



Loss of APOBEC1 RNA-editing function in microglia exacerbates age-related CNS pathophysiology

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Microglia (MG), a heterogeneous population of phagocytic cells, play important roles in central nervous system (CNS) homeostasis and neural plasticity. Under steady-state conditions, MG maintain homeostasis by producing antiinflammatory cytokines and neurotrophic factors, support myelin production, and remove synapses and cellular debris, as well as participating in “cross-correction,” a process that supplies neurons with key factors for executing autophagy-lysosomal function. As sentinels for the immune system, MG also detect “danger” signals (pathogenic or traumatic insult), become activated, produce proinflammatory cytokines, and recruit monocytes and dendritic cells to the site of damage through a breached blood–brain barrier or via brain lymphatics. Failure to effectively resolve MG activation can be problematic and can lead to chronic inflammation, a condition proposed to underlie CNS pathophysiology in heritable brain disorders and age-related neurodegenerative and cognitive decline. Here, we show that APOBEC1-mediated RNA editing occurs within MG and is key to maintaining their resting status. Like bone marrow-derived macrophages, RNA editing in MG leads to overall changes in the abundance of edited proteins that coordinate the function of multiple cellular pathways. Conversely, mice lacking the APOBEC1 editing function in MG display evidence of dysregulation, with progressive age-related signs of neurodegeneration, characterized by clustering of activated MG, aberrant myelination, increased inflammation, and lysosomal anomalies that culminate in behavioral and motor deficiencies. Collectively, our study identifies posttranscriptional modification by RNA editing as a critical regulatory mechanism of vital cellular functions that maintain overall brain health.

microglia | RNA editing | lysosome | aging | neuroinflammation

Microglia (MG) represent the most abundant cells of the mononuclear phagocyte system in the central nervous system (CNS) (1). Although they are considered immune cells by some, and act as immune sentinels within the brain, it is increasingly clear that the primary function of MG is to maintain brain homeostasis. During neural development, MG play a crucial role by eliminating redundant neurons that do not establish functional circuits and by pruning dendritic spines that are not receiving inputs from synaptic contacts (2). In the juvenile and adult steady-state CNS, MG support synaptic plasticity and neurogenesis, remove damaged myelin fragments, break down and recycle lipids, and remove dead cells and cellular debris without eliciting inflammation (3–6). During infection or injury, MG are responsible for phagocytosis and elimination of microbes and cellular debris, as well as secreting many soluble factors, such as chemoattractants, cytokines, and neurotrophic factors, that contribute to various aspects of immune responses and tissue repair in the CNS (7–9).

Brain homeostasis can also be disrupted during aging and neurodegenerative diseases. Indeed, chronic inflammation is a component of many age-related neurodegenerative diseases,

such as Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (10). In addition, acute brain insults, such as chemical toxicity, seizures, and stroke, as well as genetic metabolic diseases, such as A-beta lipoprotein anemia, lysosomal storage diseases (LSDs), and cerebral vascular disease, are all characterized by increased inflammation and manifestation of CNS degeneration and abnormal behavior (11–14). Each of these disease states is initiated by malfunction(s) in different intracellular pathways, but they all share universal components of inflammation, including activation of MG, as well as the release of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6) and reactive oxygen and nitrogen species (7). These data led to the popular hypothesis that MG dysfunction is an important contributing factor in age-related and inherited brain disorders (15). Conversely, it also has been postulated that stimuli that induce and sustain inflammation during aging arise from an overload of misfolded protein aggregates and undigested cellular debris, and may be related to

Significance

Microglia (MG) play important roles in brain homeostasis and neuronal plasticity, and are recruited by the immune system to orchestrate inflammatory responses to danger signals. We find that, within MG, an RNA-editing function performed by the deaminase APOBEC1 and its obligate cofactor affects protein expression levels necessary for harmonious function within these cells and the cells they support. Genetic inactivation of *Apoec1* leads to dysregulation and resultant age-related neurodegeneration in the central nervous system, characterized by increased inflammation, aberrant myelination, and neuronal and MG lysosomal anomalies, culminating in progressive cognitive and motor decline. These data provide powerful evidence supporting the critical role of APOBEC1-mediated RNA editing in maintaining the balance between the homeostatic and activated immune functions of MG.

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the impairment of lysosomal clearance mechanisms (e.g., autophagy and/or phagocytosis) within the MG populations (16, 17).

In bone marrow-derived macrophages (BMDMs), RNA editing mediated by the cytidine deaminase APOBEC1 results in changes in the abundance of proteins that belong to important pathways related to macrophage function [including migration and phagocytosis [Rayon-Estrada et al. (18)]. While APOBEC1 was first described for its role in lipid metabolism through editing of apolipoprotein B-100 in the small intestine enterocytes, it is now clear that it edits a large number of transcripts in the intestine, liver, macrophages, and dendritic cells (18–21). In the present study, we demonstrate that in the brain, *Apobec1* and its obligate cofactor *Rbm47* are expressed solely in MG and function as an RNA-editing enzyme complex. Within MG, APOBEC1 edits multiple mRNA transcripts, and similar to BMDMs (18), editing of target transcripts [e.g., the transcript encoding lysosomal membrane protein-2 (LAMP2)] leads to changes in protein abundance and altered cellular function (e.g., phagocytosis). Conversely, the lack of APOBEC1 within MG promotes a proinflammatory environment in the brains of middle-aged *Apobec1*^{-/-} mice that is accompanied by progressive CNS pathophysiology. This neuropathology is characterized by abnormal myelination, lysosomal anomalies within MG and neurons, and behavioral deficits reflective of age-related neural degeneration.

Results

***Apobec1* Expression Is Found Primarily in MG.** The brain is composed of many cell types, each of which expresses a distinct repertoire of genes. Recently, a complete dataset of the transcriptomes of all major neuronal, glial, and vascular brain cell types became available (22). Whereas some RNA-editing enzymes (e.g., adenosine deaminases acting on RNA; ADAR) are ubiquitously expressed in all cell lineages present within the brain, querying this dataset revealed that *Apobec1* and its obligate cofactor *Rbm47* (23) are most prevalently transcribed within MG derived from postnatal day 17 brain (22) (Fig. 1 *A* and *B*). To confirm and extend those findings, we used fluorescent activated cell sorting (FACS) to isolate two major populations of adult-derived MG we had previously identified in the steady state (24), during aging (25), and following immune challenge (26, 27). The two populations selected by gating on CD45^{int}CD11b^{hi}CD11c^{neg} (henceforth termed CD11c^{neg} MG) and CD45^{int}CD11b^{hi}CD11c⁺ (henceforth termed CD11c⁺ MG) were present in comparable numbers in *wildtype* and *Apobec1*^{-/-} mice (Fig. S1 *A* and *B*). Isolated CD11c⁺ MG and CD11c^{neg} MG populations were subsequently

subjected to RNA-sequencing (RNA-seq) and qRT-PCR to examine the expression of *Apobec1* and *Rbm47*. These experiments revealed the presence of both transcripts within these cells isolated from *wildtype* mice (Fig. S1 *C* and *D*), confirming the findings shown in Fig. 1 *A* and *B* and extending those to adult-derived MG (22).

Neuroinflammation and Ultrastructural Pathology in *Apobec1*^{-/-} Mouse Brains. We then examined whether the loss of this RNA-editing enzyme impacts the distribution or morphology of MG in the *Apobec1*^{-/-} mouse CNS. Serial sections from age- and sex-matched *wildtype* and *Apobec1*^{-/-} brains were labeled with Iba-1 antibody, a marker for MG. Our results revealed the presence of Iba1⁺ MG clusters throughout the brain's parenchyma, suggesting ongoing inflammation (Fig. 1*C*). This finding was further supported by qRT-PCR analysis of *Apobec1*^{-/-} cortex (CTX) and hippocampus (HP) tissue, which demonstrated significant increases in the mRNA levels of proinflammatory cytokines (*IL-1β*, *IL-6*, and *TNF-α*) (Fig. 1*D*). Collectively, these data support the conclusion that the loss of APOBEC1 leads to activated MG and to disruption of brain homeostasis.

Elevated levels of inflammatory cytokines are linked with impaired myelination in the brain (28). We therefore examined the *Apobec1*^{-/-} mouse brains for defects in myelination at the light and ultrastructural levels. Using Black Gold staining for myelinated fibers, we noted that the *Apobec1*^{-/-} mouse exhibits progressive demyelination, resulting in aberrant myelinated fibers in several brain regions showing neuroinflammation, including the hippocampal CA2/CA3a region (Fig. 2*A*). Further ultrastructural analysis of this region revealed many anomalies in *Apobec1*^{-/-} mice, ranging from deficient and loosely wrapped myelin (Fig. 2*B* and *C*) to increased numbers of small myelinated fibers compared with *wildtype* brains (Fig. 2*D*), a phenomenon often associated with pathological changes in demyelinating diseases (29).

Ultrastructural examination of the HPs of *Apobec1*^{-/-} and wild-type mice revealed an additional disturbance in neuronal architecture characterized by a striking accumulation and clustering of lysosomes and autophagosomes (Fig. 3). Immunofluorescence levels of LAMP1 and microtubule-associated protein 1A/1B–light chain 3-II (used as a marker of autophagosomes) were also significantly increased in *Apobec1*^{-/-} brains, confirming the changes in the abundance of autophagosomes and lysosomes seen on EM micrographs (Fig. S2 *A* and *B*).

Behavioral Defects in *Apobec1*^{-/-} Mice. Given that increased inflammation in the brain has been associated with symptoms of

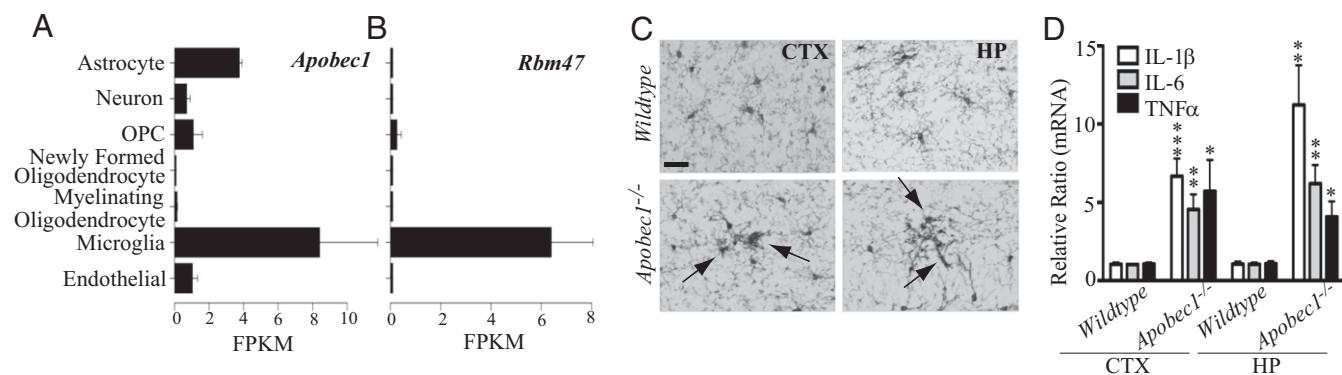


Fig. 1. *Apobec1* is expressed in the brain, and its deletion is associated with a proinflammatory phenotype. (*A* and *B*) Expression of *Apobec1* and *Rbm47* in different neural cell types was calculated from RNA-seq (22). FPKM, fragments per kilobase of transcript per million mapped reads; OPC, oligodendrocyte progenitor cells. (*C*) CTX and HP slices from *wildtype* and *Apobec1*^{-/-} mice were immunolabeled with Iba1 to detect MG. Arrows point to MG clusters only detected in *Apobec1*^{-/-} brains. (Scale bar: 25 μm.) (*D*) Transcript levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α were measured in the CTX and HP of the *Apobec1*^{-/-} and wild-type mice using qRT-PCR. Each bar represents the mean ± SEM ($n = 5$ per group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. wild-type (one-way ANOVA with Neuman–Keuls analysis).

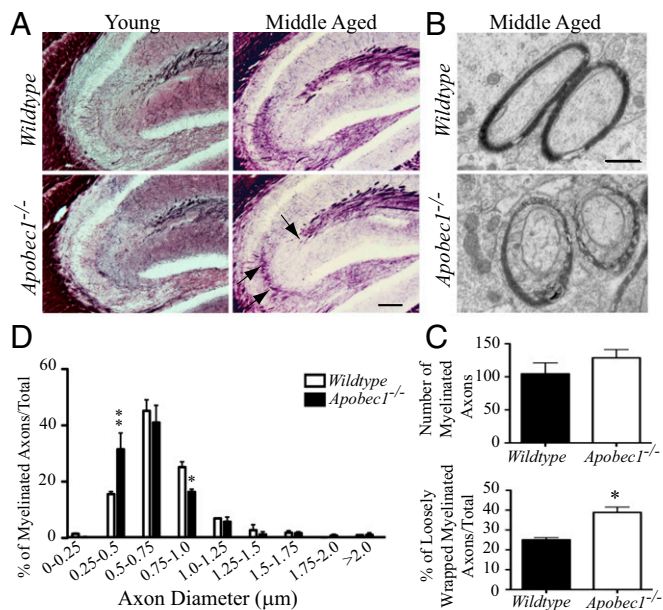


Fig. 2. *Apobec1*^{-/-} mice have reduced myelin in the HP. (A) Black Gold staining revealed reduced myelin in the CA2/3a region of HPs in 9- to 12-month-old (middle-aged) but not 3-month-old (young) *Apobec1*^{-/-} mice compared with *wildtype* mice. (Scale bar: 200 μm.) (B) EM detected defective and loosely wrapped myelin in the CA2/3a stratum radiatum of middle-aged *wildtype* and *Apobec1*^{-/-} mice. (Scale bar: 100 nm.) (C) Myelin diameter was calculated using a G-ratio (the diameter of the axon divided by the diameter of the axon plus the myelin sheath). **P* < 0.05 (Student's *t* test analysis). (D) Numbers of myelinated axons sorted by diameter were compared between *wildtype* and *Apobec1*^{-/-} brains (*n* = 3 mice per genotype). **P* < 0.05; ***P* < 0.01 (Student's *t* test analysis).

neuropsychiatric and neurodegenerative disorders (30), we also evaluated the behavior of young and middle-aged *Apobec1*^{-/-} male mice, compared with their age- and sex-matched *wildtype* littermates. Whereas there was no significant difference in anxiety phenotypes between the two genotypes in the younger cohort, the middle-aged *Apobec1*^{-/-} mice clearly displayed a significant increase in anxiety in both the elevated plus maze and open-field tests (31) (Fig. 4*A* and *B* and Fig. S3*A* and *B*). When we tested for depression using the sucrose splash test (32) in the same cohort of mice, again, only the middle-aged *Apobec1*^{-/-} mice showed significant signs of a depressive phenotype (Fig. 4*C* and Fig. S3*C*; *P* < 0.05). Collectively, these data indicate that loss of APOBEC1 is associated with increased depression and anxiety in middle-aged mice.

The same cohorts were further subjected to two separate tests that evaluate memory function: the novel object recognition test and the Y-maze for spontaneous alternation as a measure of working memory (33). The results clearly demonstrate, a significant deficit in the object recognition test in the middle-aged *Apobec1*^{-/-} cohort compared with *wildtype* mice (Fig. 4*D*). Interestingly, no differences in performance were found in the Y-maze test, indicating that the memory defect in these middle-aged animals is limited to recognition memory and does not affect working memory (Fig. S3*D*).

Finally, the mice were subjected to the extension reflex test (34). The middle-aged *Apobec1*^{-/-} mice had an abnormal hind-limb reflex response, indicating a significant loss in this motor function (Fig. 4*E* and Movies S1 and S2). This defect might be attributed to abnormal myelin detected in the brains of *Apobec1*^{-/-} mice and suggests that the pathology extends to other regions of the brain or spinal cord. Furthermore, changes in hind-limb motor behavior have been noted in a mouse with a *Cln7/Mfsd8* gene

disruption, which recapitulates CLN7 disease, a human neurodegenerative LSD (35).

Identification of APOBEC1-Edited Transcripts in Purified MG. In view of the extensive pathology and behavior anomalies we observed in *Apobec1*^{-/-} mice, we tested whether the loss of APOBEC1-mediated RNA editing could help explain some of these defects. We therefore performed RNA-seq on CD11c⁺ and CD11c^{neg} MG sorted from *wildtype* and *Apobec1*^{-/-} brains using the Illumina platform and used the datasets to comparatively infer editing rates for CD11c⁺ and CD11c^{neg} MG, sorted from *wildtype* and *Apobec1*^{-/-} HP and CTX. We have previously identified robust APOBEC1-mediated editing by looking for C-to-T changes in aligned RNA-seq reads (with respect to the reference genome) that occur exclusively in *wildtype* cells (i.e., the same C-to-T changes are not found in *Apobec1*^{-/-} editing-deficient cells) (20). However, as we have shown, the editing rates of specific coordinates can vary significantly between individual cells within a population, even when that population is highly homogeneous (20). When populations are heterogeneous (as is the case here) and/or editing only occurs in a very small subset of the cells queried, editing rates observed from bulk RNA-seq generally appear very low and occur below the stringent thresholds used for cell types that edit more uniformly (i.e., macrophages, enterocytes). This makes orthogonal validation of any sites identified essential.

To validate editing in the CD11c⁺ and CD11c^{neg} MG populations, we used an amplicon-based, deep-sequencing approach in which we generated a number of amplicons representative of potentially edited transcripts. We were able to validate a number of editing events (Table S1). Since there were some editing events we could not validate (Table S1, footnote), we estimate that these are either true false-positive results or hallmarks demonstrating that editing occurs only in a very small population of cells at steady state, which would potentially be amplified under stimulation conditions (e.g., editing rates for *B2m* and *Lamp2* are greatly increased in virally infected CD11c⁺ MG) (Fig. S4).

To examine whether the lack of APOBEC1-mediated RNA editing in MG affects mRNA levels, we compared *Apobec1*^{-/-} and *wildtype* CD11c⁺ and CD11c^{neg} MG RNA-seq datasets for gene expression differences. Comparisons using GFOLD analysis (36) revealed that loss of APOBEC1 results in gene expression differences in both the CD11c⁺ and CD11c^{neg} MG, with a greater number of differentially expressed transcripts found in the CD11c⁺ cells, and with the larger group of these genes predicted to be involved in metabolism (Table S2).

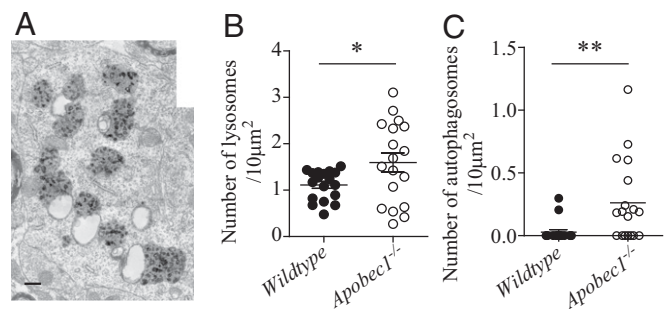


Fig. 3. *Apobec1*^{-/-} mice have lysosome-associated pathology in hippocampal CA2/3a pyramidal cell neurons. (A) EM showed an accumulation of lysosomes in CA2 pyramidal cell somata. (Scale bar: 100 nm.) The number of lysosomes (B) and autophagosomes (C) was quantified per 10 μm² of cytoplasmic area in *wildtype* and *Apobec1*^{-/-} mice. Each dot-plot represents the mean ± SEM (*n* = 3 mice per genotype; *n* = 6 somata per mouse). **P* < 0.05; ***P* < 0.01 vs. *wildtype* mice (Student's *t* test analysis).

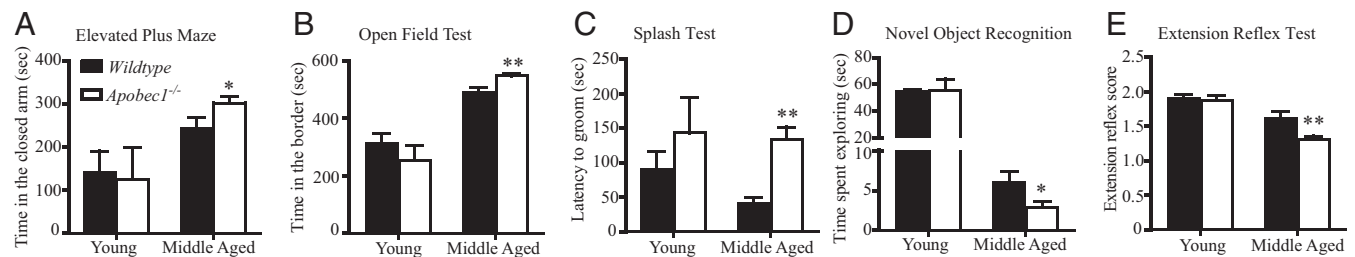


Fig. 4. *Apobec1*^{-/-} mice display anxiety- and depression-like behaviors, as well as cognitive and motor deficits. Increased anxiety in *Apobec1*^{-/-} mice was calculated based on time spent in the closed arm of the elevated plus maze (A) and border of the open-field test (B). Each bar represents the mean \pm SEM ($n = 7$ –12 mice per genotype). * $P < 0.05$; ** $P < 0.01$ vs. *wildtype* mice (Student's t test). Movement did not correlate with either measure ($r^2 = 0.13$). (C) Depressive-like behavior was measured using the sucrose splash test. Data show latency to groom; each bar represents the mean \pm SEM ($n = 7$ –12 mice per genotype). ** $P < 0.01$ (Student's t test). (D) Novel object recognition test was used to evaluate memory retention. Data show time spent investigating the novel object. Each bar represents the mean \pm SEM ($n = 7$ –10 mice per genotype). * $P < 0.05$ (Student's t test). (E) Extension reflex test was used to measure motor deficits. Data show extension reflex scores; each bar represents the mean \pm SEM ($n = 7$ –12 mice per genotype). ** $P < 0.01$ (Student's t test).

Lack of APOBEC1-Mediated Editing in MG Affects LAMP2 Protein Levels.

One of the transcripts edited in MG was *Lamp2*, which encodes an important functional component of lysosomal membranes (37). As a proof of concept, we focused on the effect of editing on *Lamp2*. We found that in BMDMs, APOBEC1 editing in 3'UTRs results in changes in protein abundance (18). Utilizing luciferase assays as an assessment of protein levels and comparing “unedited” (“*Apobec1*^{-/-}”) and edited (“wild-type”) versions of the 3'UTR of *Lamp2* (mutated at the DNA level to reflect RNA editing events found in vivo), we demonstrate herein that loss of editing decreases LAMP2 protein abundance (Fig. 5A). Immunofluorescent labeling of LAMP2 in *Apobec1*^{-/-} and *wildtype* brains further confirmed that the lack of editing significantly decreases the levels of this lysosomal protein in vivo (Fig. 5B and C). Triple immunolabeling with LAMP2, Iba1, and NeuN revealed a decrease in LAMP2 immunofluorescence in *Apobec1*^{-/-} neurons (NeuN⁺) compared with wild-type neurons (Fig. S5). Small cell bodies and a high nucleus-to-cytosol ratio of Iba1⁺ MG hindered the analysis of LAMP2 immunofluorescence in these cells. Therefore, to accurately assess changes in the levels of LAMP2 in MG, we used flow cytometry analysis, which revealed that the CD11c^{neg} subset of the MG population expresses lower levels of LAMP2 (Fig. 5D).

Apobec1^{-/-} MG Are Poor Phagocytes Compared with Wild-Type MG.

LAMP2 is an integral part of the lysosomal membrane involved in fusion with phagosomes and autophagosomes (37, 38). Thus, to test whether lack of APOBEC1-mediated editing in MG affects its cellular function, we measured the phagocytic activity of CD45^{int}/CD11b^{hi} MG isolated from *Apobec1*^{-/-} and wild-type brains. Using an assay based on the uptake of fluorescence-conjugated bacterial particles, we demonstrated that the phagocytic function of *Apobec1*^{-/-} MG is compromised (Fig. 6A). This result is in contrast to data obtained from *Apobec1*^{-/-} BMDMs, which display increases in phagocytic activity (18). This discrepancy can be explained by the differences in developmental origin, as well as developmental stage, at which these cells were tested (such mechanisms could include loss or gain of binding of specific sets of RNA binding proteins that promote or disallow ribosomal loading; reviewed in refs. 39, 40). Moreover, the differences between editing events and edited transcripts identified in BMDMs (18) and MG also reflect the high level of functional heterogeneity between these two cell populations.

Mutations in the *Lamp2* gene can cause Danon disease, which is an LSD characterized by the accumulation of autophagic vesicles (41–43). This led us to examine the accumulation of these vesicles in *Apobec1*^{-/-} brains. Our ultrastructural analysis of the HPs from *Apobec1*^{-/-} mice also shows lysosomal accumulation in neurons (Fig. 3A and B). Thus, we extended our analysis and found the clustering of aberrant lysosomes in *Apobec1*^{-/-} MG as well (Fig. 6B). Together, these data suggest that APOBEC1-mediated

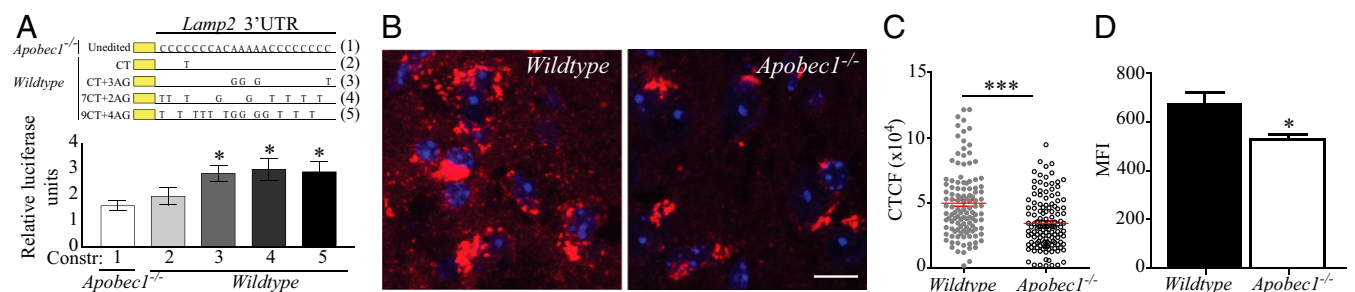


Fig. 5. RNA editing in MG affects LAMP2 protein levels. (A) Effect of editing on LAMP2 protein production was measured by cloning mutated forms of the *Lamp2* 3'UTR (reflective of a range of RNA editing found in *wildtype* cells) in a dual-luciferase-expressing construct (Constr.) (Renilla/firefly depicted by yellow rectangles). Renilla levels (normalized using firefly luminescence) are shown for each of the indicated constructs [unedited (Constr. 1) and a range of edited 3'UTRs (Constr. 2–5) are depicted at the top of the histogram]. Each bar represents the mean \pm SEM. * $P < 0.05$ (Student's t test). AG, A-to-G edit; CT, C-to-T edit. (B) Immunofluorescent labeling was used to detect LAMP2 in middle-aged *wildtype* and *Apobec1*^{-/-} mouse cortical slices. The tissue is counterstained with DAPI (blue) to better visualize individual cells. (Scale bar: 10 μ m.) (C) Quantification of LAMP2 immunofluorescence levels in middle-aged *wildtype* and *Apobec1*^{-/-} cortical and midbrain slices expressed as corrected total cell fluorescence (CTCF). Each dot-plot represents the mean \pm SEM. *** $P < 0.001$ (Student's t test). (D) Expression of LAMP2 in *Apobec1*^{-/-} and *wildtype* CD11c^{neg} MG was analyzed by flow cytometry. Each bar represents the mean \pm SEM ($n = 3$ mice per genotype). ** $P < 0.01$ (Student's t test). MFI, mean fluorescence intensity.

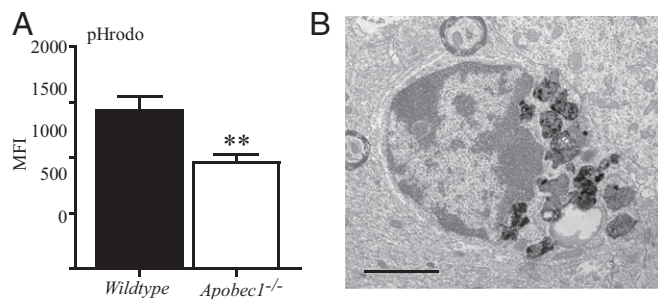


Fig. 6. *Apobec1*^{-/-} MG display lysosomal dysfunction and are deficient in phagocytic activity. (A) Phagocytic activity of *Apobec1*^{-/-} and *wildtype* MG was measured based on uptake of pHrodo-green. Data show mean fluorescent intensity (MFI) of pHrodo particles (488 nm) within the primary MG population pregated on CD45^{int}/CD11b^{hi}. Each bar represents the mean \pm SEM ($n = 6$ mice per genotype). $***P < 0.01$ (Student's *t* test). (B) Electron micrograph of MG with aberrant lysosomes in the HP from an *Apobec1*^{-/-} mouse. Identification of the MG was by characteristic clumped chromatin beneath the nuclear envelope (53). (Scale bar: 2 μ m.)

RNA editing of transcripts for lysosomal proteins (i.e., LAMP2) is fundamental for proper lysosomal function in MG, and may affect the accumulation and function of lysosomes in neurons as well in a non-cell-autonomous manner.

Discussion

The neuropathology and related behavioral deficits in the *Apobec1*^{-/-} mouse model described in this study are strong indicators that APOBEC1-mediated RNA editing in MG is required for CNS homeostasis and normal neurological and behavioral function throughout the life span. Given that *Apobec1* expression is highest in MG, and since all other neural cell types lack the appropriate cofactors (Fig. 1A), this specificity of expression points to a direct role for editing in MG function. However, the diversity of the MG population (i.e., developmental history, activation state) may determine the degree to which individual cell function is affected by RNA editing. In concert with the expression data, we also identified APOBEC1-mediated RNA-editing events within multiple transcripts in CD11c⁺ and CD11c^{neg} MG. As proof of concept, we focused our investigation on one of these edited transcripts, *Lamp2*. Interestingly, mutations in *Lamp2* underlie Danon disease, a lysosomal glycogen storage disease characterized by accumulation of autophagosomes in heart and skeletal muscle (41–43). Moreover, the lack of LAMP2 in genetically modified mouse brain leads to inflammation and abnormal behavior, including motor and learning deficits (44). Thus, our observations in the middle-aged *Apobec1*^{-/-} mice, such as increased neuroinflammation, aberrant accumulation of lysosomes and autophagosomes, and behavioral deficits, led us to hypothesize that LAMP2 is regulated by RNA editing. In support of this hypothesis, we found that the absence of APOBEC1-mediated editing decreases LAMP2 protein levels in an in vitro assay as well as in *Apobec1*^{-/-} brains. Although these data suggest that the neuropathological effect of APOBEC1 deletion may be mediated through LAMP2, we do not exclude the contributions of other edited transcripts we have not tested. Moreover, RNA-seq analysis of *Apobec1*^{-/-} and wild-type MG revealed changes in the levels of transcripts, which are not APOBEC1 substrates. Further studies are needed to determine how these changes in gene expression relate to RNA-editing events and contribute to MG function.

Although our results indicate that the APOBEC1 editing complex is functional only in MG, we found decreased LAMP2 protein

levels, aberrant accumulation of lysosomes and autophagosomes in neurons, and impaired myelin structure in the brains of *Apobec1*^{-/-} mice. Moreover, the impaired motor and cognitive behaviors displayed by *Apobec1*^{-/-} mice also point to deficiencies in neuronal function and myelination. These data provide a clear example that RNA editing may have important functional consequences in a non-cell-autonomous manner. Defective lysosomal, autophagic, and phagocytic function of MG is known to contribute to brain pathophysiology during aging and in multiple disease models, such as ALS, AD, PD, and LSD, through insufficient clearance of protein aggregates and cellular debris (16, 17, 45–47). Non-cell-autonomous effects of MG dysfunction on neighboring cells can also be explained by the compromised neuroprotective role of MG that lose the ability to maintain a “healthy” environment for neurons (48). During homeostasis, direct communication between neural cell types requires the intercellular transfer of bioactive cargos composed of lipids, proteins, RNA, or even organelles (i.e., lysosomes). The transfer of this cargo is carried out by structures such as extracellular vesicles and tunneling nanotubes (49, 50). Interestingly, the transfer of these bioactive cargos causes the recipient cell to incorporate the functional properties of the cargo into its own cellular machinery. While the transfer and incorporation mechanisms are not fully understood, the utility of the principles behind them (termed “cross-correction”) has been employed for treatment of several forms of LSD, with some success using healthy BMDMs (50, 51).

It is noteworthy that the neuropathologies and behavioral impairment in *Apobec1*^{-/-} mice described in this study are not observed in young (3-mo-old) mice, but become evident in middle age (at 9 to 12 mo of age), a time when *wildtype* mice are asymptomatic for overt signs of aging. This indicates that molecular and cellular perturbations precipitated by the lack of APOBEC1-mediated RNA editing in the brain are cumulative and lead to a progressive phenotype of premature aging. Collectively, these data indicate that RNA editing by APOBEC1 is an essential regulatory mechanism of MG function and is critical for overall brain homeostasis and healthy aging.

Finally, given that standard genome-wide association studies (which catalog DNA changes) have not been particularly successful in predicting disease progression or manifestations of neurodegenerative or psychiatric disorders, our results, which link aberrant RNA editing and neuropathophysiology, reveal the possibility that RNA editing, as a heritable but epitranscriptomic marker, might be a useful predictor for diseases of the brain.

Materials and Methods

All experimental procedures were approved by the Rockefeller University Animal Care and Use Committee, and adhere to NIH *Guidelines for the Care and Use of Experimental Animals* (52). Details of behavior tests, immunohistochemistry and Black Gold staining, EM, quantitative PCR, flow cytometry and FACS, RNA-seq and gene expression analysis, high-throughput amplicon sequencing of edited fragments and validation of editing targets, the luciferase assay, and the phagocytosis assay are provided in *SI Methods*. *Supporting Information* is available in the online version of the paper.

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