717]

Ahmed ME, Iyer S, Thangavel R, Kempuraj D, Selvakumar GP, Raikwar SP, Zaheer S, Zaheer A (2017) Co-Localization of Glia Maturation Factor with NLRP3 Inflammasome and Autophagosome Markers in Human Alzheimer's Disease Brain. J Alzheimers Dis 60:1143–1160. [PubMed: 28984607]

Akter K, Lanza EA, Martin SA, Myronyuk N, Rua M, Raffa RB (2011) Diabetes mellitus and Alzheimer's disease: shared pathology and treatment? Br J Clin Pharmacol 71:365–376. [PubMed: 21284695]

Allen F, Crepaldi L, Alsinet C, Strong AJ, Kleshchevnikov V, De Angeli P, Palenikova P, Khodak A, Kiselev V, Kosicki M, Bassett AR, Harding H, Galanty Y, Munoz-Martinez F, Metzakopian E, Jackson SP, Parts L (2018) Predicting the mutations generated by repair of Cas9-induced double-strand breaks. Nat Biotechnol. DOI: 10.1038/nbt.4317.

Allende ML, Cook EK, Larman BC, Nugent A, Brady JM, Golebiowski D, Sena-Esteves M, Tifft CJ, Proia RL (2018) Cerebral organoids derived from Sandhoff disease-induced pluripotent stem cells exhibit impaired neurodifferentiation. J Lipid Res 59:550–563. [PubMed: 29358305]

An H, Skelt L, Notaro A, Highley JR, Fox AH, La Bella V, Buchman VL, Shelkovnikova TA (2019) ALS-linked FUS mutations confer loss and gain of function in the nucleus by promoting excessive formation of dysfunctional paraspeckles. Acta Neuropathol Commun 7:7. DOI: 10.1186/s40478-019-0658-x [PubMed: 30642400]

Arias-Fuenzalida J, Jarazo J, Qing X, Walter J, Gomez-Giro G, Nickels SL, Zaehres H, Scholer HR, Schwamborn JC (2017) FACS-Assisted CRISPR-Cas9 Genome Editing Facilitates Parkinson's Disease Modeling. Stem Cell Reports. DOI: 10.1016/j.stemcr.2017.08.026.

Arkinson C, Walden H (2018) Parkin function in Parkinson's disease. Science 360:267–268. [PubMed: 29674580]

Arthur LL, Chung JJ, Jankirama P, Keefer KM, Kolotilin I, Pavlovic-Djuranovic S, Chalker DL, Grbic V, Green R, Menassa R, True HL, Skeath JB, Djuranovic S (2017) Rapid generation of hypomorphic mutations. Nat Commun 8:14112. DOI: 10.1038/ncomms14112. [PubMed: 28106166]

Bae S, Park J, Kim JS (2014) Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics 30:1473–1475. [PubMed: 24463181]

Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR, Nance M, Ross CA, Scahill RI, Wetzel R, Wild EJ, Tabrizi SJ (2015) Huntington disease. Nat Rev Dis Primers 1:15005. DOI: 10.1038/nrdp.2015.5. [PubMed: 27188817]

Becher B, Spath S, Goverman J (2017) Cytokine networks in neuroinflammation. Nat Rev Immunol 17:49–59. [PubMed: 27916979]

Bhinge A, Namboori SC, Zhang X, VanDongen AMJ, Stanton LW (2017) Genetic Correction of SOD1 Mutant iPSCs Reveals ERK and JNK Activated AP1 as a Driver of Neurodegeneration in Amyotrophic Lateral Sclerosis. Stem Cell Reports 8:856–869. [PubMed: 28366453]

Boland B, Yu WH, Corti O, Mollereau B, Henriques A, Bezard E, Pastores GM, Rubinsztein DC, Nixon RA, Duchen MR, Mallucci GR, Kroemer G, Levine B, Eskelinen EL, Mochel F, Spedding M, Louis C, Martin OR, Millan MJ (2018) Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. Nat Rev Drug Discov 17:660–688. [PubMed: 30116051]

Bolukbasi MF, Gupta A, Wolfe SA (2016) Creating and evaluating accurate CRISPR-Cas9 scalpels for genomic surgery. Nat Methods 13:41–50. [PubMed: 26716561]

Breinig M, Schweitzer AY, Herianto AM, Revia S, Schaefer L, Wendler L, Cobos Galvez A, Tschaharganeh DF (2019) Multiplexed orthogonal genome editing and transcriptional activation by Cas12a. Nat Methods 16:51–54. [PubMed: 30559432]

Burstein D, Harrington LB, Strutt SC, Probst AJ, Anantharaman K, Thomas BC, Doudna JA, Banfield JF (2017) New CRISPR-Cas systems from uncultivated microbes. Nature 542:237–241. [PubMed: 28005056]

Cameron P et al. (2017) Mapping the genomic landscape of CRISPR-Cas9 cleavage. Nat Methods 14:600–606. [PubMed: 28459459]

Cao J, Hou J, Ping J, Cai D (2018) Advances in developing novel therapeutic strategies for Alzheimer's disease. Mol Neurodegener 13:64. DOI: 10.1186/s13024-018-0299-8. [PubMed: 30541602]

Chakrabarti AM, Henser-Brownhill T, Monserrat J, Poetsch AR, Luscombe NM, Scaffidi P (2018) Target-Specific Precision of CRISPR-Mediated Genome Editing. Mol Cell. DOI: 10.1016/j.molcel.2018.11.031.

Chang AN, Liang Z, Dai HQ, Chapdelaine-Williams AM, Andrews N, Bronson RT, Schwer B, Alt FW (2018) Neural blastocyst complementation enables mouse forebrain organogenesis. Nature 563:126–130. [PubMed: 30305734]

Chatterjee P, Jakimo N, Jacobson JM (2018) Minimal PAM specificity of a highly similar SpCas9 ortholog. Sci Adv 4:eaau0766. DOI: 10.1126/sciadv.aau0766. [PubMed: 30397647]

Chen Y, Singh Dolt K, Kriek M, Baker T, Downey P, Drummond NJ, Canham MA, Natalwala A, Rosser S, Kunath T (2018) Engineering synucleinopathy-resistant human dopaminergic neurons by CRISPR-mediated deletion of the SNCA gene. Eur J Neurosci. DOI: 10.1111/ejn.14286.

Cheng-Hathaway PJ, Reed-Geaghan EG, Jay TR, Casali BT, Bemiller SM, Puntambekar SS, von Saucken VE, Williams RY, Karlo JC, Moutinho M, Xu G, Ransohoff RM, Lamb BT, Landreth GE (2018) The Trem2 R47H variant confers loss-of-function-like phenotypes in Alzheimer's disease. Mol Neurodegener 13:29. DOI: 10.1186/s13024-018-0262-8. [PubMed: 29859094]

Cheng M, Jin X, Mu L, Wang F, Li W, Zhong X, Liu X, Shen W, Liu Y, Zhou Y (2016) Combination of the clustered regularly interspaced short palindromic repeats (CRISPR)-associated 9 technique with the piggybac transposon system for mouse in utero electroporation to study cortical development. J Neurosci Res 94:814–824. [PubMed: 27317429]

Chiang TW, le Sage C, Larrieu D, Demir M, Jackson SP (2016) CRISPR-Cas9(D10A) nickase-based genotypic and phenotypic screening to enhance genome editing. Sci Rep 6:24356. DOI: 10.1038/srep24356. [PubMed: 27079678]

Chitnis T, Weiner HL (2017) CNS inflammation and neurodegeneration. J Clin Invest 127:3577–3587. [PubMed: 28872464]

Cho SW, Kim S, Kim Y, Kweon J, Kim HS, Bae S, Kim JS (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res 24:132–141. [PubMed: 24253446]

Chung CY et al. (2013) Identification and rescue of alpha-synuclein toxicity in Parkinson patient-derived neurons. Science 342:983–987. [PubMed: 24158904]

Claes C, Van Den Daele J, Boon R, Schouteden S, Colombo A, Monasor LS, Fiers M, Ordovas L, Nami F, Bohrmann B, Tahirovic S, De Strooper B, Verfaillie CM (2018) Human stem cell-derived monocytes and microglia-like cells reveal impaired amyloid plaque clearance upon heterozygous or homozygous loss of TREM2. Alzheimers Dement. DOI: 10.1016/j.jalz.2018.09.006.

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339:819–823. [PubMed: 23287718]

Coppola G et al. (2012) Evidence for a role of the rare p.A152T variant in MAPT in increasing the risk for FTD-spectrum and Alzheimer's diseases. Hum Mol Genet 21:3500–3512. [PubMed: 22556362]

Costa AS, Agostini S, Guerini FR, Mancuso R, Zanzottera M, Ripamonti E, Racca V, Nemni R, Clerici M (2017) Modulation of Immune Responses to Herpes Simplex Virus Type 1 by IFNL3 and IRF7 Polymorphisms: A Study in Alzheimer's Disease. J Alzheimers Dis 60:1055–1063. [PubMed: 28984602]

Cox DBT, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, Zhang F (2017) RNA editing with CRISPR-Cas13. Science. DOI: 10.1126/science.aaq0180.

Craft S, Watson GS (2004) Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol 3:169–178. [PubMed: 14980532]

Cromer MK, Vaidyanathan S, Ryan DE, Curry B, Lucas AB, Camarena J, Kaushik M, Hay SR, Martin RM, Steinfeld I, Bak RO, Dever DP, Hendel A, Bruhn L, Porteus MH (2018) Global Transcriptional Response to CRISPR/Cas9-AAV6-Based Genome Editing in CD34(+) Hematopoietic Stem and Progenitor Cells. Mol Ther 26:2431–2442. [PubMed: 30005866]

de Solis CA, Ho A, Holehonnur R, Ploski JE (2016) The Development of a Viral Mediated CRISPR/Cas9 System with Doxycycline Dependent gRNA Expression for Inducible In vitro and In vivo

Genome Editing. Front Mol Neurosci 9:70. DOI: 10.3389/fnmol.2016.00070. [PubMed: 27587996]

Decker JM, Kruger L, Sydow A, Dennissen FJ, Siskova Z, Mandelkow E, Mandelkow EM (2016) The Tau/A152T mutation, a risk factor for frontotemporal-spectrum disorders, leads to NR2B receptor-mediated excitotoxicity. EMBO Rep 17:552–569. [PubMed: 26931569]

Deneault E et al. (2018) Complete Disruption of Autism-Susceptibility Genes by Gene Editing Predominantly Reduces Functional Connectivity of Isogenic Human Neurons. Stem Cell Reports 11:1211–1225. [PubMed: 30392976]

di Domenico A, Carola G, Calatayud C, Pons-Espinal M, Munoz JP, Richaud-Patin Y, Fernandez-Carasa I, Gut M, Faella A, Parameswaran J, Soriano J, Ferrer I, Tolosa E, Zorzano A, Cuervo AM, Raya A, Consiglio A (2019) Patient-Specific iPSC-Derived Astrocytes Contribute to Non-Cell-Autonomous Neurodegeneration in Parkinson's Disease. Stem Cell Reports. DOI: 10.1016/j.stemcr.2018.12.011.

Dickson DW, Heckman MG, Murray ME, Soto AI, Walton RL, Diehl NN, van Gerpen JA, Uitti RJ, Wszolek ZK, Ertekin-Taner N, Knopman DS, Petersen RC, Graff-Radford NR, Boeve BF, Bu G, Ferman TJ, Ross OA (2018) APOE epsilon4 is associated with severity of Lewy body pathology independent of Alzheimer pathology. Neurology 91:e1182–e1195. [PubMed: 30143564]

Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, Washicosky KJ, Gyorgy B, Breakefield XO, Tanzi RE, Moir RD (2018) Alzheimer's Disease-Associated beta-Amyloid Is Rapidly Seeded by Herpesviridae to Protect against Brain Infection. Neuron 99:56–63 e53. [PubMed: 30001512]

Esvelt KM, Mali P, Braff JL, Moosburner M, Yaung SJ, Church GM (2013) Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. Nat Methods 10:1116–1121. [PubMed: 24076762]

Fratta P et al. (2018) Mice with endogenous TDP-43 mutations exhibit gain of splicing function and characteristics of amyotrophic lateral sclerosis. EMBO J 37. DOI: 10.15252/embj.201798684.

Frederiksen HR, Holst B, Ramakrishna S, Muddashetty R, Schmid B, Freude K (2018) Generation of two iPSC lines with either a heterozygous V717I or a heterozygous KM670/671NL mutation in the APP gene. Stem Cell Res 34:101368. DOI: 10.1016/j.scr.2018.101368. [PubMed: 30634129]

Frock RL, Hu J, Meyers RM, Ho YJ, Kii E, Alt FW (2015) Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. Nat Biotechnol 33:179–186. [PubMed: 25503383]

Fu Y, Sander JD, Reyon D, Cascio VM, Joung JK (2014) Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol 32:279–284. [PubMed: 24463574]

Fung TC, Olson CA, Hsiao EY (2017) Interactions between the microbiota, immune and nervous systems in health and disease. Nat Neurosci 20:145–155. [PubMed: 28092661]

Gaj T, Ojala DS, Ekman FK, Byrne LC, Limsirichai P, Schaffer DV (2017a) In vivo genome editing improves motor function and extends survival in a mouse model of ALS. Sci Adv 3:eaar3952. DOI: 10.1126/sciadv.aar3952. [PubMed: 29279867]

Gaj T, Staahl BT, Rodrigues GMC, Limsirichai P, Ekman FK, Doudna JA, Schaffer DV (2017b) Targeted gene knock-in by homology-directed genome editing using Cas9 ribonucleoprotein and AAV donor delivery. Nucleic Acids Res 45:e98. DOI: 10.1093/nar/gkx154. [PubMed: 28334779]

Gao L, Cox DBT, Yan WX, Manteiga JC, Schneider MW, Yamano T, Nishimasu H, Nureki O, Crosetto N, Zhang F (2017) Engineered Cpf1 variants with altered PAM specificities. Nat Biotechnol 35:789–792. [PubMed: 28581492]

Garcia-Leon JA, Cabrera-Socorro A, Eggermont K, Swijsen A, Terryn J, Fazal R, Nami F, Ordovas L, Quiles A, Lluis F, Serneels L, Wierda K, Sierksma A, Kreir M, Pestana F, Van Damme P, De Strooper B, Thorrez L, Ebneth A, Verfaillie CM (2018) Generation of a human induced pluripotent stem cell-based model for tauopathies combining three microtubule-associated protein TAU mutations which displays several phenotypes linked to neurodegeneration. Alzheimers Dement 14:1261–1280. [PubMed: 30036493]

Garcia-Reitboeck P, Phillips A, Piers TM, Villegas-Llerena C, Butler M, Mallach A, Rodrigues C, Arber CE, Heslegrave A, Zetterberg H, Neumann H, Neame S, Houlden H, Hardy J, Pocock JM (2018) Human Induced Pluripotent Stem Cell-Derived Microglia-Like Cells Harboring TREM2

Missense Mutations Show Specific Deficits in Phagocytosis. Cell Rep 24:2300–2311. [PubMed: 30157425]

Garneau JE, Dupuis ME, Villion M, Romero DA, Barrangou R, Boyaval P, Fremaux C, Horvath P, Magadan AH, Moineau S (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468:67–71. [PubMed: 21048762]

Garza JC, Qi X, Gjeluci K, Leussis MP, Basu H, Reis SA, Zhao WN, Piguel NH, Penzes P, Haggarty SJ, Martens GJ, Poelmans G, Petryshen TL (2018) Disruption of the psychiatric risk gene Ankyrin 3 enhances microtubule dynamics through GSK3/CRMP2 signaling. Transl Psychiatry 8:135. DOI: 10.1038/s41398-018-0182-y. [PubMed: 30046097]

Gentil BJ, O'Ferrall E, Chalk C, Santana LF, Durham HD, Massie R (2017) A New Mutation in FIG4 Causes a Severe Form of CMT4J Involving TRPV4 in the Pathogenic Cascade. J Neuropathol Exp Neurol 76:789–799. [PubMed: 28859335]

Gessler DJ, Li D, Xu H, Su Q, Sanmiguel J, Tuncer S, Moore C, King J, Matalon R, Gao G (2017) Redirecting N-acetylaspartate metabolism in the central nervous system normalizes myelination and rescues Canavan disease. JCI Insight 2:e90807. DOI: 10.1172/jci.insight.90807. [PubMed: 28194442]

Giaime E, Tong Y, Wagner LK, Yuan Y, Huang G, Shen J (2017) Age-Dependent Dopaminergic Neurodegeneration and Impairment of the Autophagy-Lysosomal Pathway in LRRK-Deficient Mice. Neuron 96:796–807 e796. [PubMed: 29056298]

Goedert M, Jakes R, Spillantini MG, Hasegawa M, Smith MJ, Crowther RA (1996) Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. Nature 383:550–553. [PubMed: 8849730]

Gonatopoulos-Pournatzis T, Wu M, Braunschweig U, Roth J, Han H, Best AJ, Raj B, Aregger M, O'Hanlon D, Ellis JD, Calarco JA, Moffat J, Gingras AC, Blencowe BJ (2018) Genome-wide CRISPR-Cas9 Interrogation of Splicing Networks Reveals a Mechanism for Recognition of Autism-Misregulated Neuronal Microexons. Mol Cell 72:510–524 e512. [PubMed: 30388412]

Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F (2018) Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 360:439–444. [PubMed: 29449508]

Gopalappa R, Suresh B, Ramakrishna S, Kim HH (2018) Paired D10A Cas9 nickases are sometimes more efficient than individual nucleases for gene disruption. Nucleic Acids Res 46:e71. DOI: 10.1093/nar/gky222. [PubMed: 29584876]

Gordon R, Albornoz EA, Christie DC, Langley MR, Kumar V, Mantovani S, Robertson AAB, Butler MS, Rowe DB, O'Neill LA, Kanthasamy AG, Schroder K, Cooper MA, Woodruff TM (2018) Inflammasome inhibition prevents alpha-synuclein pathology and dopaminergic neurodegeneration in mice. Sci Transl Med 10. DOI: 10.1126/scitranslmed.aah4066.

Guerreiro R et al. (2018) Investigating the genetic architecture of dementia with Lewy bodies: a two-stage genome-wide association study. Lancet Neurol 17:64–74. [PubMed: 29263008]

Guilinger JP, Thompson DB, Liu DR (2014) Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat Biotechnol 32:577–582. [PubMed: 24770324]

Guo W et al. (2017) HDAC6 inhibition reverses axonal transport defects in motor neurons derived from FUS-ALS patients. Nat Commun 8:861. DOI: 10.1038/s41467-017-00911-y. [PubMed: 29021520]

Gyorgy B, Loov C, Zaborowski MP, Takeda S, Kleinstiver BP, Commins C, Kastanenka K, Mu D, Volak A, Giedraitis V, Lannfelt L, Maguire CA, Joung JK, Hyman BT, Breakefield XO, Ingelsson M (2018) CRISPR/Cas9 Mediated Disruption of the Swedish APP Allele as a Therapeutic Approach for Early-Onset Alzheimer's Disease. Mol Ther Nucleic Acids 11:429–440. [PubMed: 29858078]

Haney MS et al. (2018) Identification of phagocytosis regulators using magnetic genome-wide CRISPR screens. Nat Genet 50:1716–1727. [PubMed: 30397336]

Harold D et al. (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41:1088–1093. [PubMed: 19734902]

Harrington LB, Burstein D, Chen JS, Paez-Espino D, Ma E, Witte IP, Cofsky JC, Kyrpides NC, Banfield JF, Doudna JA (2018) Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. Science 362:839–842. [PubMed: 30337455]

Hautbergue GM et al. (2017) SRSF1-dependent nuclear export inhibition of C9ORF72 repeat transcripts prevents neurodegeneration and associated motor deficits. Nat Commun 8:16063. DOI: 10.1038/ncomms16063. [PubMed: 28677678]

Heman-Ackah SM, Bassett AR, Wood MJ (2016) Precision Modulation of Neurodegenerative Disease-Related Gene Expression in Human iPSC-Derived Neurons. Sci Rep 6:28420. [PubMed: 27341390]

Heman-Ackah SM, Manzano R, Hoozemans JJM, Scheper W, Flynn R, Haerty W, Cowley SA, Bassett AR, Wood MJA (2017) Alpha-synuclein induces the unfolded protein response in Parkinson's disease SNCA triplication iPSC-derived neurons. Hum Mol Genet 26:4441–4450. [PubMed: 28973645]

Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A, Axt D, Remus A, Tzeng TC, Gelpi E, Halle A, Korte M, Latz E, Golenbock DT (2013) NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493:674–678. [PubMed: 23254930]

Hirano H, Gootenberg JS, Horii T, Abudayyeh OO, Kimura M, Hsu PD, Nakane T, Ishitani R, Hatada I, Zhang F, Nishimasu H, Nureki O (2016) Structure and Engineering of Francisella novicida Cas9. Cell 164:950–961. [PubMed: 26875867]

Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP Kotzbauer PT, Miller TM, Papy-Garcia D, Diamond MI (2013) Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. Proc Natl Acad Sci U S A 110:E3138–3147. [PubMed: 23898162]

Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. Science 327:167–170. [PubMed: 20056882]

Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, Zeina CM, Gao X, Rees HA, Lin Z, Liu DR (2018a) Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature 556:57–63. [PubMed: 29512652]

Hu S, Knowlton RC, Watson BO, Glanowska KM, Murphy GG, Parent JM, Wang Y (2018b) Somatic Depdc5 deletion recapitulates electroclinical features of human focal cortical dysplasia type IIA. Ann Neurol 84:140–146. [PubMed: 30080265]

Huang YA, Zhou B, Wernig M, Sudhof TC (2017) ApoE2, ApoE3, and ApoE4 Differentially Stimulate APP Transcription and Abeta Secretion. Cell 168:427–441 e421. [PubMed: 28111074]

Hughes J, Dawson R, Tea M, McAninch D, Piltz S, Jackson D, Stewart L, Ricos MG, Dibbens LM, Harvey NL, Thomas P (2017) Knockout of the epilepsy gene Depdc5 in mice causes severe embryonic dysmorphology with hyperactivity of mTORC1 signalling. Sci Rep 7:12618. DOI: 10.1038/s41598-017-12574-2. [PubMed: 28974734]

Hung COY, Livesey FJ (2018) Altered gamma-Secretase Processing of APP Disrupts Lysosome and Autophagosome Function in Monogenic Alzheimer's Disease. Cell Rep 25:3647–3660 e3642. [PubMed: 30590039]

Imamura K, Sahara N, Kanaan NM, Tsukita K, Kondo T, Kutoku Y, Ohsawa Y, Sunada Y, Kawakami K, Hotta A, Yawata S, Watanabe D, Hasegawa M, Trojanowski JQ, Lee VM, Suhara T, Higuchi M, Inoue H (2016) Calcium dysregulation contributes to neurodegeneration in FTLD patient iPSC-derived neurons. Sci Rep 6:34904. DOI: 10.1038/srep34904. [PubMed: 27721502]

Inoue K, Oliveira LMA, Abeliovich A (2017) CRISPR Transcriptional Activation Analysis Unmasks an Occult gamma-Secretase Processivity Defect in Familial Alzheimer's Disease Skin Fibroblasts. Cell Rep 21:1727–1736. [PubMed: 29141208]

Inoue N, Ogura S, Kasai A, Nakazawa T, Ikeda K, Higashi S, Isotani A, Baba K, Mochizuki H, Fujimura H, Ago Y, Hayata-Takano A, Seiriki K, Shintani Y, Shintani N, Hashimoto H (2018) Knockdown of the mitochondria-localized protein p13 protects against experimental parkinsonism. EMBO Rep 19. DOI: 10.15252/embr.201744860.

Jiang S, Wen N, Li Z, Dube U, Del Aguila J, Budde J, Martinez R, Hsu S, Fernandez MV, Cairns NJ, Dominantly Inherited Alzheimer N, International FTDGC, Harari O, Cruchaga C, Karch CM

(2018) Integrative system biology analyses of CRISPR-edited iPSC-derived neurons and human brains reveal deficiencies of presynaptic signaling in FTLD and PSP. Transl Psychiatry 8:265. DOI: 10.1038/s41398-018-0319-z. [PubMed: 30546007]

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821. [PubMed: 22745249]

Jo A, Ham S, Lee GH, Lee YI, Kim S, Lee YS, Shin JH, Lee Y (2015) Efficient Mitochondrial Genome Editing by CRISPR/Cas9. Biomed Res Int 2015:305716. DOI: 10.1155/2015/305716. [PubMed: 26448933]

Kaczmarczyk L, Mende Y, Zevnik B, Jackson WS (2016) Manipulating the Prion Protein Gene Sequence and Expression Levels with CRISPR/Cas9. PLoS One 11:e0154604. [PubMed: 27128441]

Kaeser SA, Herzig MC, Coomaraswamy J, Kilger E, Selenica ML, Winkler DT, Staufenbiel M, Levy E, Grubb A, Jucker M (2007) Cystatin C modulates cerebral beta-amyloidosis. Nat Genet 39:1437–1439. [PubMed: 18026102]

Kalebic N, Taverna E, Tavano S, Wong FK, Suchold D, Winkler S, Huttner WB, Sarov M (2016) CRISPR/Cas9-induced disruption of gene expression in mouse embryonic brain and single neural stem cells in vivo. EMBO Rep 17:338–348. [PubMed: 26758805]

Kalhor R, Mali P, Church GM (2017) Rapidly evolving homing CRISPR barcodes. Nat Methods 14:195–200. [PubMed: 27918539]

Kalhor R, Kalhor K, Mejia L, Leeper K, Graveline A, Mali P, Church GM (2018) Developmental barcoding of whole mouse via homing CRISPR. Science 361. DOI: 10.1126/science.aat9804.

Kandimalla R, Thirumala V, Reddy PH (2017) Is Alzheimer's disease a Type 3 Diabetes? A critical appraisal. Biochim Biophys Acta 1863:1078–1089.

Kantor B, Tagliafierro L, Gu J, Zamora ME, Ilich E, Grenier C, Huang ZY, Murphy S, Chiba-Falek O (2018) Downregulation of SNCA Expression by Targeted Editing of DNA Methylation: A Potential Strategy for Precision Therapy in PD. Mol Ther 26:2638–2649. [PubMed: 30266652]

Kara E, Ling H, Pittman AM, Shaw K, de Silva R, Simone R, Holton JL, Warren JD, Rohrer JD, Xiromerisiou G, Lees A, Hardy J, Houlden H, Revesz T (2012) The MAPT p.A152T variant is a risk factor associated with tauopathies with atypical clinical and neuropathological features. Neurobiol Aging 33:2231 e2237–2231 e2214.

Kempuraj D, Khan MM, Thangavel R, Xiong Z, Yang E, Zaheer A (2013) Glia maturation factor induces interleukin-33 release from astrocytes: implications for neurodegenerative diseases. J Neuroimmune Pharmacol 8:643–650. [PubMed: 23397250]

Kempuraj D, Thangavel R, Yang E, Pattani S, Zaheer S, Santillan DA, Santillan MK, Zaheer A (2015) Dopaminergic Toxin 1-Methyl-4-Phenylpyridinium, Proteins alpha-Synuclein and Glia Maturation Factor Activate Mast Cells and Release Inflammatory Mediators. PLoS One 10:e0135776. [PubMed: 26275153]

Kempuraj D, Selvakumar GP, Zaheer S, Thangavel R, Ahmed ME, Raikwar S, Govindarajan R, Iyer S, Zaheer A (2018a) Cross-Talk between Glia, Neurons and Mast Cells in Neuroinflammation Associated with Parkinson's Disease. J Neuroimmune Pharmacol 13:100–112. [PubMed: 28952015]

Kempuraj D, Selvakumar GP, Thangavel R, Ahmed ME, Zaheer S, Kumar KK, Yelam A, Kaur H, Dubova I, Raikwar SP, Iyer SS, Zaheer A (2018b) Glia Maturation Factor and Mast Cell-Dependent Expression of Inflammatory Mediators and Proteinase Activated Receptor-2 in Neuroinflammation. J Alzheimers Dis 66:1117–1129. [PubMed: 30372685]

Kempuraj D, Thangavel R, Selvakumar GP, Ahmed ME, Zaheer S, Raikwar SP, Zahoor H, Saeed D, Dubova I, Giler G, Herr S, Iyer SS, Zaheer A (2018c) Mast Cell Proteases Activate Astrocytes and Glia-Neurons and Release Interleukin-33 by Activating p38 and ERK1/2 MAPKs and NF-kappaB. Mol Neurobiol. DOI: 10.1007/s12035-018-1177-7.

Khan MM, Kempuraj D, Zaheer S, Zaheer A (2014a) Glia maturation factor deficiency suppresses 1-methyl-4-phenylpyridinium-induced oxidative stress in astrocytes. J Mol Neurosci 53:590–599. [PubMed: 24430624]

Khan MM, Zaheer S, Nehman J, Zaheer A (2014b) Suppression of glia maturation factor expression prevents 1-methyl-4-phenylpyridinium (MPP(+))-induced loss of mesencephalic dopaminergic neurons. Neuroscience 277:196–205. [PubMed: 25016212]

Khan MM, Zaheer S, Thangavel R, Patel M, Kempuraj D, Zaheer A (2015) Absence of glia maturation factor protects dopaminergic neurons and improves motor behavior in mouse model of parkinsonism. Neurochem Res 40:980–990. [PubMed: 25754447]

Kichev A, Eede P, Gressens P, Thornton C, Hagberg H (2017) Implicating Receptor Activator of NF-kappaB (RANK)/RANK Ligand Signalling in Microglial Responses to Toll-Like Receptor Stimuli. Dev Neurosci 39:192–206. [PubMed: 28402971]

Kim D, Bae S, Park J, Kim E, Kim S, Yu HR, Hwang J, Kim JI, Kim JS (2015) Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. Nat Methods 12:237–243, 231 p following 243. [PubMed: 25664545]

Kim E, Koo T, Park SW, Kim D, Kim K, Cho HY, Song DW, Lee KJ, Jung MH, Kim S, Kim JH, Kim JH, Kim JS (2017) In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni. Nat Commun 8:14500. DOI: 10.1038/ncomms14500. [PubMed: 28220790]

Kim S, Kim D, Cho SW, Kim J, Kim JS (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. Genome Res 24:1012–1019. [PubMed: 24696461]

Kim S, Yun SP, Lee S, Umanah GE, Bandaru VVR, Yin X, Rhee P, Karuppagounder SS, Kwon SH, Lee H, Mao X, Kim D, Pandey A, Lee G, Dawson VL, Dawson TM, Ko HS (2018) GBA1 deficiency negatively affects physiological alpha-synuclein tetramers and related multimers. Proc Natl Acad Sci U S A 115:798–803. [PubMed: 29311330]

Kleinberger G, Brendel M, Mracsko E, Wefers B, Groeneweg L, Xiang X, Focke C, Deussing M, Suarez-Calvet M, Mazaheri F, Parhizkar S, Pettkus N, Wurst W, Feederle R, Bartenstein P, Mueggler T, Arzberger T, Knuesel I, Rominger A, Haass C (2017) The FTD-like syndrome causing TREM2 T66M mutation impairs microglia function, brain perfusion, and glucose metabolism. EMBO J 36:1837–1853. [PubMed: 28559417]

Kleinstiver BP, Prew MS, Tsai SQ, Nguyen NT, Topkar VV, Zheng Z, Joung JK (2015a) Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition. Nat Biotechnol 33:1293–1298. [PubMed: 26524662]

Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, Joung JK (2016) High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529:490–495. [PubMed: 26735016]

Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzales AP, Li Z, Peterson RT, Yeh JR, Aryee MJ, Joung JK (2015b) Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature 523:481–485. [PubMed: 26098369]

Kolli N, Lu M, Maiti P, Rossignol J, Dunbar GL (2017) CRISPR-Cas9 Mediated Gene-Silencing of the Mutant Huntingtin Gene in an In Vitro Model of Huntington's Disease. Int J Mol Sci 18.

Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533:420–424. [PubMed: 27096365]

Konermann S, Lotfy P, Brideau NJ, Oki J, Shokhirev MN, Hsu PD (2018) Transcriptome Engineering with RNA-Targeting Type VI-D CRISPR Effectors. Cell 173:665–676 e614. [PubMed: 29551272]

Kramer NJ, Haney MS, Morgens DW, Jovicic A, Couthouis J, Li A, Ousey J, Ma R, Bieri G, Tsui CK, Shi Y, Hertz NT, Tessier-Lavigne M, Ichida JK, Bassik MC, Gitler AD (2018) CRISPR-Cas9 screens in human cells and primary neurons identify modifiers of C9ORF72 dipeptide-repeat-protein toxicity. Nat Genet 50:603–612. [PubMed: 29507424]

Krasemann S et al. (2017) The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. Immunity 47:566–581 e569. [PubMed: 28930663]

Krauthausen M, Kummer MP, Zimmermann J, Reyes-Irisarri E, Terwel D, Bulic B, Heneka MT, Muller M (2015) CXCR3 promotes plaque formation and behavioral deficits in an Alzheimer's disease model. J Clin Invest 125:365–378. [PubMed: 25500888]

Kristen H, Sastre I, Munoz-Galdeano T, Recuero M, Aldudo J, Bullido MJ (2018) The lysosome system is severely impaired in a cellular model of neurodegeneration induced by HSV-1 and oxidative stress. Neurobiol Aging 68:5–17. [PubMed: 29689425]

Kuliyev E, Gingras S, Guy CS, Howell S, Vogel P, Pelletier S (2018) Overlapping Role of SCYL1 and SCYL3 in Maintaining Motor Neuron Viability. J Neurosci. DOI: 10.1523/JNEUROSCI.2282-17.2018.

Kumar DK, Choi SH, Washicosky KJ, Eimer WA, Tucker S, Ghofrani J, Lefkowitz A, McColl G, Goldstein LE, Tanzi RE, Moir RD (2016) Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. Sci Transl Med 8:340ra372. DOI: 10.1126/scitranslmed.aaf1059.

Kumar N, Stanford W, de Solis C, Aradhana, Abraham ND, Dao TJ, Thaseen S, Sairavi A, Gonzalez CU, Ploski JE (2018) The Development of an AAV-Based CRISPR SaCas9 Genome Editing System That Can Be Delivered to Neurons in vivo and Regulated via Doxycycline and Cre-Recombinase. Front Mol Neurosci 11:413. DOI: 10.3389/fnmol.2018.00413. [PubMed: 30483052]

Labbe C et al. (2015) Role for the microtubule-associated protein tau variant p.A152T in risk of alpha-synucleinopathies. Neurology 85:1680–1686. [PubMed: 26333800]

Lagier-Tourenne C et al. (2012) Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. Nat Neurosci 15:1488–1497. [PubMed: 23023293]

Lall D, Baloh RH (2017) Microglia and C9orf72 in neuroinflammation and ALS and frontotemporal dementia. J Clin Invest 127:3250–3258. [PubMed: 28737506]

Lambert JC et al. (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41:1094–1099. [PubMed: 19734903]

Langley MR, Ghaisas S, Ay M, Luo J, Palanisamy BN, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG (2017) Manganese exposure exacerbates progressive motor deficits and neurodegeneration in the MitoPark mouse model of Parkinson's disease: Relevance to gene and environment interactions in metal neurotoxicity. Neurotoxicology. DOI: 10.1016/j.neuro.2017.06.002.

Lazic D, Sagare AP, Nikolakopoulou AM, Griffin JH, Vassar R, Zlokovic BV (2019) 3K3A-activated protein C blocks amyloidogenic BACE1 pathway and improves functional outcome in mice. J Exp Med. DOI: 10.1084/jem.20181035.

Lazzarotto CR, Nguyen NT, Tang X, Malagon-Lopez J, Guo JA, Aryee MJ, Joung JK, Tsai SQ (2018) Defining CRISPR-Cas9 genome-wide nuclease activities with CIRCLE-seq. Nat Protoc 13:2615–2642. [PubMed: 30341435]

Lee CM, Cradick TJ, Bao G (2016) The Neisseria meningitidis CRISPR-Cas9 System Enables Specific Genome Editing in Mammalian Cells. Mol Ther 24:645–654. [PubMed: 26782639]

Lee JK, Jeong E, Lee J, Jung M, Shin E, Kim YH, Lee K, Jung I, Kim D, Kim S, Kim JS (2018a) Directed evolution of CRISPR-Cas9 to increase its specificity. Nat Commun 9:3048. DOI: 10.1038/s41467-018-05477-x. [PubMed: 30082838]

Lee MH, Siddoway B, Kaeser GE, Segota I, Rivera R, Romanow WJ, Liu CS, Park C, Kennedy G, Long T, Chun J (2018b) Somatic APP gene recombination in Alzheimer's disease and normal neurons. Nature 563:639–645. [PubMed: 30464338]

Li L, Roh JH, Chang EH, Lee Y, Lee S, Kim M, Koh W, Chang JW, Kim HJ, Nakanishi M, Barker RA, Na DL, Song J (2018) iPSC Modeling of Presenilin1 Mutation in Alzheimer's Disease with Cerebellar Ataxia. Exp Neurobiol 27:350–364. [PubMed: 30429645]

Li M, Zhao H, Ananiev GE, Musser MT, Ness KH, Maglaque DL, Saha K, Bhattacharyya A, Zhao X (2017) Establishment of Reporter Lines for Detecting Fragile X Mental Retardation (FMR1) Gene Reactivation in Human Neural Cells. Stem Cells 35:158–169. [PubMed: 27422057]

Lim R, Zaheer A (1991) Structure and function of glia maturation factor beta. Adv Exp Med Biol 296:161–164. [PubMed: 1781325]

Lim R, Miller JF, Zaheer A (1989) Purification and characterization of glia maturation factor beta: a growth regulator for neurons and glia. Proc Natl Acad Sci U S A 86:3901–3905. [PubMed: 2726756]

Lim R, Zaheer A, Lane WS (1990) Complete amino acid sequence of bovine glia maturation factor beta. Proc Natl Acad Sci U S A 87:5233–5237. [PubMed: 2196564]

Lin Y, Cradick TJ, Brown MT, Deshmukh H, Ranjan P, Sarode N, Wile BM, Vertino PM, Stewart FJ, Bao G (2014) CRISPR/Cas9 systems have off-target activity with insertions or deletions between target DNA and guide RNA sequences. Nucleic Acids Res 42:7473–7485. [PubMed: 24838573]

Lin YT et al. (2018) APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types. Neuron. DOI: 10.1016/j.neuron.2018.05.008.

Liu J, Gao C, Chen W, Ma W, Li X, Shi Y, Zhang H, Zhang L, Long Y, Xu H, Guo X, Deng S, Yan X, Yu D, Pan G, Chen Y, Lai L, Liao W, Li Z (2016a) CRISPR/Cas9 facilitates investigation of neural circuit disease using human iPSCs: mechanism of epilepsy caused by an SCN1A loss-of-function mutation. Transl Psychiatry 6:e703. DOI: 10.1038/tp.2015.203. [PubMed: 26731440]

Liu JJ, Orlova N, Oakes BL, Ma E, Spinner HB, Baney KLM, Chuck J, Tan D, Knott GJ, Harrington LB, Al-Shayeb B, Wagner A, Brotzmann J, Staahl BT, Taylor KL, Desmarais J, Nogales E, Doudna JA (2019) CasX enzymes comprise a distinct family of RNA-guided genome editors. Nature. DOI: 10.1038/s41586-019-0908-x.

Liu Y, Yu C, Daley TP, Wang F, Cao WS, Bhate S, Lin X, Still C 2nd, Liu H, Zhao D, Wang H, Xie XS, Ding S, Wong WH, Wernig M, Qi LS (2018) CRISPR Activation Screens Systematically Identify Factors that Drive Neuronal Fate and Reprogramming. Cell Stem Cell 23:758–771 e758. [PubMed: 30318302]

Liu Z et al. (2016b) Autism-like behaviours and germline transmission in transgenic monkeys overexpressing MeCP2. Nature 530:98–102. [PubMed: 26808898]

Lopez-Erauskin J et al. (2018) ALS/FTD-Linked Mutation in FUS Suppresses Intra-axonal Protein Synthesis and Drives Disease Without Nuclear Loss-of-Function of FUS. Neuron 100:816–830 e817. [PubMed: 30344044]

Lu IL, Chen C, Tung CY, Chen HH, Pan JP, Chang CH, Cheng JS, Chen YA, Wang CH, Huang CW, Kang YN, Chang HY, Li LL, Chang KP, Shih YH, Lin CH, Kwan SY, Tsai JW (2018) Identification of genes associated with cortical malformation using a transposon-mediated somatic mutagenesis screen in mice. Nat Commun 9:2498. DOI: 10.1038/s41467-018-04880-8. [PubMed: 29950674]

Magadan AH, Dupuis ME, Villion M, Moineau S (2012) Cleavage of phage DNA by the Streptococcus thermophilus CRISPR3-Cas system. PLoS One 7:e40913. [PubMed: 22911717]

Malankhanova TB, Malakhova AA, Medvedev SP, Zakian SM (2017) Modern Genome Editing Technologies in Huntington's Disease Research. J Huntingtons Dis 6:19–31. [PubMed: 28128770]

Mali P, Aach J, Stranges PB, Esvelt KM, Moosburner M, Kosuri S, Yang L, Church GM (2013a) CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat Biotechnol 31:833–838. [PubMed: 23907171]

Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM (2013b) RNA-guided human genome engineering via Cas9. Science 339:823–826. [PubMed: 23287722]

Malkki H (2016) Huntington disease: Selective deactivation of Huntington disease mutant allele by CRISPR-Cas9 gene editing. Nat Rev Neurol 12:614–615.

Marthaler AG, Schmid B, Tubsuwan A, Poulsen UB, Engelbrecht AF, Mau-Holzmann UA, Hyttel P, Nielsen JE, Nielsen TT, Holst B (2016a) Generation of an isogenic, gene-corrected control cell line of the spinocerebellar ataxia type 2 patient-derived iPSC line H196. Stem Cell Res 16:162–165. [PubMed: 27345804]

Marthaler AG, Tubsuwan A, Schmid B, Poulsen UB, Engelbrecht AF, Mau-Holzmann UA, Hyttel P, Nielsen TT, Nielsen JE, Holst B (2016b) Generation of an isogenic, gene-corrected control cell line of the spinocerebellar ataxia type 2 patient-derived iPSC line H266. Stem Cell Res 16:202–205. [PubMed: 27345815]

Marthaler AG, Schmid B, Tubsuwan A, Poulsen UB, Engelbrecht AF, Mau-Holzmann UA, Hyttel P, Nielsen JE, Nielsen TT, Holst B (2016c) Generation of an isogenic, gene-corrected control cell line of the spinocerebellar ataxia type 2 patient-derived iPSC line H271. Stem Cell Res 16:180–183. [PubMed: 27345809]

Marzi SJ, Leung SK, Ribarska T, Hannon E, Smith AR, Pishva E, Poschmann J, Moore K, Troakes C, Al-Sarraj S, Beck S, Newman S, Lunnon K, Schalkwyk LC, Mill J (2018) A histone acetylome-wide association study of Alzheimer's disease identifies disease-associated H3K27ac differences in the entorhinal cortex. Nat Neurosci 21:1618–1627. [PubMed: 30349106]

May PC, Lampert-Etchells M, Johnson SA, Poirier J, Masters JN, Finch CE (1990) Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. Neuron 5:831–839. [PubMed: 1702645]

Mehrabian M, Brethour D, MacIsaac S, Kim JK, Gunawardana CG, Wang H, Schmitt-Ulms G (2014) CRISPR-Cas9-based knockout of the prion protein and its effect on the proteome. PLoS One 9:e114594. [PubMed: 25490046]

Menzies FM et al. (2017) Autophagy and Neurodegeneration: Pathogenic Mechanisms and Therapeutic Opportunities. Neuron 93:1015–1034. [PubMed: 28279350]

Merienne N, Vachey G, de Longprez L, Meunier C, Zimmer V, Perriard G, Canales M, Mathias A, Herrgott L, Beltraminelli T, Maulet A, Dequesne T, Pythoud C, Rey M, Pellerin L, Brouillet E, Perrier AL, du Pasquier R, Deglon N (2017) The Self-Inactivating KamiCas9 System for the Editing of CNS Disease Genes. Cell Rep 20:2980–2991. [PubMed: 28930690]

Mi W, Pawlik M, Sastre M, Jung SS, Radvinsky DS, Klein AM, Sommer J, Schmidt SD, Nixon RA, Mathews PM, Levy E (2007) Cystatin C inhibits amyloid-beta deposition in Alzheimer's disease mouse models. Nat Genet 39:1440–1442. [PubMed: 18026100]

Mikuni T, Nishiyama J, Sun Y, Kamasawa N, Yasuda R (2016) High-Throughput, High-Resolution Mapping of Protein Localization in Mammalian Brain by In Vivo Genome Editing. Cell 165:1803–1817. [PubMed: 27180908]

Minami SS, Min SW, Krabbe G, Wang C, Zhou Y, Asgarov R, Li Y, Martens LH, Elia LP, Ward ME, Mucke L, Farese RV Jr., Gan L (2014) Progranulin protects against amyloid beta deposition and toxicity in Alzheimer's disease mouse models. Nat Med 20:1157–1164. [PubMed: 25261995]

Mohle L, Mattei D, Heimesaat MM, Bereswill S, Fischer A, Alutis M, French T, Hambardzumyan D, Matzinger P, Dunay IR, Wolf SA (2016) Ly6C(hi) Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. Cell Rep 15:1945–1956. [PubMed: 27210745]

Moller HD, Lin L, Xiang X, Petersen TS, Huang J, Yang L, Kjeldsen E, Jensen UB, Zhang X, Liu X, Xu X, Wang J, Yang H, Church GM, Bolund L, Regenberg B, Luo Y (2018) CRISPR-C: circularization of genes and chromosome by CRISPR in human cells. Nucleic Acids Res 46:e131. DOI: 10.1093/nar/gky767. [PubMed: 30551175]

Montagna C, Petris G, Casini A, Maule G, Franceschini GM, Zanella I, Conti L, Arnoldi F, Burrone OR, Zentilin L, Zacchigna S, Giacca M, Cereseto A (2018) VSV-G-Enveloped Vesicles for Traceless Delivery of CRISPR-Cas9. Mol Ther Nucleic Acids 12:453–462. [PubMed: 30195783]

Monteys AM, Ebanks SA, Keiser MS, Davidson BL (2017) CRISPR/Cas9 Editing of the Mutant Huntingtin Allele In Vitro and In Vivo. Mol Ther 25:12–23. [PubMed: 28129107]

Mor DE, Tsika E, Mazzulli JR, Gould NS, Kim H, Daniels MJ, Doshi S, Gupta P, Grossman JL, Tan VX, Kalb RG, Caldwell KA, Caldwell GA, Wolfe JH, Ischiropoulos H (2017) Dopamine induces soluble alpha-synuclein oligomers and nigrostriatal degeneration. Nat Neurosci 20:1560–1568. [PubMed: 28920936]

Moreno-Gonzalez I, Edwards Iii G, Salvadores N, Shahnawaz M, Diaz-Espinoza R, Soto C (2017) Molecular interaction between type 2 diabetes and Alzheimer's disease through cross-seeding of protein misfolding. Mol Psychiatry 22:1327–1334. [PubMed: 28044060]

Moreno CL, Della Guardia L, Shnyder V, Ortiz-Virumbrales M, Kruglikov I, Zhang B, Schadt EE, Tanzi RE, Noggle S, Buettner C, Gandy S (2018) iPSC-derived familial Alzheimer's PSEN2 (N141I) cholinergic neurons exhibit mutation-dependent molecular pathology corrected by insulin signaling. Mol Neurodegener 13:33. [PubMed: 29945658]

Myhrvold C et al. (2018) Field-deployable viral diagnostics using CRISPR-Cas13. Science 360:444–448. [PubMed: 29700266]

Nagata K, Takahashi M, Matsuba Y, Okuyama-Uchimura F, Sato K, Hashimoto S, Saito T, Saido TC (2018) Generation of App knock-in mice reveals deletion mutations protective against Alzheimer's disease-like pathology. Nat Commun 9:1800. DOI: 10.1038/s41467-018-04238-0. [PubMed: 29728560]

Nelles DA, Fang MY, O'Connell MR, Xu JL, Markmiller SJ, Doudna JA, Yeo GW (2016) Programmable RNA Tracking in Live Cells with CRISPR/Cas9. Cell 165:488–496. [PubMed: 26997482]

Neuner SM, Heuer SE, Huentelman MJ, O'Connell KMS, Kaczorowski CC (2018) Harnessing Genetic Complexity to Enhance Translatability of Alzheimer's Disease Mouse Models: A Path toward Precision Medicine. Neuron. DOI: 10.1016/j.neuron.2018.11.040.

Nguyen AD, Nguyen TA, Zhang J, Devireddy S, Zhou P, Karydas AM, Xu X, Miller BL, Rigo F, Ferguson SM, Huang EJ, Walther TC, Farese RV Jr., (2018) Murine knockin model for progranulin-deficient frontotemporal dementia with nonsense-mediated mRNA decay. Proc Natl Acad Sci U S A 115:E2849–E2858. [PubMed: 29511098]

Nishida K, Arazoe T, Yachie N, Banno S, Kakimoto M, Tabata M, Mochizuki M, Miyabe A, Araki M, Hara KY, Shimatani Z, Kondo A (2016) Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science 353. DOI: 10.1126/science.aaf8729.

Nishimasu H et al. (2018) Engineered CRISPR-Cas9 nuclease with expanded targeting space. Science 361:1259–1262. [PubMed: 30166441]

Nishiyama J (2018) Genome editing in the mammalian brain using the CRISPR-Cas system. Neurosci Res. DOI: 10.1016/j.neures.2018.07.003.

Nishiyama J, Mikuni T, Yasuda R (2017) Virus-Mediated Genome Editing via Homology-Directed Repair in Mitotic and Postmitotic Cells in Mammalian Brain. Neuron. DOI: 10.1016/j.neuron.2017.10.004.

Nozawa K, Hayashi A, Motohashi J, Takeo YH, Matsuda K, Yuzaki M (2018) Cellular and Subcellular Localization of Endogenous Neuroligin-1 in the Cerebellum. Cerebellum 17:709–721. [PubMed: 30046996]

Obara Y, Imai T, Sato H, Takeda Y, Kato T, Ishii K (2017) Midnolin is a novel regulator of parkin expression and is associated with Parkinson's Disease. Sci Rep 7:5885. DOI: 10.1038/s41598-017-05456-0. [PubMed: 28724963]

Ortiz-Virumbrales M, Moreno CL, Kruglikov I, Marazuela P, Sproul A, Jacob S, Zimmer M, Paull D, Zhang B, Schadt EE, Ehrlich ME, Tanzi RE, Arancio O, Noggle S, Gandy S (2017) CRISPR/Cas9-Correctable mutation-related molecular and physiological phenotypes in iPSC-derived Alzheimer's PSEN2 N141I neurons. Acta Neuropathol Commun 5:77. DOI: 10.1186/s40478-017-0475-z. [PubMed: 29078805]

Ouellet DL, Cherif K, Rousseau J, Tremblay JP (2017) Deletion of the GAA repeats from the human frataxin gene using the CRISPR-Cas9 system in YG8R-derived cells and mouse models of Friedreich ataxia. Gene Ther 24:265–274. [PubMed: 28024081]

Ouyang S, Xie Y, Xiong Z, Yang Y, Xian Y, Ou Z, Song B, Chen Y, Xie Y, Li H, Sun X (2018) CRISPR/Cas9-Targeted Deletion of Polyglutamine in Spinocerebellar Ataxia Type 3-Derived Induced Pluripotent Stem Cells. Stem Cells Dev 27:756–770. [PubMed: 29661116]

Palazon-Riquelme P, Worboys JD, Green J, Valera A, Martin-Sanchez F, Pellegrini C, Brough D, Lopez-Castejon G (2018) USP7 and USP47 deubiquitinases regulate NLRP3 inflammasome activation. EMBO Rep 19. DOI: 10.15252/embr.201744766.

Park CY, Halevy T, Lee DR, Sung JJ, Lee JS, Yanuka O, Benvenisty N, Kim DW (2015) Reversion of FMR1 Methylation and Silencing by Editing the Triplet Repeats in Fragile X iPSC-Derived Neurons. Cell Rep 13:234–241. [PubMed: 26440889]

Pascual-Caro C, Berrocal M, Lopez-Guerrero AM, Alvarez-Barrientos A, Pozo-Guisado E, Gutierrez-Merino C, Mata AM, Martin-Romero FJ (2018) STIM1 deficiency is linked to Alzheimer's disease and triggers cell death in SH-SY5Y cells by upregulation of L-type voltage-operated Ca(2+) entry. J Mol Med (Berl) 96:1061–1079. [PubMed: 30088035]

Petersen MA et al. (2017) Fibrinogen Activates BMP Signaling in Oligodendrocyte Progenitor Cells and Inhibits Remyelination after Vascular Damage. Neuron. DOI: 10.1016/j.neuron.2017.10.008.

Pinto BS, Saxena T, Oliveira R, Mendez-Gomez HR, Cleary JD, Denes LT, McConnell O, Arboleda J, Xia G, Swanson MS, Wang ET (2017) Impeding Transcription of Expanded Microsatellite Repeats by Deactivated Cas9. Mol Cell 68:479–490 e475. [PubMed: 29056323]

Platt RJ, Zhou Y, Slaymaker IM, Shetty AS, Weisbach NR, Kim JA, Sharma J, Desai M, Sood S, Kempton HR, Crabtree GR, Feng G, Zhang F (2017) Chd8 Mutation Leads to Autistic-like Behaviors and Impaired Striatal Circuits. Cell Rep 19:335–350. [PubMed: 28402856]

Potting C, Crochemore C, Moretti F, Nigsch F, Schmidt I, Manneville C, Carbone W, Knehr J, DeJesus R, Lindeman A, Maher R, Russ C, McAllister G, Reece-Hoyes JS, Hoffman GR, Roma G, Muller M, Sailer AW, Helliwell SB (2018) Genome-wide CRISPR screen for PARKIN regulators reveals transcriptional repression as a determinant of mitophagy. Proc Natl Acad Sci U S A 115:E180–E189. [PubMed: 29269392]

Prasuhn J, Martensson CU, Krajka V, Klein C, Rakovic A (2017) Genome-Edited, TH-expressing Neuroblastoma Cells as a Disease Model for Dopamine-Related Disorders: A Proof-of-Concept Study on DJ-1-deficient Parkinsonism. Front Cell Neurosci 11:426. DOI: 10.3389/fncel.2017.00426. [PubMed: 29379417]

Pugazhenthi S, Qin L, Reddy PH (2017) Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. Biochim Biophys Acta 1863:1037–1045.

Quadri M et al. (2018) LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study. Lancet Neurol 17:597–608. [PubMed: 29887161]

Raikwar SP, Thangavel R, Dubova I, Selvakumar GP, Ahmed ME, Kempuraj D, Zaheer SA, Iyer SS, Zaheer A (2018) Targeted Gene Editing of Glia Maturation Factor in Microglia: a Novel Alzheimer's Disease Therapeutic Target. Mol Neurobiol 10.1007/s12035-018-1068-y.

Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F (2013a) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8:2281–2308. [PubMed: 24157548]

Ran FA, Hsu PD, Lin CY, Gootenberg JS, Konermann S, Trevino AE, Scott DA, Inoue A, Matoba S, Zhang Y, Zhang F (2013b) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 154:1380–1389. [PubMed: 23992846]

Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F (2015) In vivo genome editing using Staphylococcus aureus Cas9. Nature 520:186–191. [PubMed: 25830891]

Rannals MD, Page SC, Campbell MN, Gallo RA, Mayfield B, Maher BJ (2016a) Neurodevelopmental models of transcription factor 4 deficiency converge on a common ion channel as a potential therapeutic target for Pitt Hopkins syndrome. Rare Dis 4:e1220468. DOI: 10.1080/21675511.2016.1220468. [PubMed: 28032012]

Rannals MD, Hamersky GR, Page SC, Campbell MN, Briley A, Gallo RA, Phan BN, Hyde TM, Kleinman JE, Shin JH, Jaffe AE, Weinberger DR, Maher BJ (2016b) Psychiatric Risk Gene Transcription Factor 4 Regulates Intrinsic Excitability of Prefrontal Neurons via Repression of SCN10a and KCNQ1. Neuron 90:43–55. [PubMed: 26971948]

Ransohoff RM (2016) How neuroinflammation contributes to neurodegeneration. Science 353:777–783. [PubMed: 27540165]

Readhead B, Haure-Mirande JV, Funk CC, Richards MA, Shannon P, Haroutunian V, Sano M, Liang WS, Beckmann ND, Price ND, Reiman EM, Schadt EE, Ehrlich ME, Gandy S, Dudley JT (2018) Multiscale Analysis of Independent Alzheimer's Cohorts Finds Disruption of Molecular, Genetic, and Clinical Networks by Human Herpesvirus. Neuron 99:64–82 e67. DOI: 10.1016/j.neuron.2018.05.023. [PubMed: 29937276]

Rees HA, Liu DR (2018) Base editing: precision chemistry on the genome and transcriptome of living cells. Nat Rev Genet 19:770–788. [PubMed: 30323312]

Ribierre T, Deleuze C, Bacq A, Baldassari S, Marsan E, Chipaux M, Muraca G, Roussel D, Navarro V, Leguern E, Miles R, Baulac S (2018) Second-hit mosaic mutation in mTORC1 repressor DEPDC5 causes focal cortical dysplasia-associated epilepsy. J Clin Invest 128:2452–2458. [PubMed: 29708508]

Rice HC, de Malmazet D, Schreurs A, Frere S, Van Molle I, Volkov AN, Creemers E, Vertkin I, Nys J, Ranaivoson FM, Comoletti D, Savas JN, Remaut H, Balschun D, Wierda KD, Slutsky I, Farrow K, De Strooper B, de Wit J (2019) Secreted amyloid-beta precursor protein functions as a GABABR1a ligand to modulate synaptic transmission. Science 363. DOI: 10.1126/science.aao4827.

Richter F, Fonfara I, Bouazza B, Schumacher CH, Bratovic M, Charpentier E, Moglich A (2016) Engineering of temperature- and light-switchable Cas9 variants. Nucleic Acids Res 44:10003–10014. [PubMed: 27744350]

Robbins JP, Perfect L, Ribe EM, Maresca M, Dangla-Valls A, Foster EM, Killick R, Nowosiad P, Reid MJ, Polit LD, Nevado AJ, Ebner D, Bohlooly YM, Buckley N, Pangalos MN, Price J, Lovestone S (2018) Clusterin Is Required for beta-Amyloid Toxicity in Human iPSC-Derived Neurons. Front Neurosci 12:504. [PubMed: 30090055]

Rosas-Vidal LE, Lozada-Miranda V, Cantres-Rosario Y, Vega-Medina A, Melendez L, Quirk GJ (2018) Alteration of BDNF in the medial prefrontal cortex and the ventral hippocampus impairs extinction of avoidance. Neuropsychopharmacology 43:2636–2644. [PubMed: 30127343]

Rossidis AC, Stratigis JD, Chadwick AC, Hartman HA, Ahn NJ, Li H, Singh K, Coons BE, Li L, Lv W, Zoltick PW, Alapati D, Zacharias W, Jain R, Morrisey EE, Musunuru K, Peranteau WH (2018) In utero CRISPR-mediated therapeutic editing of metabolic genes. Nat Med 24:1513–1518. [PubMed: 30297903]

Ryu JK et al. (2018) Fibrin-targeting immunotherapy protects against neuroinflammation and neurodegeneration. Nat Immunol 19:1212–1223. [PubMed: 30323343]

Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32:347–355. [PubMed: 24584096]

Sanson KR, Hanna RE, Hegde M, Donovan KF, Strand C, Sullender ME, Vaimberg EW, Goodale A, Root DE, Piccioni F, Doench JG (2018) Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. Nat Commun 9:5416. DOI: 10.1038/s41467-018-07901-8. [PubMed: 30575746]

Sasaguri H, Nagata K, Sekiguchi M, Fujioka R, Matsuba Y, Hashimoto S, Sato K, Kurup D, Yokota T, Saido TC (2018) Introduction of pathogenic mutations into the mouse Psen1 gene by Base Editor and Target-AID. Nat Commun 9:2892. DOI: 10.1038/s41467-018-05262-w. [PubMed: 30042426]

Schirer Y, Malishkevich A, Ophir Y, Lewis J, Giladi E, Gozes I (2014) Novel marker for the onset of frontotemporal dementia: early increase in activity-dependent neuroprotective protein (ADNP) in the face of Tau mutation. PLoS One 9:e87383. [PubMed: 24489906]

Schmid-Burgk JL, Chauhan D, Schmidt T, Ebert TS, Reinhardt J, Endl E, Hornung V (2016) A Genome-wide CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Screen Identifies NEK7 as an Essential Component of NLRP3 Inflammasome Activation. J Biol Chem 291:103–109. [PubMed: 26553871]

Selvakumar GP, Iyer SS, Kempuraj D, Ahmed ME, Thangavel R, Dubova I, Raikwar SP, Zaheer S, Zaheer A (2018a) Molecular Association of Glia Maturation Factor with the Autophagic Machinery in Rat Dopaminergic Neurons: a Role for Endoplasmic Reticulum Stress and MAPK Activation. Mol Neurobiol. DOI: 10.1007/s12035-018-1340-1.

Selvakumar GP, Iyer SS, Kempuraj D, Raju M, Thangavel R, Saeed D, Ahmed ME, Zahoor H, Raikwar SP, Zaheer S, Zaheer A (2018b) Glia Maturation Factor Dependent Inhibition of Mitochondrial PGC-1alpha Triggers Oxidative Stress-Mediated Apoptosis in N27 Rat Dopaminergic Neuronal Cells. Mol Neurobiol. DOI: 10.1007/s12035-018-0882-6.

Selvaraj BT et al. (2018) C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca(2+)-permeable AMPA receptor-mediated excitotoxicity. Nat Commun 9:347. DOI: 10.1038/s41467-017-02729-0. [PubMed: 29367641]

Seo J, Kritskiy O, Watson LA, Barker SJ, Dey D, Raja WK, Lin YT, Ko T, Cho S, Penney J, Silva MC, Sheridan SD, Lucente D, Gusella JF, Dickerson BC, Haggarty SJ, Tsai LH (2017) Inhibition of p25/Cdk5 Attenuates Tauopathy in Mouse and iPSC Models of Frontotemporal Dementia. J Neurosci 37:9917–9924. [PubMed: 28912154]

Sepulcre J, Grothe MJ, d'Oleire Uquillas F, Ortiz-Teran L, Diez I, Yang HS, Jacobs HIL, Hanseeuw BJ, Li Q, El-Fakhri G, Sperling RA, Johnson KA (2018) Neurogenetic contributions to amyloid beta and tau spreading in the human cortex. Nat Med 24:1910–1918. [PubMed: 30374196]

Shen B, Zhang W, Zhang J, Zhou J, Wang J, Chen L, Wang L, Hodgkins A, Iyer V, Huang X, Skarnes WC (2014) Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. Nat Methods 11:399–402. [PubMed: 24584192]

Shi CH et al. (2018a) Disrupted structure and aberrant function of CHIP mediates the loss of motor and cognitive function in preclinical models of SCAR16. PLoS Genet 14:e1007664. [PubMed: 30222779]

Shi Y et al. (2018b) Haploinsufficiency leads to neurodegeneration in C9ORF72 ALS/FTD human induced motor neurons. Nat Med 24:313–325. [PubMed: 29400714]

Shin JW, Kim KH, Chao MJ, Atwal RS, Gillis T, MacDonald ME, Gusella JF, Lee JM (2016) Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. Hum Mol Genet 25:4566–4576. [PubMed: 28172889]

Shinmyo Y, Kawasaki H (2017) CRISPR/Cas9-Mediated Gene Knockout in the Mouse Brain Using In Utero Electroporation. Curr Protoc Neurosci 79:3 32 31–33 32 11.

Shinmyo Y, Tanaka S, Tsunoda S, Hosomichi K, Tajima A, Kawasaki H (2016) CRISPR/Cas9-mediated gene knockout in the mouse brain using in utero electroporation. Sci Rep 6:20611. DOI: 10.1038/srep20611. [PubMed: 26857612]

Shinmyo Y, Terashita Y, Dinh Duong TA, Horiike T, Kawasumi M, Hosomichi K, Tajima A, Kawasaki H (2017) Folding of the Cerebral Cortex Requires Cdk5 in Upper-Layer Neurons in Gyrencephalic Mammals. Cell Rep 20:2131–2143. [PubMed: 28854363]

Shinohara M, Sato N (2017) Bidirectional interactions between diabetes and Alzheimer's disease. Neurochem Int 108:296–302. [PubMed: 28551028]

Shmakov S, Abudayyeh OO, Makarova KS, Wolf YI, Gootenberg JS, Semenova E, Minakhin L, Joung J, Konermann S, Severinov K, Zhang F, Koonin EV (2015) Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems. Mol Cell 60:385–397. [PubMed: 26593719]

Shyng C, Nelvagal HR, Dearborn JT, Tyynela J, Schmidt RE, Sands MS, Cooper JD (2017) Synergistic effects of treating the spinal cord and brain in CLN1 disease. Proc Natl Acad Sci U S A 114:E5920–E5929. [PubMed: 28673981]

Silva MC, Cheng C, Mair W, Almeida S, Fong H, Biswas MHU, Zhang Z, Huang Y, Temple S, Coppola G, Geschwind DH, Karydas A, Miller BL, Kosik KS, Gao FB, Steen JA, Haggarty SJ (2016) Human iPSC-Derived Neuronal Model of Tau-A152T Frontotemporal Dementia Reveals Tau-Mediated Mechanisms of Neuronal Vulnerability. Stem Cell Reports 7:325–340. [PubMed: 27594585]

Simhadri VL, McGill J, McMahon S, Wang J, Jiang H, Sauna ZE (2018) Prevalence of Pre-existing Antibodies to CRISPR-Associated Nuclease Cas9 in the USA Population. Mol Ther Methods Clin Dev 10:105–112. [PubMed: 30073181]

Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F (2016) Rationally engineered Cas9 nucleases with improved specificity. Science 351:84–88. [PubMed: 26628643]

Smargon AA, Cox DB, Pyzocha NK, Zheng K, Slaymaker IM, Gootenberg JS, Abudayyeh OA, Essletzbichler P, Shmakov S, Makarova KS, Koonin EV, Zhang F (2017) Cas13b Is a Type VI-B CRISPR-Associated RNA-Guided RNase Differentially Regulated by Accessory Proteins Csx27 and Csx28. Mol Cell 65:618–630 e617. [PubMed: 28065598]

Sparling DP, Yu J, Kim K, Zhu C, Brachs S, Birkenfeld AL, Pajvani UB (2016) Adipocyte-specific blockade of gamma-secretase, but not inhibition of Notch activity, reduces adipose insulin sensitivity. Mol Metab 5:113–121. [PubMed: 26909319]

Staahl BT, Benekareddy M, Coulon-Bainier C, Banfal AA, Floor SN, Sabo JK, Urnes C, Munares GA, Ghosh A, Doudna JA (2017) Efficient genome editing in the mouse brain by local delivery of engineered Cas9 ribonucleoprotein complexes. Nat Biotechnol 35:431–434. [PubMed: 28191903]

Stanley M, Macauley SL, Holtzman DM (2016) Changes in insulin and insulin signaling in Alzheimer's disease: cause or consequence? J Exp Med 213:1375–1385. [PubMed: 27432942]

Steinberg S et al. (2015) Loss-of-function variants in ABCA7 confer risk of Alzheimer's disease. Nat Genet 47:445–447. [PubMed: 25807283]

Stopschinski BE, Holmes BB, Miller GM, Manon VA, Vaquer-Alicea J, Prueitt WL, Hsieh-Wilson LC, Diamond MI (2018) Specific glycosaminoglycan chain length and sulfation patterns are required for cell uptake of tau versus alpha-synuclein and beta-amyloid aggregates. J Biol Chem 293:10826–10840. [PubMed: 29752409]

Strecker J, Jones S, Koopal B, Schmid-Burgk J, Zetsche B, Gao L, Makarova KS, Koonin EV, Zhang F (2019) Engineering of CRISPR-Cas12b for human genome editing. Nat Commun 10:212. DOI: 10.1038/s41467-018-08224-4. [PubMed: 30670702]

Suarez-Calvet M et al. (2018) CSF progranulin increases in the course of Alzheimer's disease and is associated with sTREM2, neurodegeneration and cognitive decline. EMBO Mol Med 10. DOI: 10.15252/emmm.201809712.

Sun B, Zhou Y, Halabisky B, Lo I, Cho SH, Mueller-Steiner S, Devidze N, Wang X, Grubb A, Gan L (2008) Cystatin C-cathepsin B axis regulates amyloid beta levels and associated neuronal deficits in an animal model of Alzheimer's disease. Neuron 60:247–257. [PubMed: 18957217]

Sun J, Carlson-Stevermer J, Das U, Shen M, Delenclos M, Snead AM, Koo SY, Wang L, Qiao D, Loi J, Petersen AJ, Stockton M, Bhattacharyya A, Jones MV, Zhao X, McLean PJ, Sproul AA, Saha K, Roy S (2019) CRISPR/Cas9 editing of APP C-terminus attenuates beta-cleavage and promotes alpha-cleavage. Nat Commun 10:53. DOI: 10.1038/s41467-018-07971-8. [PubMed: 30604771]

Sydow A, Hochgrafe K, Konen S, Cadinu D, Matenia D, Petrova O, Joseph M, Dennissen FJ, Mandelkow EM (2016) Age-dependent neuroinflammation and cognitive decline in a novel Ala152Thr-Tau transgenic mouse model of PSP and AD. Acta Neuropathol Commun 4:17. DOI: 10.1186/s40478-016-0281-z. [PubMed: 26916334]

Tagliafierro L, Chiba-Falek O (2016) Up-regulation of SNCA gene expression: implications to synucleinopathies. Neurogenetics 17:145–157. [PubMed: 26948950]

Tchasovnikarova IA, Timms RT, Douse CH, Roberts RC, Dougan G, Kingston RE, Modis Y, Lehner PJ (2017) Hyperactivation of HUSH complex function by Charcot-Marie-Tooth disease mutation in MORC2. Nat Genet 49:1035–1044. [PubMed: 28581500]

Thambisetty M et al. (2010) Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. Arch Gen Psychiatry 67:739–748. [PubMed: 20603455]

Thangavel R, Stolmeier D, Yang X, Anantharam P, Zaheer A (2012) Expression of glia maturation factor in neuropathological lesions of Alzheimer's disease. Neuropathol Appl Neurobiol 38:572–581. [PubMed: 22035352]

Thangavel R, Kempuraj D, Stolmeier D, Anantharam P, Khan M, Zaheer A (2013) Glia maturation factor expression in entorhinal cortex of Alzheimer's disease brain. Neurochem Res 38:1777–1784. [PubMed: 23715664]

Thangavel R, Kempuraj D, Zaheer S, Raikwar S, Ahmed ME, Selvakumar GP, Iyer SS, Zaheer A (2017) Glia Maturation Factor and Mitochondrial Uncoupling Proteins 2 and 4 Expression in the Temporal Cortex of Alzheimer's Disease Brain. Front Aging Neurosci 9:150. DOI: 10.3389/fnagi.2017.00150 [PubMed: 28572767]

Thangavel R, Bhagavan SM, Ramaswamy SB, Surpur S, Govindarajan R, Kempuraj D, Zaheer S, Raikwar S, Ahmed ME, Selvakumar GP, Iyer SS, Zaheer A (2018) Co-Expression of Glia Maturation Factor and Apolipoprotein E4 in Alzheimer's Disease Brain. J Alzheimers Dis 61:553–560. [PubMed: 29172001]

Tropak MB, Yonekawa S, Karumuthil-Melethil S, Thompson P, Wakarchuk W, Gray SJ, Walia JS, Mark BL, Mahuran D (2016) Construction of a hybrid beta-hexosaminidase subunit capable of forming stable homodimers that hydrolyze GM2 ganglioside in vivo. Mol Ther Methods Clin Dev 3:15057. [PubMed: 26966698]

Trujillo CA, Muotri AR (2018) Brain Organoids and the Study of Neurodevelopment. Trends Mol Med. DOI: 10.1016/j.molmed.2018.09.005.

Tsai SQ, Joung JK (2016) Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. Nat Rev Genet 17:300–312. [PubMed: 27087594]

Tsai SQ, Nguyen NT, Malagon-Lopez J, Topkar VV, Aryee MJ, Joung JK (2017) CIRCLE-seq: a highly sensitive in vitro screen for genome-wide CRISPR-Cas9 nuclease off-targets. Nat Methods 14:607–614. [PubMed: 28459458]

Tsai SQ, Wyvekens N, Khayter C, Foden JA, Thapar V, Reyon D, Goodwin MJ, Aryee MJ, Joung JK (2014) Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. Nat Biotechnol 32:569–576. [PubMed: 24770325]

Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar VV, Thapar V, Wyvekens N, Khayter C, Iafrate AJ, Le LP, Aryee MJ, Joung JK (2015) GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nat Biotechnol 33:187–197. [PubMed: 25513782]

Tsuang D et al. (2013) APOE epsilon4 increases risk for dementia in pure synucleinopathies. JAMA Neurol 70:223–228. [PubMed: 23407718]

Tsunekawa Y, Terhune RK, Fujita I, Shitamukai A, Suetsugu T, Matsuzaki F (2016) Developing a de novo targeted knock-in method based on in utero electroporation into the mammalian brain. Development 143:3216–3222. [PubMed: 27578183]

Tulloch J, Leong L, Chen S, Keene CD, Millard SP, Shutes-David A, Lopez OL, Kofler J, Kaye JA, Woltjer R, Nelson PT, Neltner JH, Jicha GA, Galasko D, Masliah E, Leverenz JB, Yu CE, Tsuang D (2018) APOE DNA methylation is altered in Lewy body dementia. Alzheimers Dement 14:889–894. [PubMed: 29544979]

Tycko J, Myer VE, Hsu PD (2016) Methods for Optimizing CRISPR-Cas9 Genome Editing Specificity. Mol Cell 63:355–370. [PubMed: 27494557]

Uemura T, Mori T, Kurihara T, Kawase S, Koike R, Satoga M, Cao X, Li X, Yanagawa T, Sakurai T, Shindo T, Tabuchi K (2016) Fluorescent protein tagging of endogenous protein in brain neurons using CRISPR/Cas9-mediated knock-in and in utero electroporation techniques. Sci Rep 6:35861. DOI: 10.1038/srep35861. [PubMed: 27782168]

Vakulskas CA, Dever DP, Rettig GR, Turk R, Jacobi AM, Collingwood MA, Bode NM, McNeill MS, Yan S, Camarena J, Lee CM, Park SH, Wiebking V, Bak RO, Gomez-Ospina N, Pavel-Dinu M, Sun W, Bao G, Porteus MH, Behlke MA (2018) A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. Nat Med 24:1216–1224. [PubMed: 30082871]

Valdez C, Wong YC, Schwake M, Bu G, Wszolek ZK, Krainc D (2017) Progranulin-mediated deficiency of cathepsin D results in FTD and NCL-like phenotypes in neurons derived from FTD patients. Hum Mol Genet 26:4861–4872. [PubMed: 29036611]

Vannocci T, Faggianelli N, Zaccagnino S, della Rosa I, Adinolfi S, Pastore A (2015) A new cellular model to follow Friedreich's ataxia development in a time-resolved way. Dis Model Mech 8:711–719. [PubMed: 26035392]

Vesikansa A (2018) Unraveling of Central Nervous System Disease Mechanisms Using CRISPR Genome Manipulation. J Cent Nerv Syst Dis 10:1179573518787469. [PubMed: 30013417]

Wang C, Najm R, Xu Q, Jeong DE, Walker D, Balestra ME, Yoon SY, Yuan H, Li G, Miller ZA, Miller BL, Malloy MJ, Huang Y (2018a) Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. Nat Med 24:647–657. [PubMed: 29632371]

Wang G, Liu X, Gaertig MA, Li S, Li XJ (2016a) Ablation of huntingtin in adult neurons is nondeleterious but its depletion in young mice causes acute pancreatitis. Proc Natl Acad Sci U S A 113:3359–3364. [PubMed: 26951659]

Wang H, Guo W, Mitra J, Hegde PM, Vandoorne T, Eckelmann BJ, Mitra S, Tomkinson AE, Van Den Bosch L, Hegde ML (2018b) Mutant FUS causes DNA ligation defects to inhibit oxidative damage repair in Amyotrophic Lateral Sclerosis. Nat Commun 9:3683. DOI: 10.1038/s41467-018-06111-6. [PubMed: 30206235]

Wang J, Gu BJ, Masters CL, Wang YJ (2017a) A systemic view of Alzheimer disease - insights from amyloid-beta metabolism beyond the brain. Nat Rev Neurol 13:612–623. [PubMed: 28960209]

Wang P, Mokhtari R, Pedrosa E, Kirschenbaum M, Bayrak C, Zheng D, Lachman HM (2017b) CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in cerebral organoids derived from iPS cells. Mol Autism 8:11. DOI: 10.1186/s13229-017-0124-1. [PubMed: 28321286]

Wang P, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W, Lachman HM, Zheng D (2015) CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. Mol Autism 6:55. DOI: 10.1186/s13229-015-0048-6. [PubMed: 26491539]

Wang X, Cao C, Huang J, Yao J, Hai T, Zheng Q, Wang X, Zhang H, Qin G, Cheng J, Wang Y, Yuan Z, Zhou Q, Wang H, Zhao J (2016b) One-step generation of triple gene-targeted pigs using CRISPR/Cas9 system. Sci Rep 6:20620. [PubMed: 26857844]

Wang Y, Ji T, Nelson AD, Glanowska K, Murphy GG, Jenkins PM, Parent JM (2018c) Critical roles of alphaII spectrin in brain development and epileptic encephalopathy. J Clin Invest 128:760–773. [PubMed: 29337302]

Wiedenheft B, Sternberg SH, Doudna JA (2012) RNA-guided genetic silencing systems in bacteria and archaea. Nature 482:331–338. [PubMed: 22337052]

Wild EJ, Tabrizi SJ (2017) Therapies targeting DNA and RNA in Huntington's disease. Lancet Neurol 16:837–847. [PubMed: 28920889]

Wyvekens N, Topkar VV, Khayter C, Joung JK, Tsai SQ (2015) Dimeric CRISPR RNA-Guided FokI-dCas9 Nucleases Directed by Truncated gRNAs for Highly Specific Genome Editing. Hum Gene Ther 26:425–431. [PubMed: 26068112]

Xiang X, Piers TM, Wefers B, Zhu K, Mallach A, Brunner B, Kleinberger G, Song W, Colonna M, Herms J, Wurst W, Pocock JM, Haass C (2018) The Trem2 R47H Alzheimer's risk variant impairs splicing and reduces Trem2 mRNA and protein in mice but not in humans. Mol Neurodegener 13:49. DOI: 10.1186/s13024-018-0280-6. [PubMed: 30185230]

Xie N, Gong H, Suhl JA, Chopra P, Wang T, Warren ST (2016) Reactivation of FMR1 by CRISPR/Cas9-Mediated Deletion of the Expanded CGG-Repeat of the Fragile X Chromosome. PLoS One 11:e0165499. [PubMed: 27768763]

Xu C, Lu Z, Luo Y, Liu Y, Cao Z, Shen S, Li H, Liu J, Chen K, Chen Z, Yang X, Gu Z, Wang J (2018) Targeting of NLRP3 inflammasome with gene editing for the amelioration of inflammatory diseases. Nat Commun 9:4092. DOI: 10.1038/s41467-018-06522-5. [PubMed: 30291237]

Xu X, Tay Y, Sim B, Yoon SI, Huang Y, Ooi J, Utami KH, Ziaei A, Ng B, Radulescu C, Low D, Ng AY, Loh M, Venkatesh B, Ginhoux F, Augustine GJ, Pouladi MA (2017) Reversal of Phenotypic Abnormalities by CRISPR/Cas9-Mediated Gene Correction in Huntington Disease Patient-Derived Induced Pluripotent Stem Cells. Stem Cell Reports 8:619–633. [PubMed: 28238795]

Yan WX, Chong S, Zhang H, Makarova KS, Koonin EV, Cheng DR, Scott DA (2018) Cas13d Is a Compact RNA-Targeting Type VI CRISPR Effector Positively Modulated by a WYL-Domain-Containing Accessory Protein. Mol Cell 70:327–339 e325. [PubMed: 29551514]

Yan WX, Mirzazadeh R, Garnerone S, Scott D, Schneider MW, Kallas T, Custodio J, Wernersson E, Li Y, Gao L, Federova Y, Zetsche B, Zhang F, Bienko M, Crosetto N (2017) BLISS is a versatile and quantitative method for genome-wide profiling of DNA double-strand breaks. Nat Commun 8:15058. DOI: 10.1038/ncomms15058. [PubMed: 28497783]

Yang S, Chang R, Yang H, Zhao T, Hong Y, Kong HE, Sun X, Qin Z, Jin P, Li S, Li XJ (2017) CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. J Clin Invest 127:2719–2724. [PubMed: 28628038]

Yao X, Liu X, Zhang Y, Li Y, Zhao C, Yao S, Wei Y (2017) Gene Therapy of Adult Neuronal Ceroid Lipofuscinoses with CRISPR/Cas9 in Zebrafish. Hum Gene Ther 28:588–597. [PubMed: 28478735]

Yeh FL, Wang Y, Tom I, Gonzalez LC, Sheng M (2016) TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia. Neuron 91:328–340. [PubMed: 27477018]

Young JE, Boulanger-Weill J, Williams DA, Woodruff G, Buen F, Revilla AC, Herrera C, Israel MA, Yuan SH, Edland SD, Goldstein LS (2015) Elucidating molecular phenotypes caused by the SORL1 Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. Cell Stem Cell 16:373–385. [PubMed: 25772071]

Zafra MP, Schatoff EM, Katti A, Foronda M, Breinig M, Schweitzer AY, Simon A, Han T, Goswami S, Montgomery E, Thibado J, Kastenhuber ER, Sanchez-Rivera FJ, Shi J, Vakoc CR, Lowe SW,

Tschaharganeh DF, Dow LE (2018) Optimized base editors enable efficient editing in cells, organoids and mice. Nat Biotechnol. DOI: 10.1038/nbt.4194.

Zaheer A, Yorek MA, Lim R (2001) Effects of glia maturation factor overexpression in primary astrocytes on MAP kinase activation, transcription factor activation, and neurotrophin secretion. Neurochem Res 26:1293–1299. [PubMed: 11885780]

Zaheer A, Mathur SN, Lim R (2002) Overexpression of glia maturation factor in astrocytes leads to immune activation of microglia through secretion of granulocyte-macrophage-colony stimulating factor. Biochem Biophys Res Commun 294:238–244. [PubMed: 12051700]

Zaheer A, Zaheer S, Thangavel R, Wu Y, Sahu SK, Yang B (2008) Glia maturation factor modulates beta-amyloid-induced glial activation, inflammatory cytokine/chemokine production and neuronal damage. Brain Res 1208:192–203. [PubMed: 18395194]

Zaheer A, Zaheer S, Sahu SK, Knight S, Khosravi H, Mathur SN, Lim R (2007a) A novel role of glia maturation factor: induction of granulocyte-macrophage colony-stimulating factor and pro-inflammatory cytokines. J Neurochem 101:364–376. [PubMed: 17250654]

Zaheer S, Thangavel R, Sahu SK, Zaheer A (2011) Augmented expression of glia maturation factor in Alzheimer's disease. Neuroscience 194:227–233. [PubMed: 21835226]

Zaheer S, Wu Y, Bassett J, Yang B, Zaheer A (2007b) Glia maturation factor regulation of STAT expression: a novel mechanism in experimental autoimmune encephalomyelitis. Neurochem Res 32:2123–2131. [PubMed: 17551829]

Zaheer S, Thangavel R, Wu Y, Khan MM, Kempuraj D, Zaheer A (2013) Enhanced expression of glia maturation factor correlates with glial activation in the brain of triple transgenic Alzheimer's disease mice. Neurochem Res 38:218–225. [PubMed: 23086473]

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163:759–771. [PubMed: 26422227]

Zhang Y, Schmid B, Nielsen TT, Nielsen JE, Clausen C, Hyttel P, Holst B, Freude KK (2016) Generation of a human induced pluripotent stem cell line via CRISPR-Cas9 mediated integration of a site-specific heterozygous mutation in CHMP2B. Stem Cell Res 17:148–150. [PubMed: 27558613]

Zhang Y et al. (2017) Patient iPSC-Derived Neurons for Disease Modeling of Frontotemporal Dementia with Mutation in CHMP2B. Stem Cell Reports 8:648–658. [PubMed: 28216144]

Zhou H et al. (2018a) In vivo simultaneous transcriptional activation of multiple genes in the brain using CRISPR-dCas9-activator transgenic mice. Nat Neurosci 21:440–446. [PubMed: 29335603]

Zhou M, Hu Z, Qiu L, Zhou T, Feng M, Hu Q, Zeng B, Li Z, Sun Q, Wu Y, Liu X, Wu L, Liang D (2018b) Seamless Genetic Conversion of SMN2 to SMN1 via CRISPR/Cpf1 and Single-Stranded Oligodeoxynucleotides in Spinal Muscular Atrophy Patient-Specific Induced Pluripotent Stem Cells. Hum Gene Ther 29:1252–1263. [PubMed: 29598153]

Zhou W, Ma D, Sun AX, Tran HD, Ma DL, Singh BK, Zhou J, Zhang J, Wang D, Zhao Y, Yen PM, Goh E, Tan EK (2018c) PD-linked CHCHD2 mutations impair CHCHD10 and MICOS complex leading to mitochondria dysfunction. Hum Mol Genet. DOI: 10.1093/hmg/ddy413.

Zhou X, Xin J, Fan N, Zou Q, Huang J, Ouyang Z, Zhao Y, Zhao B, Liu Z, Lai S, Yi X, Guo L, Esteban MA, Zeng Y, Yang H, Lai L (2015) Generation of CRISPR/Cas9-mediated gene-targeted pigs via somatic cell nuclear transfer. Cell Mol Life Sci 72:1175–1184. [PubMed: 25274063]

Zhu XX, Zhong YZ, Ge YW, Lu KH, Lu SS (2018) CRISPR/Cas9-Mediated Generation of Guangxi Bama Minipigs Harboring Three Mutations in alpha-Synuclein Causing Parkinson's Disease. Sci Rep 8:12420. DOI: 10.1038/s41598-018-30436-3. [PubMed: 30127453]

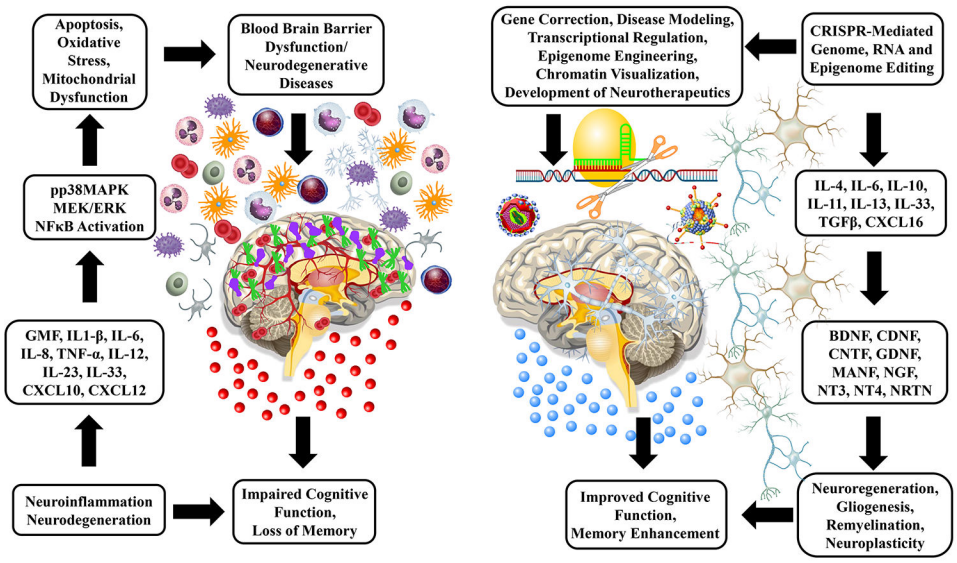

Figure 1: CRISPR-Mediated Genome Editing for Neurodegenerative Diseases:

Neuroinflammation plays a crucial role in the initiation and progression of various neurodegenerative disorders. Activation of astrocytes and microglia induces the expression of proinflammatory cytokines and chemokines including GMF, IL1-β, IL-6, IL-8, TNF, IL-12, IL-23, IL-33, CXCL10 and CXCL12. Because of neuroinflammation, there is increased phosphorylation of p38MAPK/ERK pathways, which leads to activation, and nuclear translocation of NFκB thereby causing increased oxidative stress, mitochondrial dysfunction and apoptosis. These deleterious effects ultimately lead to neurodegenerative disorders and impaired blood brain barrier. The progression of neurodegenerative cascade results in impaired cognitive function and loss of memory. CRISPR/Cas9-mediated genome editing is a powerful tool for inducing gene correction, disease modeling, transcriptional regulation, epigenome engineering, chromatin visualization as well as development of neurotherapeutics. It can be used to increase the levels of anti-inflammatory cytokines (IL-4, IL-6, IL-10, IL-11, IL-13, IL-33, TGFβ, CXCL16) and neurotrophic factors (BDNF, CDNF, GDNF, MANF, NGF, NT3, NT4, NRTN) which in turn can stimulate the proliferation and expansion of neural stem cells, neurogenesis, gliogenesis, remyelination and neural plasticity thereby ultimately leading to improved cognitive function and memory enhancement.

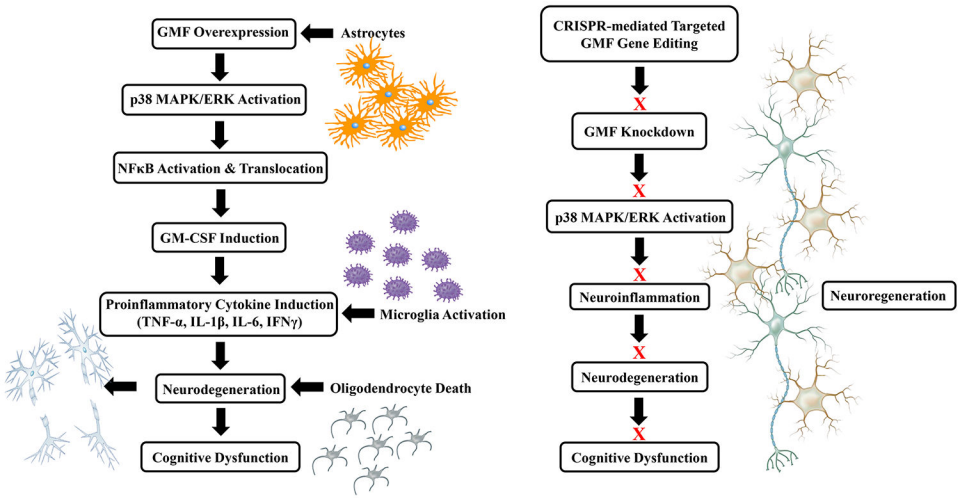

Figure 2: Role of Glia Maturation Factor (GMF) in Neuroinflammation and Neurodegenerative Diseases:

GMF is a 141 amino acid multifunctional intracellular protein, which is predominantly expressed in the central nervous system and plays a crucial role in the growth and differentiation of glia and neurons. GMF overexpression, which is observed in several neurodegenerative diseases, induces the activation of the p38MAPK/ERK signaling leading to activation and nuclear translocation of NFκB resulting in a significant increase in the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF is a proinflammatory cytokine and a potent mitogenic factor for microglia. Activation and proliferation of microglia induces the secretion of various proinflammatory cytokines/ chemokines including TNF-α, IL-1β, IL-6 and IFNγ (represented as red spheres) and leads to oxidative stress, mitochondrial dysfunction as well as apoptosis. These deleterious effects induce neurodegeneration and blood brain barrier dysfunction, which in turn lead to impaired cognitive function and loss of memory. CRISPR-mediated targeted GMF gene editing leads to GMF knockdown and very effectively blocks the proinflammatory downstream signaling pathways thereby delaying or halting the progression of neurodegenerative disorders, enhancing the proliferation and differentiation of neural stem cells, improving neurogenesis, producing neurotrophins (depicted as blue spheres) neuroplasticity and improving the cognitive function.

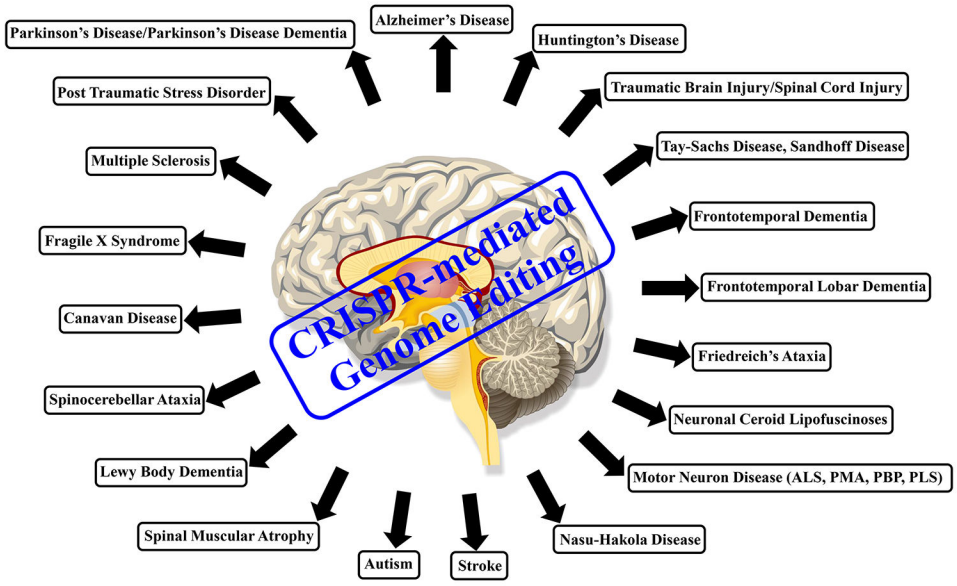

Figure 3: CRISPR-mediated Genome Editing of Neuroinflammation and Neurodegenerative Disorders:

A wide spectrum of neurodegenerative disorders can be efficiently targeted by CRISPR-mediated genome editing to either correct the genetic defect, restore the functional expression of the protein, knockdown the expression of the defective protein, as well as achieve epigenetic modifications. A wide variety of CRISPR enzymes are available to achieve desired genetic modification. Temporo-spatial regulation of precision-targeted CRISPR-mediated genome editing holds the key to successful correction of the genetic defect without any off target effects.

Table1:

CRISPR Types and their PAM Recognition Sequences

CRISPR	Species	PAM Sequence	References
SaCas9	*Staphylococcus aureus*	5'NNGRRT/NNNRRT	(Ran et al., 2015)
SaCas9 KKH	*Staphylococcus aureus*	3'NNNRRT	(Kleinstiver et al., 2015a)
SpCas9	*Streptococcus pyogenes*	3'NGG	(Cong et al., 2013)
SpCas9 D1135E	*Streptococcus pyogenes*	3'NGA (Reduced NAG binding)	(Kleinstiver et al., 2015b)
SpCas9 VRER	*Streptococcus pyogenes*	3'NGCG	(Kleinstiver et al., 2015b)
SpCas9 EQR	*Streptococcus pyogenes*	3'NGAG	(Kleinstiver et al., 2015b)
SpCas9 VQR	*Streptococcus pyogenes*	3'NGAN/NGNG	(Kleinstiver et al., 2015b)
SpCas9 HF1 (N497A/ R661A/Q695A/ Q926A)	*Streptococcus pyogenes*	3'NGG (Reduced off-target effect)	(Kleinstiver et al., 2016)
Sp-nCas9 (D10A or H840H)	*Streptococcus pyogenes*	5'NGG	(Jinek et al., 2012)
Sp-nCas9 (D10A/ H840H)	*Streptococcus pyogenes*	5'NGG (Nuclease deficient)	(Jinek et al., 2012)
NmCas9	*Neisseria meningitidis*	5'NNNNGMTT	(Lee et al., 2016)
SpCas9	*Streptococcus pasteurianus*	3'NNGTGA	(Ran et al., 2015)
ScCas9	*Streptococcus canis*	5'NNG	(Chatterjee et al., 2018)
eSPCas9 (1.0/1.1)	*Streptococcus pyogenes*	5'NGG (Reduced Off-target effect)	(Slaymaker et al., 2016)
Fok1-Sp-dCas9 (D10A/H840A+FokI)	*Streptococcus pyogenes*	5'NGG	(Richter et al., 2016)
FnCas9	*Francisella novicida*	5'NGG	(Hirano et al., 2016)
FnCas9 RHA (E1369R/E1449H/ R1556A)	*Francisella novicida*	5'YG	(Hirano et al., 2016)
AsCpf1	*Acidaminococcus sp. BV3L6*	5'TTTV	(Zetsche et al., 2015)
LbCpf1	*Lachnospiraceae bacterium ND2006*	5'TTTV	(Zetsche et al., 2015)
CMtCpf1	*Candidatus Methanoplasma termitum*	Not known	(Zetsche et al., 2015)
BpCpf1	*Butyrivibrioproteoclasticus*	Not known	(Zetsche et al., 2015)
FnCpf1	*Francisella novicida*	3'TTN/KYTV/CTA	(Zetsche et al., 2015)
MbCpf1	*Moraxella bovoculi*	5'TTN	(Zetsche et al., 2015)
PdCpf1	*Prevotella disens*	Not known	(Zetsche et al., 2015)
PcCpf1	*Prophyromonas crevioricanis*	Not known	(Zetsche et al., 2015)
PbCpf1	*Parcubacteria bacterium*	Not known	(Zetsche et al., 2015)
Lb3Cpf1	*Lachnospiraceae bacterium MC2017*	Not known	(Zetsche et al., 2015)
SsCpf1	*Smithella sp.* SC_K08D17	Not known	(Zetsche et al., 2015)
EeCpf1	*Eubacterium eligens*	Not known	(Zetsche et al., 2015)
Lb2Cpf1	*Lachnospiraceae bacterium MA2020*	Not known	(Zetsche et al., 2015)
LiCpf1	*Leptospira inadai*	Not known	(Zetsche et al., 2015)

CRISPR	Species	PAM Sequence	References
PeCpf1	*Peregrinbacteria bacterium GW2011_GWA_33_10*	Not known	(Zetsche et al., 2015)
AsCpf1 RR (S542R/ K607R)	*Acidaminococcus sp.*	5'TYCV/CCCC	(Gao et al., 2017)
AsCpf1 RVR (S542R/ K548V/N552R)	*Acidaminococcus sp.*	5'TATV/TTTV (V=A,C,G)	(Gao et al., 2017)
St1Cas9	*Streptococcus thermophilus*	3'NNAGAAW/NNGGAA	(Garneau et al., 2010)
St3Cas9	*Streptococcus thermophilus*	5'NGGNG	(Magadan et al., 2012)
CjCas9	*Campylobacter jejuni*	3'NNNVRYAC/ NNNNRYAC	(Kim et al., 2017)
xCas9 3.7 TLIKDIV SpCas9	*Streptococcus pyogenes*	3'NGT/NG	(Hu et al., 2018a)
TdCas9	*Treponema denticola*	3'NAAAAC	(Esvelt et al., 2013)
Cas12a or Cpf1	*Clostridium aceticum*	5'TTTN (makes staggered cuts)	(Zetsche et al., 2015)
AacCas12b	*Alicyclobacillus acidoterrestris*	5'TTN	(Shmakov et al., 2015)
BhCas12b	*Bacillus hisashii*	5'ATTN	(Strecker et al., 2019)
PspCas13	*Prevotella buccae*	Targeted RNA knockdown	(Abudayyeh et al., 2016)
PspCas13a	*Prevotella buccae*	Targeted RNA knockdown	(Abudayyeh et al., 2016)
LwaCas13a	*Leptotrichia wadeii*	Targeted RNA knockdown	(Abudayyeh et al., 2017)
PspCas13b	*Prevotella sp.* $P_{5\text{-}125}$	Targeted RNA knockdown	(Abudayyeh et al., 2016; Smargon et al., 2017)
PspCas13c	*Prevotella buccae*	Targeted RNA knockdown	(Abudayyeh et al., 2016)
PspCas13d	*Prevotella buccae*	Targeted RNA knockdown	(Konermann et al., 2018; Yan et al., 2018)
RfxCas13d	*Ruminococcus flavefaciens*	Targeted RNA knockdown	(Konermann et al., 2018)
Cas14a	*Uncultivated Archaea bacteria*	Targets ssDNA without PAM	(Harrington et al., 2018)
Cas14b	*Uncultivated Archaea bacteria*	Targets ssDNA without PAM	(Harrington et al., 2018)
Cas14c	*Uncultivated Archaea bacteria*	Targets ssDNA without PAM	(Harrington et al., 2018)
Archael Cas9	*Candidatus Micrarchaeum acidophilum ARMAN-1, Candidatus Parvarchaeum acidiphilum ARMAN-4*	5'NGG	(Burstein et al., 2017)
plmCasX	*Planctomycetes, Deltaproteobacteria*	5'TTCN (makes staggered cuts)	(Burstein et al., 2017)
DpbCasX (D672A/ E769A/D935A)	*Planctomycetes, Deltaproteobacteria*	5'TTCN	(Liu et al., 2019)
CasY	*Katanobacteria, Kerfeldbacteria, Vogelbacteria, Parcubacteria, Komeilibacteria*	5'TA	(Burstein et al., 2017)

Table 2:

Select Neurodegenerative Disorders and Targets for Genome Editing

Neurodegenerative Disease	Genetic Basis	Pathological Findings	Interventions/ Therapeutic Targets	References
Alzheimer's Disease	ABCA7, AKAP9, APOE ε4, Mutations in PSEN1, PSEN2, APP (T48P, L52P, K53N), TREM2 variants 97C→A, 140C→T, 185C→T, 259C→T, 469C→T, 632C→T, CD33, CLU, SORL1, BIN, CD2AP, PICALM, PLD3, CASS4, CELF1, DSG2, FERMT2, DRB5/ HLA-DRB1, INPP5D, MEF2C, NME8, PTK2B, SLC24H4-RIN3, SQSTM1, TTC3, UNC5C, ZCWPW1	Amyloid plaques, neurofibrillary tangles, tau hyperphosphorylation , GMF overexpression, mitochondrial dysfunction, neuroinflammation, neurodegeneration	PSEN1[a], PSEN2[b], APP[c], GMF[d], TREM2[e], APOE4[f], BACE1[g], [EXT1, EXT2, EXTL3, NDST1, 6-O-sulfotransferase (HS6ST2)][h], ACVR1[i], NLRP3[j], Cystatin C[k], Progranulin[l], TM2D3[m], CLU[n], CR1[o], SORL1[p], ABCA7[q], CXCR3[r], Fibrin[s], Fmn2[t], STIM1[u], TAU[v]	(Sasaguri et al., 2018)[a], (Ortiz-Virumbrales et al., 2017)[b], (Frederiksen et al., 2018; Gyorgy et al., 2018; Hung and Livesey, 2018; Nagata et al., 2018; Sun et al., 2019)[c], (Raikwar et al., 2018)[d], (Krasemann et al., 2017; Cheng-Hathaway et al., 2018; Claes et al., 2018; Xiang et al., 2018)[e], (Huang et al., 2017; Lin et al., 2018; Wang et al., 2018a)[f], (Inoue et al., 2017)[g], (Stopschinski et al., 2018)[h], (Petersen et al., 2017)[i], (Heneka et al., 2013; Schmid-Burgk et al., 2016; Xu et al., 2018)[j], (Kaeser et al., 2007; Mi et al., 2007; Sun et al., 2008)[k], (Minami et al., 2014; Suarez-Calvet et al., 2018)[l], (Haney et al., 2018)[m], (Robbins et al., 2018)[n], (Marzi et al., 2018)[o], (Young et al., 2015)[p], (Steinberg et al., 2015)[q], (Krauthausen et al., 2015)[r], (Ryu et al., 2018)[s], (Agis-Balboa et al., 2017)[t], (Pascual-Caro et al., 2018)[u], (Garcia-Leon et al., 2018)[v]
Parkinson's Disease	Mutations in SNCA, PARK2, PINK1, UCHL1, DJ1, LRRK2, SIPAiL2, INPP5F, microRNA4697 , GCH1, DDRGK1, EIF4G1, VPS35, VPS13C, SQSTM1, TREM2 variants 140C→T	GMF overexpression, mitochondrial dysfunction, neuroinflammation, neurodegeneration, Lewy bodies in brain stem, minimal striatal α-synuclein pathology, resting tremor, stiffness, slowness and walking/balance problems	GMF[aa], NLRP3[bb], GBA1[cc], TFAM[dd], P13[ee], LRRK1[ff], PARKIN +MIDN[gg], CHCHD2[hh], PINK1[ii], SNCA/ α-Synuclein[jj], DJ1[kk]	(Kempuraj et al., 2018b; Kempuraj et al., 2018a; Selvakumar et al., 2018a)[aa], (Gordon et al., 2018)[bb], (Kim et al., 2018)[cc], (Langley et al., 2017)[dd], (Inoue et al., 2018)[ee], (Giaime et al., 2017)[ff], (Wang et al., 2016b; Obara et al., 2017; Potting et al., 2018)[gg], (Zhou et al., 2018c)[hh], (Zhou et al., 2015)[ii], (Tagliafierro and Chiba-Falek, 2016; Arias-Fuenzalida et al., 2017; Heman-Ackah et al., 2017; Mor et al., 2017; Chen et al., 2018; Kantor et al., 2018; Zhu et al., 2018)[jj], (Prasuhn et al., 2017)[kk]
Parkinson's Disease Dementia	GBA mutations, MAPT H1 variant, $COMT_{val}158_{met}$ polymorphism, APOE-ε4,	Diffuse Lewy body proteins, neurofibrillary tangles, senile plaques, extensive striatal α-synuclein pathology, postural instability, gait abnormalities, rapid motor decline, frequent falls,	SNCA*, GBA[#], LRP10[%]	(Tagliafierro and Chiba-Falek, 2016; Heman-Ackah et al., 2017; Chen et al., 2018; Kantor et al., 2018)*, (Kim et al., 2018)[#], (Quadri et al., 2018)[%]

Neurodegenerative Disease	Genetic Basis	Pathological Findings	Interventions/ Therapeutic Targets	References
Lewy Body Disease	SNCA, SNCB, BCHE, APOE, GBA	Lewy body proteins, neuritic plaques, neurofibrillary tangles, resting tremor, bradykinesia, stiffness, slowness and walking/ balance problems, memory/ thinking problems, visual hallucinations, excessive day time drowsiness, unpredictable fluctuating levels of attention or alertness,	APOE^[@], SNCA[@], GBA[@], GABRB3[@], BCL7CI/STX1B[@]	(Tsuang et al., 2013; Dickson et al., 2018; Tulloch et al., 2018)^, (Chen et al., 2018; Guerreiro et al., 2018)[@]
Amyotrophic Lateral Sclerosis	TREM2 variants 140C→T,	Degeneration of and loss of the large motor neurons in the anterior horn of the spinal cord, spongiosis, Bunina bodies positive for Cystatin c in the motor neurons of the spinal cord and brain stem, activated microglia, neuroinflammation, TDP-43	C9ORF72[+], SOD1[§], TREM2[Δ], FUS[●], SRSF1[■], TDP-43[♦]	(Kramer et al., 2018; Selvaraj et al., 2018; Shi et al., 2018b)[+], (Bhinge et al., 2017)[§], (Krasemann et al., 2017)[Δ], (Lagier-Tourenne et al., 2012)[●], (Hautbergue et al., 2017)[■], (Fratta et al., 2018)[♦]
Frontotemporal Dementia	TREM2 variants 42+3 delAGG, 97C→A, 113A→G, 140C→T, 197C→T, 257A→T, 286C→A, 598G→A, 632C→T, Progranulin, ADNP, FUS, CHMP2B, C9ORF72, Cystatin C, MAPT		TREM2[α], Progranulin[β], ADNP[γ], FUS[δ], CHMP2B[ε], C9ORF72[ε], MAPT[ζ]	(Valdez et al., 2017)[α], (Nguyen et al., 2018)[β], (Schirer et al., 2014)[γ], (Lopez-Erauskin et al., 2018)[δ], (Zhang et al., 2016; Zhang et al., 2017; Kramer et al., 2018)[ε], (Coppola et al., 2012)[ζ]
Frontotemporal Lobar Dementia	TREM2 variants 42+3 delAGG, 97C→A, 113A→G, 140C→T, 197C→T, 257A→T, 286C→A, 598G→A, 632C→T, MAPT		TREM21[‡], MAPT[∞]	(Valdez et al., 2017)[‡], (Imamura et al., 2016; Jiang et al., 2018)[∞]
Huntington's Disease	More than 39 polyglutamine repeats in HTT, SQSTM1, H3K4me3	Polyglutamine expansion in the first exon of huntingtin	HTT exon 1[⊥]	(Malkki, 2016; Shin et al., 2016; Kolli et al., 2017; Malankhanova et al., 2017; Wild and Tabrizi, 2017; Xu et al., 2017; Yang et al., 2017)[⊥]
Multiple Sclerosis/ Experiment al autoimmune encephalomyelitis	HLA-DRB1* 15:01, IL-7R, DDX39B, IL2RA, TNFRF1A, EVI5, IRE1α-XBP1	Neuroinflammation, prickiling or tingling sensation (paresthesia), numbness, pain, itching, Lhermitte sign, tremors, muscle stiffness (spasticity), exaggerated reflexes (hyperreflexia), weakness or partial paralysis of the limb muscles, difficulty walking, poor bladder control	Fibrin[!!]	(Petersen et al., 2017; Ryu et al., 2018)[!!]
Autism	ADNP, ANK2, ARID1B, ASH1L, ASXL3, CACNA1H, CHD2, CHD8, CNTN4, CNTNAP2, CTNND2, DSCAM, DYRK1A, GABRB3, GRIN2B, KATNAL2, KDM5B, MECP2, MYT1L, NLGN3, NRXN1, POGZ, PTCHD1, PTEN, RELN, SCN2A, SHANK2, SHANK3, SYNGAP1, TBR1	Impaired ability to interact with other people, difficulty understanding and using non-verbal cues, reduced eye contact and social interaction, repetitive behavior, preservation, difficulty tolerating sensory stimuli, mild to moderate intellectual disability,	CHD8[◄], MECP2[►], (AFF2/ FMR2, ANOS1, ASTN2, ATRX, CACNA1C, CHD8, DLGAP2, KCNQ2, SCN2A, TENM1)[◙], SRRM4[○]	(Wang et al., 2015; Platt et al., 2017; Wang et al., 2017b)[◄], (Liu et al., 2016b)[►], (Deneault et al., 2018)[◙], (Gonatopoulos-Pournatzis et al., 2018)[○]

Neurodegenerative Disease	Genetic Basis	Pathological Findings	Interventions/ Therapeutic Targets	References
Fragile X Syndrome	Expansion of CGG repeat in FMR1 gene	Delayed development of speech, intellectual disability, cognitive impairment	FMR1[♫]	(Park et al., 2015; Xie et al., 2016; Li et al., 2017)[♫]
Charcot Marie Tooth Disease	AARS, ABHD12, AIFM1, ARHGEF10, ATP1A1, ATP7A, BAG3, BSCL2, CNTNAP1, COA7, COX6A1, DCTN1, DCTN2, DHTKD1, DNAJB2, DNM2, DNMT1, DRP2, EGR2, FGD4, FIG4, GARS, GDAP1, GJB1, GNB4, HARS, HINT1, HK1, HSPB1, HSPB3, HSPB8, IGHMBP2, INF2, JPH1, KARS, KIF1B, KIF5A, LITAF, LMNA, LRSAM1, MARS, MCM3AP, MED25, MFN2, MME, MORC2, MPV17, MPZ, MT-ATP6, MTMR2, NAGLU, NDRG1, NEFH, NEFL, PDK3, PLEKHG5, PMP22, PRPS1, PRX, PTRH2, RAB7A, SBF1, SBF2, SCO2, SETX, SGPL1, SH3TC2, SIGMAR1, SLC5A46, SPG11, SPTLC1, SURF1, TRIM2, TRPV4, VCP, WARS, YARS, Chromosome 17	Muscle atrophy in feet, abnormalities in myelin, fibers or axons	MORC2[Ω], FIG4[Σ]	(Tchasovnikaro va et al., 2017)[Ω], (Gentil et al., 2017)[Σ]
Spinocerebellar Ataxia 1-3	ATXN1, ATXN2, ATXN3, CACNA1A,	Numbness, tingling or pain in arms and legs (sensory neuropathy, dystonia, atrophy, fasciculations, chorea etc.	SCA2[Ψ], SCA3[ω], SCAR16[κ]	(Marthaler et al., 2016a, c; Marthaler et al., 2016b)[Ψ], (Ouyang et al., 2018)[ω], (Shi et al., 2018a)[κ]
Spinal Muscular Atrophy I-III	SMN1	Hypotonia,	SMN1[λ]	(Zhou et al., 2018b)[λ]
Motor Neuron Disease (ALS, PMA, PBP, PLS)	TDP43, FUS/TLS, Optineurin, UBQLN2, C9ORF72, SPATACSIN, SENATAXIN, ALSIN	Chronic progressive illnesses, muscle wasting, muscle weakness, fasciculations, speech problems, spasticity, swallowing problems, excessive saliva, neurodegeneration	TDP-43[μ], SCYL1[π]	(Fratta et al., 2018)[μ], (Kuliyev et al., 2018)[π]
Epilepsy	AARS, ALDH7A1, ANO3, ARV1, ARHGEF9, ALG13, BCL11A, CACNA1H, CACNB4, CDKL5, CERS1, CHD2, CHRNA2, CHRNA4, CLCN2, CNTN2, CPA6, CSTB, DEPDC5, DNM1, DOCK7, EEF1A2, EFHC1, EPM2A, FRRS1L, GAL, GUF1, GABRD, GRIK1, GRIN2A, GABRA1, GABRB3, GABRG2, GOSR2, HCN1, ITPA, KCNC1, KCNMA1, KCNQ2, KCNQ3, KCNT1, KCTD7, LGI1, LMNB2, NECAP1, NHLRC1, PCDH7, PCDH19, PLCB1, PNPO,	Convulsions, stiffness, confused memory, hippocampal sclerosis, focal cortical dysplasia, cerebellar atrophy,	DEPDC5[ρ], SCN1A[σ]	(Hughes et al., 2017)[ρ], (Liu et al., 2016a)[σ]

Neurodegenerative Disease	Genetic Basis	Pathological Findings	Interventions/ Therapeutic Targets	References
	PRDM8, PRICKLE1, PRRT2, QARS, SCARB2, SCN1A, SCN1B, SCN2A, SCN9A, SLC2A1, SLC6A1, SLC12A5, SLC13A5, SLC25A12, SLC25A22, SNN8A, SPTAN1, STAT4, ST3GAL5, STX1B, SZT2, TBC1D24, TTC21B, ZEB2, 16p11.2 microduplication, WWOX,			
Friedreich's Ataxia	GAA repeats in FXN gene	Reduced elongation and impaired transcription of FXN gene	FXN[τ]	(Vannocci et al., 2015; Ouellet et al., 2017)[τ]
Tay-Sachs Disease	HEXA	Reduced Hexosaminidase A, abnormal buildup of GM2 ganglioside in the nerve cells, seizures, vision and hearing loss, muscle weakness, cherry red spots in the eyes, loss of motor skills	HEXA[υ]	(Tropak et al., 2016)[υ]
Sandhoff Disease	HEXB	Reduced Hexosaminidase B, abnormal buildup of GM2 ganglioside in the nerve cells, neuroinflammation, gliosis, progressive neurodegeneration	HEXB[φ]	(Allende et al., 2018)[φ]
Canavan Disease	NAT8L, ASPA	Toxic accumulation of N-acetylaspartate causing diffuse spongiform white matter degeneration, disrupted development, macrocephaly, seizures, CNS vacuolization, hypomyelination	ASPA[χ]	(Gessler et al., 2017)[χ]
Batten Disease/ Neuronal Ceroid Lipofuscinoses	CLN1/PPT1, CLN2/TPP1, CLN3, CLN4, CLN5, CLN6, CLN7, CLN8, CLN9, CLN10, CLN11, CLN12, CLN13, CLN14	Lysosomal accumulation of pathological lipofuscin-like material in neurons and glia, neuron loss, early impairment of vision, progressive decline in cognitive and motor functions, seizures, progressive neuron loss	CLN1/PPT1[ω]	(Shyng et al., 2017; Yao et al., 2017)[ω]
Nasu-Hakola Disease	TYROBP, TREM2 variants, 40G→T, 97C→A, 132G→A, 233G→A, 267delG, 313delG, 377T→G, 401A→G, 482+2T→C, 588G→A, DAP12, TYROBP	Progressive presenile dementia associated with recurrent bone fractures, extensive demyelination (leukoencephalopath y), astrogliosis, accumulation of axonal spheroids, activation of microglia in the white matter of frontal temporal lobes, overexpression of gp91phox in microglia	TREM2[ϡ]	(Valdez et al., 2017; Garcia-Reitboeck et al., 2018)[ϡ]
Prion Disease	PRNP (P102L)	Prion protein in the substantia gelatinosa, spinothalamic tracts, posterior columns and nuclei and in the neuropil surrounding anterior horns (Gerstmann-Straussler-Scheineker syndrome)	PrP[T̄]	(Mehrabian et al., 2014; Kaczmarczyk et al., 2016)[T̄]

Note: Alphabets, Roman Numerals and symbols depicted in superscript in column 4 correspond with the respective references in column 5 in the **Table 2.**