The Role of Neuronal NLRP1 Inflammasome in Alzheimer's Disease: Bringing Neurons into the Neuroinflammation Game

Jeremy Kean Yi Yap[1], Benjamin Simon Pickard[2], Elaine Wan Ling Chan[3], Sook Yee Gan[4]*

[1] School of Postgraduate Studies, International Medical University, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

[2] Strathclyde Institute of Pharmacy and Biomedical Sciences**,** University of Strathclyde, Hamnett Wing, 161, Cathedral Street, Glasgow, United Kingdom, G4 0RE.

[3] Institute for Research, Development and Innovation, International Medical University, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

[4] Department of Life Sciences, School of Pharmacy, International Medical University, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

*Corresponding Author: Sook Yee Gan, Department of Life Sciences, School of Pharmacy, International Medical University, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia. Phone: +603-2731 7718; Fax: +603-8656 7229; E-mail: sookyee_gan@imu.edu.my

Jeremy Kean Yi Yap

E-mail: JEREMY.YAP@student.imu.edu.my

Benjamin Simon Pickard, PhD

E-mail: benjamin.pickard@strath.ac.uk

Elaine Wan Ling Chan, PhD

E-mail: elainechan@imu.edu.my

Sook Yee Gan, PhD

E-mail: sookyee_gan@imu.edu.my

Abstract The innate immune system and inflammatory response in the brain have critical impacts on the pathogenesis of many neurodegenerative diseases including Alzheimer's disease (AD). In the central nervous system (CNS), the innate immune response is primarily mediated by microglia. However, non-glial cells such as neurons could also partake in inflammatory response independently through inflammasome signalling. The NLR family pyrin domain-containing 1 (NLRP1) inflammasome in the CNS is primarily expressed by pyramidal neurons and oligodendrocytes. NLRP1 is activated in response to amyloid-β (Aβ) aggregates and its activation subsequently cleaves caspase-1 into its active subunits. The activated caspase-1 proteolytically processes interleukin-1β and interleukin-18 (IL-1β and IL-18) into maturation whilst co-ordinately triggers caspase-6 which is responsible for apoptosis and axonal degeneration. In addition, caspase-1 activation also induces pyroptosis, an inflammatory form of programmed cell death. Studies in murine AD models indicate that the NLRP1 inflammasome is indeed upregulated in AD and neuronal death is observed leading to cognitive decline. However, the mechanism of NLRP1 inflammasome activation in AD is particularly elusive, given its structural and functional complexities. In this review, we examine the implications of the human NLRP1 inflammasome and its signalling pathways in driving neuroinflammation in AD.

Keywords: Alzheimer's disease, Caspase-1, Interleukin-1β, Interleukin-18, Neuroinflammation, NLRP1 inflammasome

Introduction

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease which gradually damages patients' memory and cognitive functions. It is the most common cause of dementia, accounting for 60-80% of total dementia cases [1]. The clinical symptoms of AD include memory loss, behavioural fluctuations, and difficulty in speech, problem-solving and basic cognitive functions - all of which are attributed to the shrinking of select regions in the brain caused by widespread neuronal loss. As the brain continues to deteriorate, the symptoms worsen and eventually leading to death. At present, there is no available treatment capable of modifying or reversing the progression of AD. Current clinical interventions such as cholinesterase inhibitors (e.g. Donzepil, Rivastigmine, and Galantamine) for early to moderate stages of AD, and N-methyl-D-aspartate inhibitors (e.g. Memantine) for late to severe AD, are limited to symptom management and improvement of quality of life [2].

The aetiology of AD has remained elusive ever since the disease was first described. Two well-known pathological hallmarks of AD, namely senile plaques (primarily composed of amyloid-β (Aβ) peptides) and neurofibrillary tangles (NFTs, primarily composed of tau proteins), have been a major focus in contemporary AD research [3]. To cure AD, however, remains challenging as our knowledge in AD pathophysiology has not yet translated into the development of drugs that target the root cause of the disease. Several drugs designed to reduce Aβ production or aggregation such as rosiglitazone, semagacestat, tarenflurbil, tramiprosate and scyllo-inositol have failed in clinical trials either due to ineffectiveness or severe side effects [4]. As reported in 2018, there are approximately 17 potentially disease-modifying drugs in Phase III clinical trial which include 14 drugs targeting amyloid and one targeting tau [5]. In the wake of the second failure of aducanumab in a recent Phase III clinical trial, we are forced to consider novel approaches towards future AD drug development that targets beyond the conventional AD pathologies.

In recent years, neuroinflammation has received much attention as a potential driver of neurodegeneration, and its implications in AD is evident in genetic studies, neuroimaging and biopsies of patients, as reviewed in: [6,7]. As inquiries in the roles of neuroinflammation in AD gain traction, a type of multiprotein complex known as the inflammasome has garnered much interest for its part in inflammation regulation. Inflammasomes serve as platforms for the recruitment and activation of caspase-1, the *de facto* executioner of a diverse downstream inflammatory processes including the maturation of two major pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 [8]. Several types of inflammasomes have been identified within the central nervous system (CNS), of which the best characterized are the absent in melanoma 2 (AIM2), NLR-family pyrin

domain-containing 1 (NLRP1), NLR-family pyrin domain-containing 3 (NLRP3) and NLR-family CARD-containing 4 (NLRC4) inflammasomes [9]. Recently, the NLRP1 inflammasome was shown to co-activate with NLRP3 inflammasome in monocytes of AD patients [10]. In AD brains, there is approximately 25- to 30-fold increase in neuronal NLRP1 immunopositivity compared to non-AD brains [11]. As such, we propose that the NLRP1 inflammasome in neurons play a pivotal role in contributing towards neuroinflammation in AD. In this review, we consolidate the latest findings on the structure and mechanisms of regulation and activation of the NLRP1 inflammasome. Then, we examine potential roles of neuronal NLRP1 inflammasome and its corresponding signalling pathways in contributing towards neurodegeneration in AD.

The innate immune system, inflammation and inflammasomes in the CNS

The innate immune system acts as a forefront in host defence against infectious pathogens as well as non-infectious harmful stimuli. In the periphery, innate immunity is modulated by immune cells such as mast cells, dendritic cells, macrophages and neutrophils. [12,13]. These immune cells recognize molecules associated with groups of pathogens termed pathogen-associated molecular patterns (PAMPs) or with sterile tissue injuries termed danger-associated molecular patterns (DAMPs) [14]. These signals are recognized through germline-encoded pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), the retinoic acid-inducible gene-I-like receptors (RIG-I-like receptor or RLRs), C-type lectin receptors (CLRs) and the nucleotide oligomerization domain-like receptors (NOD-like receptors or NLRs), as well as a number of DNA-sensing molecules including the pyrin and HIN domain-containing (PYHIN) receptors [15,16]. When engaged, these receptors function in synergy to promote maturation and secretion of pro-inflammatory cytokines and molecules which results in inflammation and pathogen clearance.

The central nervous system (CNS) is separated from the peripheral circulatory system by the blood-brain barrier (BBB), a highly selective semi-permeable membrane barrier which shield the CNS against external injury, toxins and pathogens [17]. In addition, the sanctity of the CNS is protected by microglia and astrocytes which makes up its primary resident immune cells. The roles of these glial cells in immunity are reviewed in: [18,19]. While the primary function of these cells are to maintain homeostasis and to provide support for neuronal functions, they are capable of eliciting innate immune responses against infections and stimulate inflammation within the CNS, more commonly known as neuroinflammation. Surprisingly, even neurons are shown to be capable of participating in such responses through the expression of PRRs and the production of pro-inflammatory

cytokines [20,21]. Although inflammation is intended to be protective in nature, dysregulated inflammation can instead lead to severe tissue damage and even death. In the CNS, neuroinflammation is widely implicated in various neurogenerative diseases including AD, Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), as reviewed in: [22].

In AD, chronic neuroinflammation is believed to be the possible missing link between neuritic plaques, NFTs and neuronal death [6]. One of the cardinal signs of neuroinflammation in AD is in the patients' cytokine profile. The mRNA expression of several major cytokines (interleukins, tumour necrosis factor α converting enzyme (TACE), and transforming growth factor β1 (TGFβ1)) are significantly upregulated in the brains of AD patients compared to non-demented controls [23]. Moreover, a meta-analysis has indicated elevated levels of pro-inflammatory cytokines including tumour necrosis factor α (TNFα), TGFβ1, IL-1β and IL-18 found in the peripheral blood of AD patients further substantiates the association between inflammation and AD [24]. Interestingly, this spike in cytokine expression is only observed in late stage AD with significant cognitive decline, but not in its pre-symptomatic stages, suggesting that neuroinflammation may be a secondary response to external factors such as Aβ which rapidly escalates with greater amyloid deposition. Moreover, leaky blood-brain barrier typically observed in brains of AD patients further encourages influx of toxic molecules, pathogens, peripheral immune cells and pro-inflammatory cytokines which collectively escalates the severity of neuroinflammation [25].

In recent years, a wealth of literature points toward inflammasomes in the CNS as an important driver of AD pathogenesis through caspase-1-mediated neuroinflammatory responses against Aβ [26–29]. Inflammasomes are assembled as a cluster of PRR which recruit caspase-1 via an adaptor protein known as apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). Among the PRRs, only NLRs and PYHINs are capable of forming inflammasomes [30]. The NLRP3 inflammasome expressed in microglia is the most extensively studied of all the inflammasomes and its roles in AD is well-established [29,31,32]. While studies in inflammasomes in neurons have been relatively scant, several types namely AIM2, NLRC4 and NLRP1, have been identified and may elicit similar neuroinflammatory processes [11,33]. Within neurons, the NLRP1 inflammasome is particularly relevant in AD. A cohort study has identified four non-synonymous single nucleotide polymorphisms (SNPs) in the *NLRP1* gene are associated with increased AD susceptibility; rs2137722 (Arg1322Cys), rs3473379 (Thr995Ile), rs11657747 (Thr878Met), and rs11651595 (Thr246Ser) [34], although these data are not confirmed in the latest genome-wide association (GWA) meta-analysis [35]. However, *in vivo* and *in vitro* exposure of neurons to Aβ in AD murine models have shown to induce Nlrp1-dependent neuronal

loss [36]. In the human brain, there is a significant increase in NLRP1 mRNA, but not NLRP3, expressed in the cerebellum and cortex of AD brains compared to healthy controls, with 25- to 30- fold increase in NLRP1 immunopositive staining within the hippocampus. NLRP1, and subsequently caspase-1 activity, also co-ordinately activate caspase-6 which consequentially leads to neuroinflammation and axonal degeneration [11]. All these studies allude to NLRP1's contribution towards neurodegeneration in AD (Fig. 1).

The NLRP1 inflammasome: Structure and mechanism of activation

Although NLRP1 was the first inflammasome to be described, its properties remained particularly enigmatic as studies were hindered by numerous complications such as the uncertainty of its specific ligands and the substantial structural and functional divergence between the human NLRP1 and its murine counterpart [37,38]. Although found in a diverse mammalian species, the NLRP1 inflammasome has undergone considerable modifications among these species. For example, there are three paralogs which exist in mice for the *Nlrp1* gene, namely *Nlrp1a, Nlrp1b* and *Nlrp1c*, whilst only a single *NLRP1* gene exists in humans (Fig. 2) [39]. Moreover, alternative splicing has resulted in considerable polymorphism both within and between species. In humans, there are seven isoforms of NLRP1 that is known to exist, each with altered protein sequences at the C-terminal region. The specific activators for the NLRP1 inflammasome are also varied between the human and rodent orthologues. For example, while it is known that mouse Nlrp1b, the species that exhibit the most similarity with that of human's, is reactive towards anthrax lethal factor (LF) due to the presence of an LF cleavage site located at the N-terminal region [40], human NLRP1 does not produce a similar response. This poses a challenge in accurately modelling human NLRP1 and its related diseases in animal models.

As a member of the NLR family, the 'canonical' full-length human NLRP1 (1473 amino acids; 165.9 kDa) contains a central NLR which consists of a nucleotide oligomerization domain (NOD) to facilitate the post-activation assembly of the inflammasome core and a leucine-rich repeat (LRR) domain which acts as an autoinhibitory domain [41]. In common with the NLRP sub-family, a pyrin domain (PYD) is attached to its N-terminus [42]. Additionally, NLRP1 contains a unique 'function-to-find' (FIIND) domain which is flanked by LRR and caspase recruitment domain (CARD). The FIIND domain is only shared with another inflammasome-related protein known as CARD family member 8 (CARD), and bioinformatic analyses revealed that FIIND is structurally remarkably similar to a combination of two domains namely ZU5 and UPA [43]. It has therefore been suggested that FIIND be renamed ZU5-UPA-like domain (for convention's sake, we will continue to refer to this

domain as FIIND). The post-translational auto-proteolytic cleavage within the FIIND domain is strictly necessary for the NLRP1 activation [44]. This cleavage is initiated by deprotonation of Ser^{1213} located within the FIIND domain by a highly-conserved distal His^{1186} residue. Incidentally, an SNP near His^{1186} disrupts the cleavage within FIIND region and hence prevents NLRP1 activation. As the name suggests, the precise function of this FIIND cleavage is yet to be determined, but presumably it is important for complex formation to enable ASC and caspase-1 interactions [45].

Unlike other NLRPs including murine Nlrp1, the human NLRP1 simultaneously contains both PYD and CARD within its structure. These proteins belong to the death domain (DD) superfamily which are protein-protein interaction domains for apoptotic or inflammatory signal transduction [37,46,47]. Although the human NLRP1 contains two signal transduction domains, surprisingly the PYD of NLRP1 is dispensable whereas the CARD appears to be its *bona fide* effector domain. Crystallization and nuclear magnetic resonance spectroscopy analyses revealed structural distinctions between NLRP1's PYD compared to the PYD of other NLRs [48,49] with the most conspicuous difference being the lack of a defined $\alpha 3$ helix, one of the six conserved α-helices typical of the DD superfamily, which instead is replaced by a flexible loop. This unique structural feature may be sufficient to modify the function of NLRP1 PYD. Studies have demonstrated that the proteolytic cleavage of NLRP1 N-terminus is a general molecular mechanism for its activation [50,51]. In humans, this cleavage occurs at the unusually long linker region between PYD and NOD, resulting in a severed PYD. In a study by Chavarría-Smith *et al.*, a reconstituted human NLRP1 could be activated following an artificial cleavage on the linker sequence even with its PYD replaced with a green fluorescent protein [51]. Therefore, it is hypothesized that the N-terminal region of NLRP1 plays an auto-inhibitory role instead of inflammasome signal transduction. It is interesting to note that mouse Nlrp1 lacks a PYD which suggests the possibility that PYD is capable of participating in an independent process to supplement NLRP1-mediated inflammation in the human system.

Two known mechanisms of activation have been described for mouse Nlrp1b; N-terminal cleavage by anthrax LF and dipeptidyl peptidases 8 and 9 (DPP8 and DPP9) inhibition [52,53]. In murine macrophages, Nlrp1b is directly cleaved by anthrax LF at 44 amino acids from the N-terminus. Remarkably, it is the Nlrp1b molecule itself acts as a substrate for direct cleavage by anthrax LF, a process that is suggested to enable Nlrp1b to protect protection from anthrax toxicity by functioning as a decoy substrate that prevents LF from attacking MAPK kinases which are vital for host immunity. The cleavage of Nlrp1b generates a neo N-terminal which is targeted by ubiquitin and subsequently undergoes a proteasome-mediated proteolytic degradation known as the N-end rule pathway, which then liberates an active C-terminal fragment with potent inflammatory capacity,

consisting of the UPA-like domain attached to the CARD [54,55]. Presumably, it is for this reason that FIIND cleavage is crucial for the C-terminal UPA-CARD fragment to escape this degradation process. It is surprising, however, that studies in reconstituted human NLRP1 suggested that full-length protein degradation is not necessary for its activation, although FIIND autoproteolysis and N-terminal cleavage is [44,51]. This is interesting because the NOD in human NLRP1 contains Walker-A and Walker-B, two adenosine triphosphate (ATP)-binding motifs which are required for self-oligomerization and inflammasome assembly [56]. To our best knowledge, it is only the human NLRP1 that has been explicitly demonstrated to form inflammasome complex, thus raising the question of whether murine Nlrp1 is also capable of full inflammasome formation [37]. We propose that, unlike human NLRP1, it is possible that mouse Nlrp1b forms small complexes that recruits caspase-1 via CARD-CARD interaction without necessarily scaffolding into a complete inflammasome (Fig. 3).

A more recently-uncovered mechanism of NLRP1 activation involves the inhibition of DPP8 and DPP9. These are intercellular serine peptidases that perform enzymatic non-reversible N-terminal cleavage on their peptide substrates. Members of the DPP family, especially DPP8 and DPP9, have been extensively studied for their importance in regulating a variety of biologically processes, the most important of which is in the immune system. Multiple studies have found that DPP8 and DPP9 inhibition elicits a host of anti-tumour immune responses including T cell activity and caspase-1-dependent pyroptosis in both immune cells and cancer cells [57–59]. This has led to the development of several small-molecule DPP inhibitors such as Val-boroPro as potential cancer therapeutics.

Two independent groups (Okondo *et al.* and de Vasconcelos *et al.*) have demonstrated that DPP8 and DPP9 inhibition stimulates the activation of Nlrp1b inflammasome and caspase-1 [53,60]. In mouse macrophages with DPP8 and DPP9 inhibited with Val-boroPro, pyroptosis, and concomitantly caspase-1 maturation, ASC speck assembly, and secretion of mature IL-1β and IL-18, are significantly accelerated when challenged with anthrax LF, suggesting that DPP8 and DPP9 inhibition unleashes Nlrp1b for maximum inflammatory potency [60]. Nlrp1b activation by Dpp8 and Dpp9 inhibition is also characterised by N-terminal cleavage and degradation, albeit not via the N-end rule pathway but instead an indirect stimulation of endogenous proteasomal lysis pathway [55]. These results suggest that DPP8 and DPP9 are important immune suppressor molecules that serve as regulatory checkpoints upstream of inflammasome activity. As demonstrated by Zhong *et al.* in the human system, DPP9 in particular was shown to bind to NLRP1 FIIND and subsequently processes a currently unidentified protein with inflammasome repressive abilities [61]. Both NLRP1 FIIND binding and DPP9's enzymatic function is necessary to restrict NLRP1 activity and identifying DPP9's ligands may provide insightful information on inflammasome-

mediated cellular inflammation and may prove to be useful in the development of novel anti-inflammatory compounds. FIIND binding by DPPs also reveals novel intramolecular regulatory mechanisms in inflammasomes as DPP8 and DPP9 inhibition also activate CARD8 which, like NLRP1, contains a FIIND directly upstream of a C-terminal CARD that undergoes auto-proteolysis as a *sine qua non* for its activation [43].

In the human system, NLRP1 activation initiates a structural conformational shift allows it oligomerization into the characteristic filamentous star-shaped conformation via clustering among NODs, followed by subsequent ASC speck formation and pro-caspase 1 recruitment to form the complete inflammasome complex (Fig. 4). The mechanisms of inflammasome assembly are reviewed in: [62,63]. In most other inflammasomes, the N-terminal PYD interacts with ASC via homotypic PYD-PYD attraction, which in turn recruit pro-caspase-1 (caspase-1 zymogen) via interaction between the CARD of ASC and the CARD of pro-caspase-1. As NLRP1 possesses its own CARD, it could theoretically bypass the requirement for ASC in caspase-1 recruitment. Indeed, it was observed that ASC-knockout mouse macrophages are able to produce Nlrp1b-mediated IL-1β and pyroptosis in response to anthrax LF without the occurrence of ASC-mediated speck formation and caspase-1 auto-proteolysis [64]. However, ASC speck formation could amplify inflammasome signalling by activating sufficient levels of caspase-1 for optimum downstream reactions including production of matured cytokines [65]. It is also noteworthy that in other inflammasomes, the signal transduction domain is located on the N-terminal upstream of NOD. Upon oligomerization, these inflammasomes orientate its PYD or CARD at the core of the complex to create a platform for ASC aggregation and caspase-1 activation. Since the CARD of NLRP1 is located at the C-terminal, the pattern of oligomerization of NLRP1 could differ compared to other inflammasomes. The precise structural mechanism of NLRP1 inflammasome oligomerization is currently undetermined.

Aβ activates NLRP1 inflammasome via P2X7 purinoceptor/pannexin 1 signalling

Aβ is the product of alternative sequential cleavage of the amyloid precursor protein (APP) by β-secretase (BACE1) followed by γ-secretase. The popular but controversial amyloid cascade hypothesis (ACH) posits that the heavy production and deposition of Aβ peptides aggregates into insoluble fibrils which forms the major component of neuritic plaques which drive the neurodegenerative process [66]. Scientists have also discovered that the more soluble oligomeric form of Aβ is potentially more toxic to the brain compared to its fibril counterpart [67]. Aβ oligomers and fibrils are known inducers of neuroinflammation, owing to their ability to potentiate inflammasome activation [26]. A study by Tan *et al*. provided preliminary evidences that Aβ produced in the

brains of AD transgenic mice with mutant APP and presenilin 1 genes (APPswe/PS1dE9) indeed activates the Nlrp1 inflammasome leading to neuronal loss, likely through pyroptosis [36]. These transgenic mice exhibit cognitive and memory deficits compared to wild type mice. Moreover, Nlrp1-mediated pyroptosis-like death is also observed in *in vitro* cultured rat primary neurons challenged with Aβ oligomers. As NLRP1 is primarily expressed in pyramidal neurons and oligodendrocytes (both important cell types that make up the cerebral cortex, the hippocampus, and the amygdala), Aβ-induced NLRP1-mediated pyroptosis could result in destruction of such integral regions of the brain, leading to brain shrinkage and loss of vital cognitive functions and memory typically observed in AD patients [68]. Importantly, *in vivo* silencing of Nlrp1 and caspase-1 is able to rescue neurons from programmed cell death and markedly restored spatial learning in APPswe/PS1dE9 mice [36], alluding to the indispensable role of Nlrp1 and caspase-1 in AD neurodegeneration. However, the precise mechanism by which Aβ activates the NLRP1 inflammasome remained to be elucidated.

A series of studies by Orellana *et al.* proposed that Aβ-induced neuronal death in AD brains involves P2X7/pannexin 1 interaction [69,70]. Aβ oligomers are reported to induce ATP leakage in cells and at the same time, cause an overexpression of P2X7 purinergic receptors in both microglia and hippocampal neurons [70,71]. P2X7 receptors are ATP-gated cation channel which when stimulated by high concentrations of extracellular ATP (a DAMP typically released in high concentration to the extracellular matrix by injured or inflammatory cells), activates a non-selective ionic passage, including potassium (K^+) ion efflux and calcium (Ca^{2+}) influx. Additionally, P2X7 receptors co-activate with pannexin 1, a membrane hemichannel which creates pores allowing for the passage of much larger molecules, most notably ATP and IL-1β [72]. The pannexin 1 channel is activated by high concentration of extracellular K^+ ions which effectively establishes a positive feedback activation mechanism with P2X7. More importantly, while the outcome of these findings by Orellana *et al.* demonstrated Aβ-induced neuronal death by synaptotoxicity distinct from inflammatory pathways, the K^+/Ca^{2+} imbalance brought about by P2X7/pannexin 1 signalling is also a potent activator of inflammasomes, including NLRP1 inflammasome in neurons (Fig. 4) [73,74]. P2X7/pannexin 1 activation by raised extracellular ATP has been shown to stimulate inflammasome activation through the adenosine monophosphate-activated protein kinase (AMPK) signalling pathway, which is hypothesized to further regulate membrane pore formation by P2X7 receptors [75]. In a separate study on macrophages under hypoxic conditions, a reduced cytosolic ATP content (in addition to high extracellular ATP content) also activates the Nlrp1b inflammasome via AMPK-mediated pathway [76]. In both cases, inflammasome activities are evident in caspase-1 activation and the resultant IL-1β production and cellular pyroptosis.

Implications of NLRP1-mediated caspase-1 activation in AD

Upon NLRP1 inflammasome activation, the recruited pro-caspase-1 cleaves into its P10 and P20 subunits which constitute the active enzyme. This initiates a cascade of downstream inflammatory signalling processes such as IL-1β and IL-18 maturation, caspase-6 activation and neuronal death leading to AD (Fig. 1) [77]. Caspases are a family of cysteine protease enzyme responsible for regulating physiological programmed cell death. There are three categories of caspases: apoptosis initiators (e.g. caspase-8 and caspase-9), apoptosis executioners (e.g. caspase-3, caspase-6 and caspase-7) and inflammatory caspases (e.g. caspase-1, caspase-4 and caspase-5) [78].

IL-1β and IL-18 maturation

One of the principal functions of caspase-1 is to process IL-1β and IL-18 to maturity from their respective precursors, pro-IL-1β and pro-IL-18. Both IL-1β and IL-18 belongs to the IL-1 family which, although usually recognized as potent pro-inflammatory cytokines, are in fact constitutively expressed in controlled levels in the CNS and are essential for physiological neuromodulation [79]. IL-1β is excessively produced by microglia, astrocytes and neurons in response to brain injury. The intracellular signalling pathways elicited by IL-1β within the CNS are cell type-specific [80]. In glial cells, IL-1β regulates the production and release of inflammatory cytokines (IL-1, IL-6, monocyte chemoattractant protein-1 (MCP-1), and TNFs) through the activation of nuclear factor κB (NF-κB). In hippocampal neurons, IL-1β controls synaptic function by activating the cAMP response element-binding protein (CREB), a transcription factor which plays an integral role in neuronal plasticity and memory formation, via activation of the p38 MAPK (mitogen-activated protein kinase) pathway but not the MAPK/ERK (extracellular signal-regulated kinases) pathway. Experimentally, however, these events do not persist as the phosphorylation of CREB declines after only 20 minutes. Interestingly, in a study by Soiampornkul *et al*., IL-1β could suppress CREB activation by interfering with neurotrophin 3-induced Ras/MAPK/ERK and P13-K/Akt signalling pathways even at a concentration where neuron viability is not compromised [81]. CREB suppression might affect neuronal functionality by interfering with long-term potentiation which affects synaptic plasticity, memory formation and spatial awareness, possibly contributing to symptoms of dementia at early stages of AD including mild cognitive impairment (MCI) [82]. Indeed, a controlled level of IL-1β is essential for physiological regulation of neuronal functions and is even shown to be neuroprotective, as IL-1β was reported to decrease Aβ production and ameliorate amyloid burden, possibly through amplification of microglial-mediated

amyloid clearance or encouraging non-amyloidogenic processing of APP [83,84]. Despite reduced amyloid burden, overexpression of IL-1β instead encourages tau hyperphosphorylation mediated by p38 MAPK and glycogen synthase kinase 3 β (GSK-3β) signalling pathways [85]. At the same time, IL-1β mediates neuronal-glial interaction which elevates neuronal acetylcholinesterase expression and activity which, in hippocampal neurons, is shown to impair excitatory synapses via disruption of neuroligin–neurexin junction which consequentially suppresses synaptic glutaminergic signalling [86,87].

Like IL-1β, IL-18 is another essential IL-1 cytokine which is expressed in healthy brain. Physiologically, IL-18 is shown to promote neuronal survivability by increasing the levels of brain-derived neurotrophic factor and the anti-apoptotic protein B-cell lymphoma 2 (BCL-2), whilst also inducing the expression of CREB. These processes sufficiently confer neuroprotection when cortical neurons are challenged with oxygen glucose-deprivation [88]. However, IL-18 in AD patients is likewise abnormally upregulated in neurons and glial cells, especially at the frontal lobe where most of the dopamine neurons in the cerebral cortex are located [89]. Unlike IL-1β, increased levels of IL-18 is found to colocalize with both Aβ aggregations and hyperphosphorylated tau. In a study using SH-SY5Y human neuronal model, IL-18 is shown to promote Aβ production and aggregation by upregulating BACE1 and the N-terminal fragment of presenilin 1 [90]. At the same time, IL-18 upregulates kinase activities important for tau phosphorylation, including cyclin-dependent kinase 5 (Cdk5) and GSK-3β [91]. The actual impact of IL-18 in AD susceptibility is currently a subject of debate as multiple case-control studies on two functional polymorphisms in the IL-18 promoter gene (-607 C/A and -137 G/C) have reported conflicting results [92,93]. In the hopes of consolidating these findings, a meta-analysis conducted by Luo *et al*. revealed that such polymorphisms indeed decrease the risk of AD, particularly in Asian and apolipoprotein-ε4-positive (a significant AD risk gene) population [94]. However, these studies suffer from various limitations such as publication bias and small sample sizes. As with the *NLRP1* gene, the genetic component of IL-18 in AD is not reflected in the most recent GWA study [35]. As such, larger scale studies on AD patient cytokine profiling may be required to re-evaluate the potential risks that IL-18 confers in AD.

Pyroptosis

Along with cytokine maturation, caspase-1 activation promotes an inflammatory programmed cell death known as pyroptosis, a caspase-1-dependent cell death characterized by host-mediated poration of the cell membrane which dissipates the cellular ionic gradient, producing a net increase of osmotic pressure, and cell

swelling [95]. As opposed to apoptosis, pyroptosis is characterized by membrane rupture causing the release of cytosolic contents, including DAMPs and pro-inflammatory cytokines such as ATP, IL-1β and IL-18 into the extracellular spaces to further perpetuate inflammatory responses. Recent evidence suggests that caspase-1 induces pyroptosis via cleavage of the executioner protein gasdermin D, which is a required component in inflammasome-mediated pyroptosis although it does not participate in IL-1β maturation [96]. Gasdermin D-mediated pyroptosis, however, is required for IL-1β secretion to the extracellular spaces. The activation of gasdermin D is also dependent on its cleavage by caspase-1 on the linker between its N-terminal and C-terminal domains, where the N-terminus of gasdermin D is the effector of pyroptosis and the C-terminus functions as an intramolecular inhibitor when connected to the N-terminus [97]. Pyroptosis is typically observed in macrophages as a result of NLRP3 inflammasome activation. Macrophagic pyroptosis may be beneficial to the host as the released PAMPs are further cleared by surrounding neutrophils [98]. However, pyroptosis in brain cells can be particularly detrimental because it leads to permanent loss of neurons. While Tan *et al.* have demonstrated in cultured rat cortical neurons that Aβ induces NLRP1-mediated neuronal death, the presumption that pyroptosis is involved is not sufficiently conclusive as the TUNEL assay that was performed did not rule out other forms of cell death such as apoptosis [36]. As such, there are currently no available data that directly associate Aβ and neuronal pyroptosis. It is also not known if gasdermin D is expressed in neurons. Where gasdermin D is not present, NLRP1 activation instead induces cellular apoptosis via caspase-1 cleavage of caspase-3 and caspase-7 [99]. It would be interesting therefore, to test for the effect of Aβ on possible gasdermin D activation in neurons to study the extent of neuronal pyroptosis that may be involved in AD.

Caspase-6 activation

Caspase-1 activation in human primary CNS neurons has been demonstrated to activate caspase-6 in an NLRP1-dependent manner [11]. Caspase-6 is a caspase of the apoptosis effector class which is expressed ubiquitously in the brain, including the entorhinal cortex, hippocampus and the striatum [100]. The pathophysiological role of caspase-6 is relatively complex as its function is not limited to apoptosis and does not always induce cell death when activated. Physiologically, caspase-6 plays a vital role in axon pruning during development and have apparent neuroprotective effects against ALS [101]. However, caspase-6 is also highly implicated in neurodegenerative diseases including HD and AD [102].

Caspase-6 activity can be observed in all developmental stages of AD from non-cognitive impaired individuals to cases of very severe AD [103]. The abundance of caspase-6 activity in the entorhinal cortex and hippocampus also correlates inversely with episodic memory assessments, including in non-demented individuals, and is a reliable predictor of a person's risk of developing AD [104]. Caspase-6 processes a range of substrates associated with neurodegenerative diseases including tau, APP, huntingtin and the presenilins 1 and 2 [105–108]. The presence of active caspase-6 in AD lesions including neuropil threads, neuritic plaques, and neurofibrillary tangles suggests caspase-6 is heavily involved in the production these pathologies [109]. In fact, caspase-6 is known to facilitate a number of events in early stages of AD which precede Aβ and NFT formation. For example, caspase-6 functions synergistically with caspase-3 in tau Asp^{421} cleavage when stimulated by excessive reactive oxygen species, a sign of mitochondrial dysfunction [110,111]. Additionally, tau is further cleaved on the N-terminal Asp^{13} by caspase-6, resulting in a compromised cytoskeletal stability that facilitates the formation of tau filament which could be phosphorylated by GSK-3β to produce NFTs [105]. Caspase-6 also encourages amyloidosis through cleavage of APP in a pathway distinct from that described in the ACH. As APP contains multiple caspase cleavage sites which are targeted by apoptotic caspases-3,-6,-7 and -8 independent of secretases, caspase-6 is shown to cleave APP at the C terminus, generating a 6.5 kDa fragment which could undergo further processing to produce Aβ peptides [106,112,113].

Caspase-6 activity may directly compromise neurons independent of Aβ and NFTs, possibly through aberrant apoptosis and axonal degeneration. In a study by LeBlanc *et al*., transgenic mice free from neuritic plaques and NFTs, but expressing an artificial self-activating form of human caspase-6 in the pyramidal neuron-dense CA1 subfield of the hippocampus, were observed to develop neurodegeneration and memory impairments in an age-dependent manner [114]. As such, the removal of AD pathologies alone without eliminating caspase-6 may be insufficient to halt AD progression. This presents the NLRP1 inflammasome in neurons as an attractive therapeutic target as both caspase-1-mediated neuroinflammation and caspase-6 activity may be simultaneously disrupted by eliminating the major platform from which they function.

Conclusion and future perspectives

The NLRP3 inflammasome in microglia, the resident macrophage of the CNS, have been well-established as a potent driver neuroinflammation in AD. Here, we proposed that the NLRP1 inflammasome activity in neurons is equally paramount in the grand scheme of neuroinflammation in AD. Its capability to support

a myriad of inflammatory and neurodegenerative pathways within neurons themselves makes NLRP1 a potential therapeutic target for AD intervention. Recently, a study by Yin *et al*. has successfully demonstrated that JC-124, an NLRP3 inhibitor is able to decrease Aβ deposition and reduce microglia activation in an AD mouse model [115]. This is promising news as several studies have previously identified potential NLRP1 inhibitors that functions by preventing ATP-binding and inflammasome complex formation [116], although these are yet to be tested in AD conditions. These inhibitors may someday be developed into a new class of anti-neuroinflammatory drugs that can be used in combination with Aβ or NFT-targeting therapies to rescue neurons and hopefully modify disease progression in AD patients. However, there remain many challenges to be solved before such drugs may be developed as our current knowledge in inflammasome biology, particularly in the context of AD and other neurodegenerative diseases, are still in its adolescence. Given the complexity of the NLRP1 inflammasome and the extreme dissimilarity between its human and mice orthologues, we encourage future studies on neuronal NLRP1 to take advantage of recently-developed models derived from human induced pluripotent stem cells (iPSCs) which would undoubtedly represent the human conditions more accurately and at the same time facilitate drug discovery [117–119]. Neuroinflammation of the brain is increasingly recognized as a fundamental propagator of neurodegeneration in AD. By understanding precisely how inflammasomes work in the CNS under both physiological and pathological conditions, as well as figuring out how these inflammasomes can be targeted, we are one major step closer towards developing a proper cure for AD.

Acknowledgment We would like to acknowledge the Fundamental Research Grant Scheme (FRGS/1/2016/SKK08/IMU/03/1) by the Ministry of Higher Education of Malaysia for funding this work.

Conflicts of interest The authors declare that they have no conflicts of interest.

References

1. Association A. 2017 Alzheimer's disease facts and figures. Alzheimer's Dement [Internet]. 2017;13(4):325–73. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1552526017300511

2. Yiannopoulou KG, Papageorgiou SG. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2013;6(1):19–33.

3. Polanco JC, Li C, Bodea LG, Martinez-Marmol R, Meunier FA, Götz J. Amyloid-β and tau complexity

- Towards improved biomarkers and targeted therapies. Nat Rev Neurol [Internet]. 2018;14(1):22–40. Available from: http://dx.doi.org/10.1038/nrneurol.2017.162

4. Salomone S, Caraci F, Leggio GM, Fedotova J, Drago F. New pharmacological strategies for treatment of Alzheimer's disease: Focus on disease modifying drugs. Br J Clin Pharmacol. 2012;73(4):504–17.

5. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2018. Alzheimer's Dement Transl Res Clin Interv [Internet]. 2018;4:195–214. Available from: https://doi.org/10.1016/j.trci.2018.03.009

6. Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. Alzheimer's Dement [Internet]. 2016;12(6):719–32. Available from: http://dx.doi.org/10.1016/j.jalz.2016.02.010

7. Walters A, Phillips E, Zheng R, Biju M, Kuruvilla T. Evidence for neuroinflammation in Alzheimer's disease. Prog Neurol Psychiatry. 2016;20(5):25–31.

8. Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G. The inflammasome: A caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol. 2009;10(3):241–7.

9. Walsh JG, Muruve DA, Power C. Inflammasomes in the CNS. Nat Rev Neurosci [Internet]. 2014;15(2):84–97. Available from: http://www.nature.com/doifinder/10.1038/nrn3638

10. Saresella M, La Rosa F, Piancone F, Zoppis M, Marventano I, Calabrese E, et al. The NLRP3 and NLRP1 inflammasomes are activated in Alzheimer's disease. Mol Neurodegener [Internet]. 2016;11(1):23. Available from: http://www.molecularneurodegeneration.com/content/11/1/23

11. Kaushal V, Dye R, Pakavathkumar P, Foveau B, Flores J, Hyman B, et al. Neuronal NLRP1 inflammasome activation of Caspase-1 coordinately regulates inflammatory interleukin-1-beta production and axonal degeneration-associated Caspase-6 activation. Cell Death Differ [Internet]. 2015;22(10):1676–86. Available from: http://www.nature.com/doifinder/10.1038/cdd.2015.16

12. Clark R, Kupper T. Old Meets New: The Interaction Between Innate and Adaptive Immunity. J Invest Dermatol. 2005 Oct;125(4):629–37.

13. Newton K, Dixit VM. Signaling in Innate Immunity and Inflammation. Cold Spring Harb Perspect Biol. 2012 Mar;4(3):a006049–a006049.

14. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30(1):16–34.

15. Takeuchi O, Akira S. Pattern Recognition Receptors and Inflammation. Cell [Internet]. 2010;140(6):805–20. Available from: http://dx.doi.org/10.1016/j.cell.2010.01.022

16. Schattgen SA, Fitzgerald KA. The PYHIN protein family as mediators of host defenses. Immunol Rev. 2011;243(1):109–18.

17. Daneman R, Prat A. The blood-brain barrier. Cold Spring Harb Perspect Biol. 2015 Jan;7(1):a020412.

18. Hauwel M, Furon E, Canova C, Griffiths M, Neal J, Gasque P. Innate (inherent) control of brain infection, brain inflammation and brain repair: the role of microglia, astrocytes, "protective" glial stem cells and stromal ependymal cells. Brain Res Rev. 2005 Apr;48(2):220–33.

19. Griffiths MR, Gasque P, Neal JW. The regulation of the CNS innate immune response is vital for the restoration of tissue homeostasis (repair) after acute brain injury: a brief review. Int J Inflam. 2010 Aug;2010:151097.

20. Ransohoff RM, Brown M a. Review series Innate immunity in the central nervous system. J Clin Invest [Internet]. 2012;122(4):1164–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22466658

21. Lafon M, Megret F, Lafage M, Prehaud C. The Innate Immune Facet of Brain: Human Neurons Express TLR-3 and Sense Viral dsRNA. J Mol Neurosci. 2006;29(3):185–94.

22. Frank-Cannon TC, Alto LT, McAlpine FE, Tansey MG. Does neuroinflammation fan the flame in neurodegenerative diseases? Mol Neurodegener. 2009;4(1):1–13.

23. Morimoto K, Horio J, Satoh H, Sue L, Beach T, Arita S, et al. Expression profiles of cytokines in the brains of Alzheimer's disease (AD) patients compared to the brains of non-demented patients with and without increasing AD pathology. J Alzheimer's Dis. 2011;25(1):59–76.

24. Swardfager W, Lanctt K, Rothenburg L, Wong A, Cappell J, Herrmann N. A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiatry [Internet]. 2010;68(10):930–41. Available from: http://dx.doi.org/10.1016/j.biopsych.2010.06.012

25. Sweeney MD, Sagare AP, Zlokovic B V. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Publ Gr [Internet]. 2018;14(3):133–50. Available from:

http://dx.doi.org/10.1038/nrneurol.2017.188

26. Salminen A, Ojala J, Suuronen T, Kaarniranta K, Kauppinen A. Amyloid-β oligomers set fire to inflammasomes and induce Alzheimer's pathology: Alzheimer Review Series. J Cell Mol Med. 2008;12(6A):2255–62.

27. Gold M, El Khoury J. β-amyloid, microglia, and the inflammasome in Alzheimer's disease. Vol. 37, Seminars in Immunopathology. 2015. p. 607–11.

28. Dansokho C, Heneka MT. Neuroinflammatory responses in Alzheimer's disease. J Neural Transm [Internet]. 2018;125(5):771–9. Available from: https://doi.org/10.1007/s00702-017-1831-7

29. Tan M-S, Yu J-T, Jiang T, Zhu X-C, Tan L. The NLRP3 Inflammasome in Alzheimer's Disease. Mol Neurobiol [Internet]. 2013;48(3):875–82. Available from: http://link.springer.com/10.1007/s12035-013-8475-x

30. Davis BK, Wen H, Ting JP-Y. The Inflammasome NLRs in Immunity, Inflammation, and Associated Diseases. Annu Rev Immunol. 2011 Apr;29(1):707–35.

31. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature [Internet]. 2012;493(7434):674–8. Available from: http://www.nature.com/doifinder/10.1038/nature11729

32. Sarlus H, Heneka MT. Microglia in Alzheimer ' s disease. J Clin Invest. 2017;127(9):33–5.

33. Adamczak SE, De Rivero Vaccari JP, Dale G, Brand FJ, Nonner D, Bullock M, et al. Pyroptotic neuronal cell death mediated by the AIM2 inflammasome. J Cereb Blood Flow Metab. 2014;34(4):621–9.

34. Pontillo A, Catamo E, Arosio B, Mari D, Crovella S. NALP1/NLRP1 Genetic Variants are Associated With Alzheimer Disease. Alzheimer Dis Assoc Disord. 2012;26(3):277–81.

35. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing [Internet]. Vol. 51, Nature Genetics. 2019. p. 414–30. Available from: http://www.nature.com/articles/s41588-019-0358-2

36. Tan M-S, Tan L, Jiang T, Zhu X-C, Wang H-F, Jia C-D, et al. Amyloid-β induces NLRP1-dependent

neuronal pyroptosis in models of Alzheimer's disease. Cell Death Dis [Internet]. 2014;5(8):e1382. Available from: http://www.nature.com/doifinder/10.1038/cddis.2014.348

37. Martinon F, Burns K, Tschopp J. The Inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-β. Mol Cell. 2002;10(2):417–26.

38. Chavarría-Smith J, Vance RE. The NLRP1 inflammasomes. Immunol Rev. 2015;265(1):22–34.

39. Sastalla I, Crown D, Masters SL, McKenzie A, Leppla SH, Moayeri M. Transcriptional analysis of the three Nlrp1 paralogs in mice. BMC Genomics. 2013;14(1):1–10.

40. Yu CH, Moecking J, Geyer M, Masters SL. Mechanisms of NLRP1-Mediated Autoinflammatory Disease in Humans and Mice. J Mol Biol [Internet]. 2018;430(2):142–52. Available from: http://dx.doi.org/10.1016/j.jmb.2017.07.012

41. Inohara N, Chamaillard M, McDonald C, Nuñez G. NOD-LRR PROTEINS: Role in Host-Microbial Interactions and Inflammatory Disease. Annu Rev Biochem. 2005;

42. Kersse K, Bertrand MJM, Lamkanfi M, Vandenabeele P. NOD-like receptors and the innate immune system: Coping with danger, damage and death. Cytokine Growth Factor Rev [Internet]. 2011;22(5–6):257–76. Available from: http://dx.doi.org/10.1016/j.cytogfr.2011.09.003

43. D'Osualdo A, Weichenberger CX, Wagner RN, Godzik A, Wooley J, Reed JC. CARD8 and NLRP1 undergo autoproteolytic processing through a ZU5-like domain. PLoS One. 2011;6(11).

44. Finger JN, Lich JD, Dare LC, Cook MN, Brown KK, Duraiswamis C, et al. Autolytic proteolysis within the function to find domain (FIIND) is required for NLRP1 inflammasome activity. J Biol Chem. 2012;287(30):25030–7.

45. Frew BC, Joag VR, Mogridge J. Proteolytic processing of Nlrp1b is required for inflammasome activity. PLoS Pathog. 2012;8(4).

46. Maharana J. Elucidating the interfaces involved in CARD-CARD interactions mediated by NLRP1 and Caspase-1 using molecular dynamics simulation. J Mol Graph Model [Internet]. 2018;80:7–14. Available from: http://dx.doi.org/10.1016/j.jmgm.2017.12.016

47. Park HH, Lo Y-C, Lin S-C, Wang L, Yang JK, Wu H. The Death Domain Superfamily in Intracellular Signaling of Apoptosis and Inflammation. Annu Rev Immunol [Internet]. 2007;25(1):561–86. Available

from: http://www.annualreviews.org/doi/10.1146/annurev.immunol.25.022106.141656

48. Hiller S, Kohl A, Fiorito F, Herrmann T, Wider G, Tschopp J, et al. NMR structure of the apoptosis- and inflammation-related NALP1 pyrin domain. Structure. 2003;11(10):1199–205.

49. Chu LH, Gangopadhyay A, Dorfleutner A, Stehlik C. An updated view on the structure and function of PYRIN domains. Apoptosis. 2015;20(2):157–73.

50. Chavarría-Smith J, Vance RE. Direct Proteolytic Cleavage of NLRP1B Is Necessary and Sufficient for Inflammasome Activation by Anthrax Lethal Factor. PLoS Pathog. 2013;9(6):1–4.

51. Chavarría-Smith J, Mitchell PS, Ho AM, Daugherty MD, Vance RE. Functional and Evolutionary Analyses Identify Proteolysis as a General Mechanism for NLRP1 Inflammasome Activation. PLoS Pathog. 2016;12(12):1–22.

52. Levinsohn JL, Newman ZL, Hellmich KA, Fattah R, Getz MA, Liu S, et al. Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. PLoS Pathog. 2012;8(3).

53. Okondo MC, Rao SD, Taabazuing CY, Chui AJ, Poplawski SE, Johnson DC, et al. Inhibition of Dpp8/9 Activates the Nlrp1b Inflammasome. Cell Chem Biol [Internet]. 2018;25(3):262–267.e5. Available from: https://doi.org/10.1016/j.chembiol.2017.12.013

54. Sandstrom A, Mitchell PS, Goers L, Mu EW, Lesser CF, Vance RE. Functional degradation: A mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes. Science (80-) [Internet]. 2019;1330(March):eaau1330. Available from: http://www.sciencemag.org/lookup/doi/10.1126/science.aau1330

55. Chui AJ, Okondo MC, Rao SD, Gai K, Griswold AR, Johnson DC, et al. N-terminal degradation activates the NLRP1B inflammasome. Science (80-) [Internet]. 2019;1208(March):eaau1208. Available from: http://www.sciencemag.org/lookup/doi/10.1126/science.aau1208

56. Faustin B, Lartigue L, Bruey JM, Luciano F, Sergienko E, Bailly-Maitre B, et al. Reconstituted NALP1 Inflammasome Reveals Two-Step Mechanism of Caspase-1 Activation. Mol Cell. 2007;25(5):713–24.

57. Walsh MP, Duncan B, Larabee S, Krauss A, Davis JPE, Cui Y, et al. Val-BoroPro Accelerates T Cell Priming via Modulation of Dendritic Cell Trafficking Resulting in Complete Regression of Established Murine Tumors. PLoS One. 2013;8(3):1–13.

58. Okondo MC, Johnson DC, Sridharan R, Go E Bin, Chui AJ, Wang MS, et al. DPP8 and DPP9 inhibition induces pro-caspase-1-dependent monocyte and macrophage pyroptosis. Nat Chem Biol [Internet]. 2017;13(1):46–53. Available from: http://dx.doi.org/10.1038/nchembio.2229

59. Johnson DC, Taabazuing CY, Okondo MC, Chui AJ, Rao SD, Brown FC, et al. DPP8/DPP9 inhibitor-induced pyroptosis for treatment of acute myeloid leukemia. Nat Med [Internet]. 2018;24(8):1151–6. Available from: http://dx.doi.org/10.1038/s41591-018-0082-y

60. de Vasconcelos NM, Vliegen G, Gonçalves A, De Hert E, Martín-Pérez R, Van Opdenbosch N, et al. DPP8/DPP9 inhibition elicits canonical Nlrp1b inflammasome hallmarks in murine macrophages. Life Sci Alliance. 2019;2(1):e201900313.

61. Zhong FL, Tan K-Y, Reversade B, Robinson K, Sobota RM, Reed JC, et al. Human DPP9 represses NLRP1 inflammasome and protects against autoinflammatory diseases via both peptidase activity and FIIND domain binding. J Biol Chem. 2018;293(49):18864–78.

62. Lu A, Wu H. Structural mechanisms of inflammasome assembly. FEBS J. 2015;282(3):435–44.

63. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol [Internet]. 2016;16(7):407–20. Available from: http://www.nature.com/doifinder/10.1038/nri.2016.58

64. Van Opdenbosch N, Gurung P, Vande Walle L, Fossoul A, Kanneganti TD, Lamkanfi M. Activation of the NLRP1b inflammasome independently of ASC-mediated caspase-1 autoproteolysis and speck formation. Nat Commun [Internet]. 2014;5:1–14. Available from: http://dx.doi.org/10.1038/ncomms4209

65. Dick MS, Sborgi L, Rühl S, Hiller S, Broz P. ASC filament formation serves as a signal amplification mechanism for inflammasomes. Nat Commun. 2016;7(May).

66. A. Armstrong R. A critical analysis of the 'amyloid cascade hypothesis.' Folia Neuropathol [Internet]. 2014;3(3):211–25. Available from: http://www.termedia.pl/doi/10.5114/fn.2014.45562

67. Dahlgren KN, Manelli AM, Blaine Stine W, Baker LK, Krafft GA, Ladu MJ. Oligomeric and fibrillar species of amyloid-β peptides differentially affect neuronal viability. J Biol Chem. 2002;277(35):32046–53.

68. Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, et al. Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. J Histochem Cytochem. 2007;55(5):443–52.

69. Sáez-Orellana F, Godoy PA, Bastidas CY, Silva-Grecchi T, Guzmán L, Aguayo LG, et al. ATP leakage induces P2XR activation and contributes to acute synaptic excitotoxicity induced by soluble oligomers of β-amyloid peptide in hippocampal neurons. Neuropharmacology. 2016;100:116–23.

70. Sáez-Orellana F, Fuentes-Fuentes MC, Godoy PA, Silva-Grecchi T, Panes JD, Guzmán L, et al. P2X receptor overexpression induced by soluble oligomers of amyloid beta peptide potentiates synaptic failure and neuronal dyshomeostasis in cellular models of Alzheimer's disease. Neuropharmacology. 2018;128:366–78.

71. McLarnon JG, Ryu JK, Walker DG, Choi HB. Upregulated expression of purinergic P2X7 receptor in Alzheimer disease and amyloid-β peptide-treated microglia and in peptide-injected rat hippocampus. J Neuropathol Exp Neurol. 2006;

72. Pelegrin P, Surprenant A. Pannexin-1 mediates large pore formation and interleukin-1?? release by the ATP-gated P2X7 receptor. EMBO J. 2006;25(21):5071–82.

73. Silverman WR, de Rivero Vaccari JP, Locovei S, Qiu F, Carlsson SK, Scemes E, et al. The pannexin 1 channel activates the inflammasome in neurons and astrocytes. J Biol Chem. 2009;284(27):18143–51.

74. Yaron JR, Gangaraju S, Rao MY, Kong X, Zhang L, Su F, et al. K+ regulates Ca2+ to drive inflammasome signaling: dynamic visualization of ion flux in live cells. Cell Death Dis. 2015 Oct;6(10):e1954–e1954.

75. Zha QB, Wei HX, Li CG, Liang YD, Xu LH, Bai WJ, et al. ATP-induced inflammasome activation and pyroptosis is regulated by AMP-activated protein kinase in macrophages. Front Immunol. 2016;7(DEC):1–16.

76. Liao K-C, Mogridge J. Activation of the Nlrp1b inflammasome by reduction of cytosolic ATP. Infect Immun. 2013 Feb;81(2):570–9.

77. Elliott JM, Rouge L, Wiesmann C, Scheer JM. Crystal structure of procaspase-1 zymogen domain reveals insight into inflammatory caspase autoactivation. J Biol Chem. 2009;284(10):6546–53.

78. Galluzzi L, López-Soto A, Kumar S, Kroemer G. Caspases Connect Cell-Death Signaling to Organismal Homeostasis. Immunity. 2016;44(2):221–31.

79. Vitkovic L, Bockaert J, Jacque C. "Inflammatory" cytokines' neuromodulators in normal brain? Journal of Neurochemistry. 2000.

80. Srinivasan D. Cell Type-Specific Interleukin-1 Signaling in the CNS. J Neurosci [Internet]. 2004;24(29):6482–8. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.5712-03.2004

81. Soiampornkul R, Tong L, Thangnipon W, Balazs R, Cotman CW. Interleukin-1β interferes with signal transduction induced by neurotrophin-3 in cortical neurons. Brain Res. 2008;1188(1):189–97.

82. Kandel ER. The molecular biology of memory: CAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Mol Brain [Internet]. 2012;5(1):1. Available from: Molecular Brain

83. Matousek SB, Ghosh S, Shaftel SS, Kyrkanides S, Olschowka JA, O'Banion MK. Chronic IL-1β-mediated neuroinflammation mitigates amyloid pathology in a mouse model of alzheimer's disease without inducing overt neurodegeneration. J Neuroimmune Pharmacol. 2012;7(1):156–64.

84. Tachida Y, Nakagawa K, Saito T, Saido TC, Honda T, Saito Y, et al. Interleukin-1β up-regulates TACE to enhance α-cleavage of APP in neurons: Resulting decrease in Aβ production. J Neurochem. 2008;104(5):1387–93.

85. Ghosh S, Wu MD, Shaftel SS, Kyrkanides S, LaFerla FM, Olschowka JA, et al. Sustained Interleukin-1 Overexpression Exacerbates Tau Pathology Despite Reduced Amyloid Burden in an Alzheimer's Mouse Model. J Neurosci [Internet]. 2013;33(11):5053–64. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4361-12.2013

86. Li Y, Liu L, Kang J, Sheng JG, Barger SW, Mrak RE, et al. Neuronal-glial interactions mediated by interleukin-1 enhance neuronal acetylcholinesterase activity and mRNA expression. J Neurosci [Internet]. 2000;20(1):149–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10627591

87. Dong H. Excessive Expression of Acetylcholinesterase Impairs Glutamatergic Synaptogenesis in Hippocampal Neurons. J Neurosci [Internet]. 2004;24(41):8950–60. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.2106-04.2004

88. Zhou J, Ping F feng, Lv W ting, Feng J yi, Shang J. Interleukin-18 directly protects cortical neurons by activating PI3K/AKT/NF-κB/CREB pathways. Cytokine [Internet]. 2014;69(1):29–38. Available from: http://dx.doi.org/10.1016/j.cyto.2014.05.003

89. Ojala J, Alafuzoff I, Herukka SK, van Groen T, Tanila H, Pirttilä T. Expression of interleukin-18 is increased in the brains of Alzheimer's disease patients. Neurobiol Aging. 2009;30(2):198–209.

90. Sutinen EM, Pirttil?? T, Anderson G, Salminen A, Ojala JO. Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-β production in human neuron-like cells. J Neuroinflammation. 2012;9:1–14.

91. Ojala JO, Sutinen EM, Salminen A, Pirttilä T. Interleukin-18 increases expression of kinases involved in tau phosphorylation in SH-SY5Y neuroblastoma cells. J Neuroimmunol [Internet]. 2008;205(1–2):86–93. Available from: http://dx.doi.org/10.1016/j.jneuroim.2008.09.012

92. Yu JT, Tan L, Song JH, Sun YP, Chen W, Miao D, et al. Interleukin-18 promoter polymorphisms and risk of late onset Alzheimer's disease. Brain Res [Internet]. 2009;1253(5):169–75. Available from: http://dx.doi.org/10.1016/j.brainres.2008.11.083

93. Segat L, Milanese M, Arosio B, Vergani C, Crovella S. Lack of association between Interleukin-18 gene promoter polymorphisms and onset of Alzheimer's disease. Neurobiol Aging. 2010;31(1):162–4.

94. Luo L, Li K, Wang X. Relationship between the IL-18 gene polymorphisms and Alzheimer ' s disease : a meta-analysis. 2016;9(11):22720–8.

95. Fink SL, Cookson BT. Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. Cell Microbiol. 2006;8(11):1812–25.

96. He WT, Wan H, Hu L, Chen P, Wang X, Huang Z, et al. Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. Cell Res [Internet]. 2015;25(12):1285–98. Available from: http://dx.doi.org/10.1038/cr.2015.139

97. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature. 2015;526(7575):660–5.

98. Miao EA, Leaf IA, Treuting PM, Mao DP, Dors M, Sarkar A, et al. Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. Nat Immunol [Internet].

2010;11(12):1136–42. Available from: http://dx.doi.org/10.1038/ni.1960

99. Taabazuing CY, Okondo MC, Bachovchin DA. Pyroptosis and Apoptosis Pathways Engage in Bidirectional Crosstalk in Monocytes and Macrophages. Cell Chem Biol [Internet]. 2017;24(4):507–514.e4. Available from: http://dx.doi.org/10.1016/j.chembiol.2017.03.009

100. Noël A, Zhou L, Foveau B, Sjöström PJ, LeBlanc AC. Differential susceptibility of striatal, hippocampal and cortical neurons to Caspase-6. Cell Death Differ [Internet]. 2018;1–17. Available from: http://dx.doi.org/10.1038/s41418-017-0043-x

101. Hogg MC, Mitchem MR, König H, Prehn JHM. Caspase 6 has a protective role in SOD1 G93A transgenic mice. BBA - Mol Basis Dis [Internet]. 2016;1862(6):1063–73. Available from: http://dx.doi.org/10.1016/j.bbadis.2016.03.006

102. Wang X-J, Cao Q, Zhang Y, Su X-D. Activation and Regulation of Caspase-6 and Its Role in Neurodegenerative Diseases. Annu Rev Pharmacol Toxicol [Internet]. 2015;55(1):553–72. Available from: http://www.annualreviews.org/doi/10.1146/annurev-pharmtox-010814-124414

103. Albrecht S, Bourdeau M, Bennett D, Mufson EJ, Bhattacharjee M, LeBlanc AC. Activation of caspase-6 in aging and mild cognitive impairment. Am J Pathol. 2007;170(4):1200–9.

104. Ramcharitar J, M. Afonso V, Albrecht S, A. Bennett D, LeBlanc AC. Caspase-6 activity predicts lower episodic memory ability in aged individuals. Neurobiol Aging [Internet]. 2013;34(7):1815–24. Available from: http://dx.doi.org/10.1016/j.neurobiolaging.2013.01.007

105. Horowitz PM. Early N-Terminal Changes and Caspase-6 Cleavage of Tau in Alzheimer's Disease. J Neurosci [Internet]. 2004;24(36):7895–902. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1988-04.2004

106. Pellegrini L, Passer BJ, Tabaton M, Ganjei JK, D'Adamio L. Alternative, non-secretase processing of Alzheimer's β-amyloid precursor protein during apoptosis by caspase-6 and -8. J Biol Chem. 1999;274(30):21011–6.

107. Graham RK, Deng Y, Carroll J, Vaid K, Cowan C, Pouladi MA, et al. Cleavage at the 586 Amino Acid Caspase-6 Site in Mutant huntingtin Influences Caspase-6 Activation In Vivo. J Neurosci [Internet]. 2010;30(45):15019–29. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.2071-

10.2010

108. Van De Craen M, De Jonghe C, Van Den Brande I, Declercq W, Van Gassen G, Van Criekinge W, et al. Identification of caspases that cleave presenilin-1 and presenilin-2: Five presenilin-1 (PS1) mutations do not alter the sensitivity of PS1 to caspases. FEBS Lett. 1999;445(1):149–54.

109. Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. Am J Pathol [Internet]. 2004;165(2):523–31. Available from: http://dx.doi.org/10.1016/S0002-9440(10)63317-2

110. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. J Clin Invest. 2004;114(1):121–30.

111. Zhao H, Zhao W, Lok K, Wang Z, Yin M. A synergic role of caspase-6 and caspase-3 in tau truncation at D421 induced by H2O2. Cell Mol Neurobiol. 2014;34(3):369–78.

112. LeBlanc A, Liu H, Goodyer C, Bergeron C, Hammond J. Caspase-6 role in apoptosis of human neurons, amyloidogenesis, and Alzheimer's disease. J Biol Chem. 1999;274(33):23426–36.

113. Tesco G, Koh YH, Tanzi RE. Caspase Activation Increases β-Amyloid Generation Independently of Caspase Cleavage of the β-Amyloid Precursor Protein (APP). J Biol Chem. 2003;278(46):46074–80.

114. Leblanc AC, Ramcharitar J, Afonso V, Hamel E, Bennett DA, Pakavathkumar P, et al. Caspase-6 activity in the CA1 region of the hippocampus induces age-dependent memory impairment. Cell Death Differ [Internet]. 2014;21(5):696–706. Available from: http://dx.doi.org/10.1038/cdd.2013.194

115. Yin J, Zhao F, Chojnacki JE, Fulp J, Klein WL, Zhang S, et al. NLRP3 Inflammasome Inhibitor Ameliorates Amyloid Pathology in a Mouse Model of Alzheimer's Disease. Mol Neurobiol. 2018;55(3):1977–87.

116. Harris PA, Duraiswami C, Fisher DT, Fornwald J, Hoffman SJ, Hofmann G, et al. High throughput screening identifies ATP-competitive inhibitors of the NLRP1 inflammasome. Bioorganic Med Chem Lett [Internet]. 2015;25(14):2739–43. Available from: http://dx.doi.org/10.1016/j.bmcl.2015.05.032

117. Jorfi M, D'Avanzo C, Tanzi RE, Kim DY, Irimia D. Human Neurospheroid Arrays for In Vitro Studies of Alzheimer's Disease. Sci Rep [Internet]. 2018;8(1):2450. Available from:

http://www.nature.com/articles/s41598-018-20436-8

118. Gonzalez C, Armijo E, Bravo-alegria J, Mays ABCE, Soto C. Modeling amyloid beta and tau pathology in human cerebral organoids. Mol Psychiatry [Internet]. 2018; Available from: https://www.nature.com/articles/s41380-018-0229-8

119. Park J, Wetzel I, Marriott I, Dréau D, D'Avanzo C, Kim DY, et al. A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nat Neurosci. 2018;21(7):941–51.

Figure captions

Fig. 1 The activation of NLRP1 inflammasome by AD pathology, most notably Aβ, which ignites a cascade of reactions leading to loss of synapse and functional neurons. As NLRP1 is highly expressed in pyramidal neurons, the cerebral cortex, hippocampus and amygdala are most severely affected, thus leading to manifestation of the clinical symptoms of AD

Fig. 2 Structures of NLRs that form inflammasomes. Human NLRP1 is structurally distinct from mice Nlrp1 which contains 3 paralogues and lacks PYD. NLRP1 (with the exception of mouse Nlrp1C) contains a unique function-to-find domain (FIIND) and a functional CARD on its C-terminus

Fig. 3 The activation model of mouse Nlrp1b which is characterised by ubiquitin-proteasome degradation post-N-terminal cleavage, liberating an immunoactive C-terminal UPA-CARD fragment. As a consequent of this degradation, mouse Nlrp1b is not capable of forming a complete inflammasome complex. Instead, Nlrp1b relies on CARD-CARD interaction for inflammasome-like signalling with the support of ASC

Fig. 4 NLRP1 can be activated by P2X7/pannexin 1 signalling under the influence of Aβ, prompting assembly of inflammasome. The expressions of NLRP1, pro-IL-1β and pro-IL-18 are driven by NF-κB signalling pathways. A region of the FIIND is post-translationally auto-cleaved as a requirement for its subsequent activation. NLRP1 PYD might play an auto-inhibitory role which, when cleaved, activates the receptor to allow inflammasome complex formation. ASC are required to amplify caspase-1 recruitment for optimum downstream inflammasome activities including IL-1β and IL-18 maturation

(**Fig. 1** is generated using Microsoft Word. **Fig. 2**, **Fig. 3** and **Fig. 4** are generated using ChemDraw Professional 16.0 software.)

Fig 1.

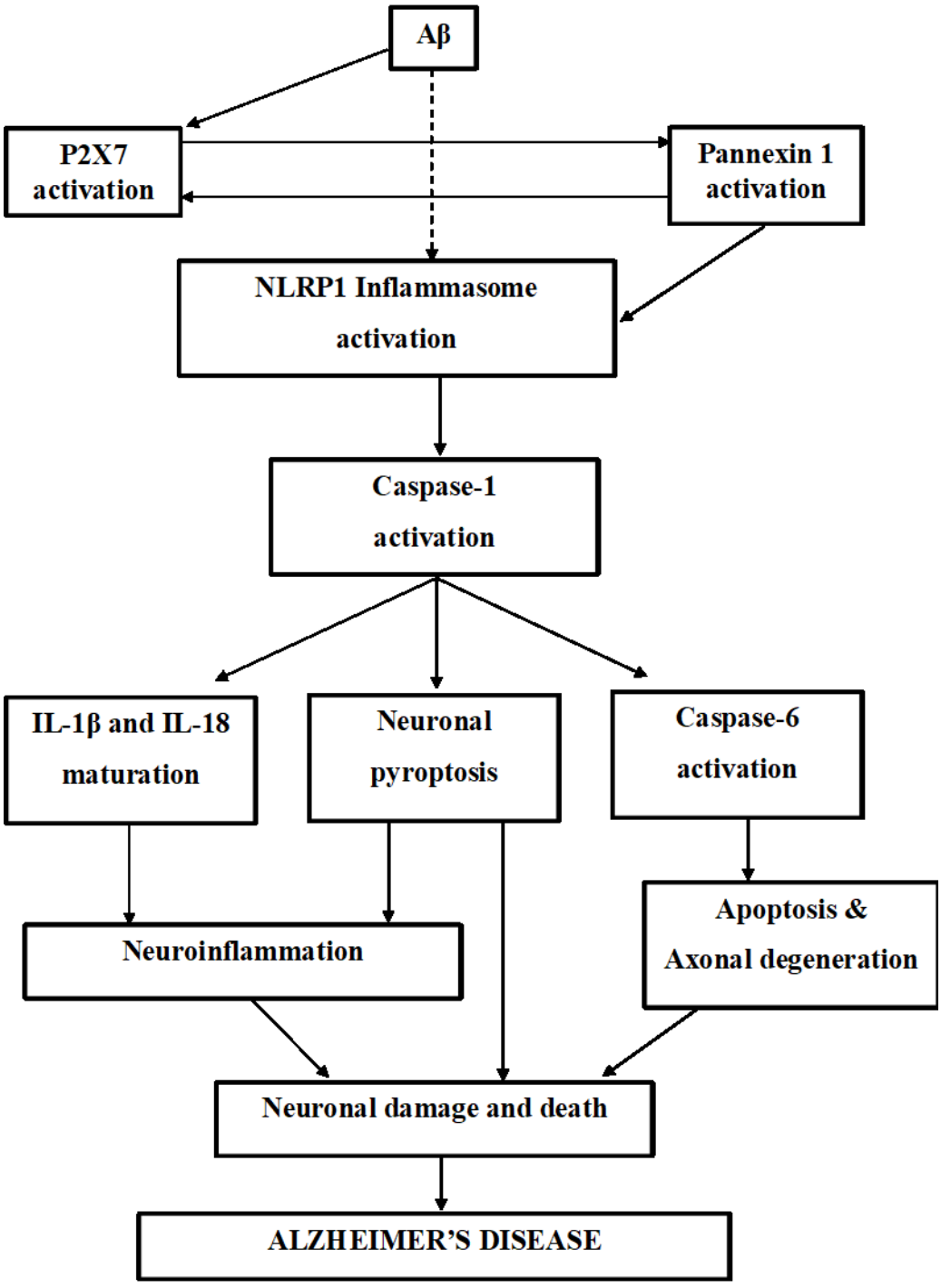

Aβ
P2X7 activation
Pannexin 1 activation
NLRP1 Inflammasome activation
Caspase-1 activation
IL-1β and IL-18 maturation
Neuronal pyroptosis
Caspase-6 activation
Neuroinflammation
Apoptosis & Axonal degeneration
Neuronal damage and death
ALZHEIMER'S DISEASE

Fig. 2.

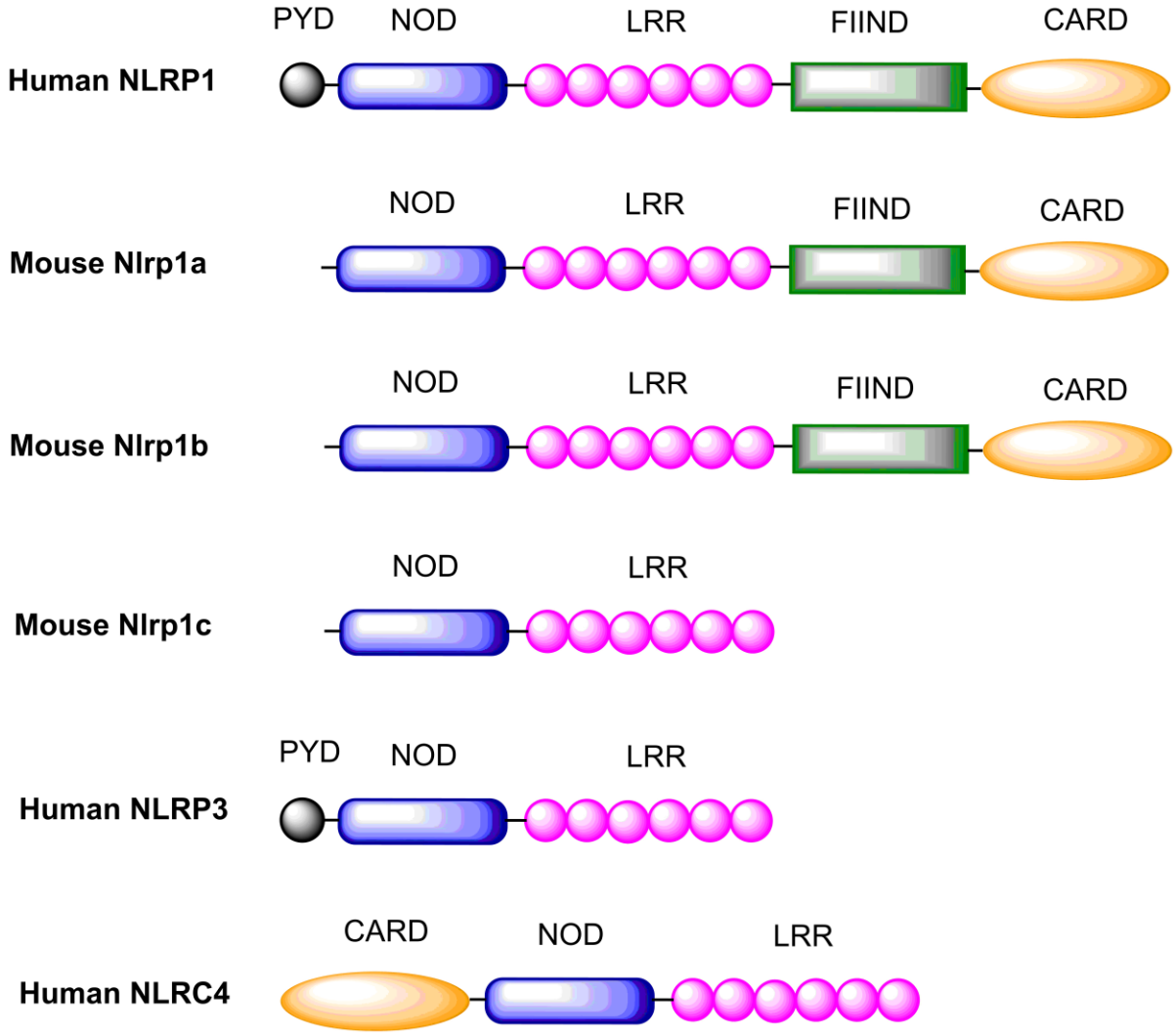

PYD
NOD
LRR
FIIND
CARD
Human NLRP1
NOD
LRR
FIIND
CARD
Mouse Nlrp1a
NOD
LRR
FIIND
CARD
Mouse Nlrp1b
NOD
LRR
Mouse Nlrp1c
PYD
NOD
LRR
Human NLRP3
CARD
NOD
LRR
Human NLRC4

Fig 3.

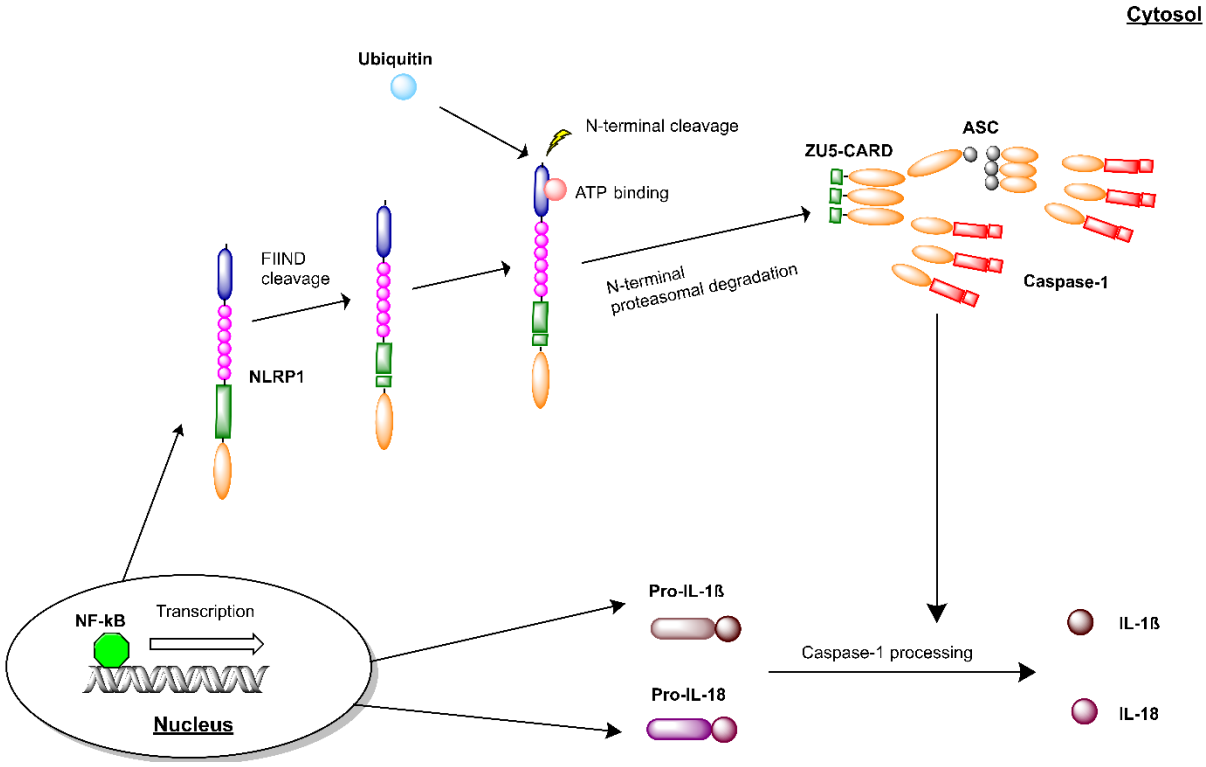
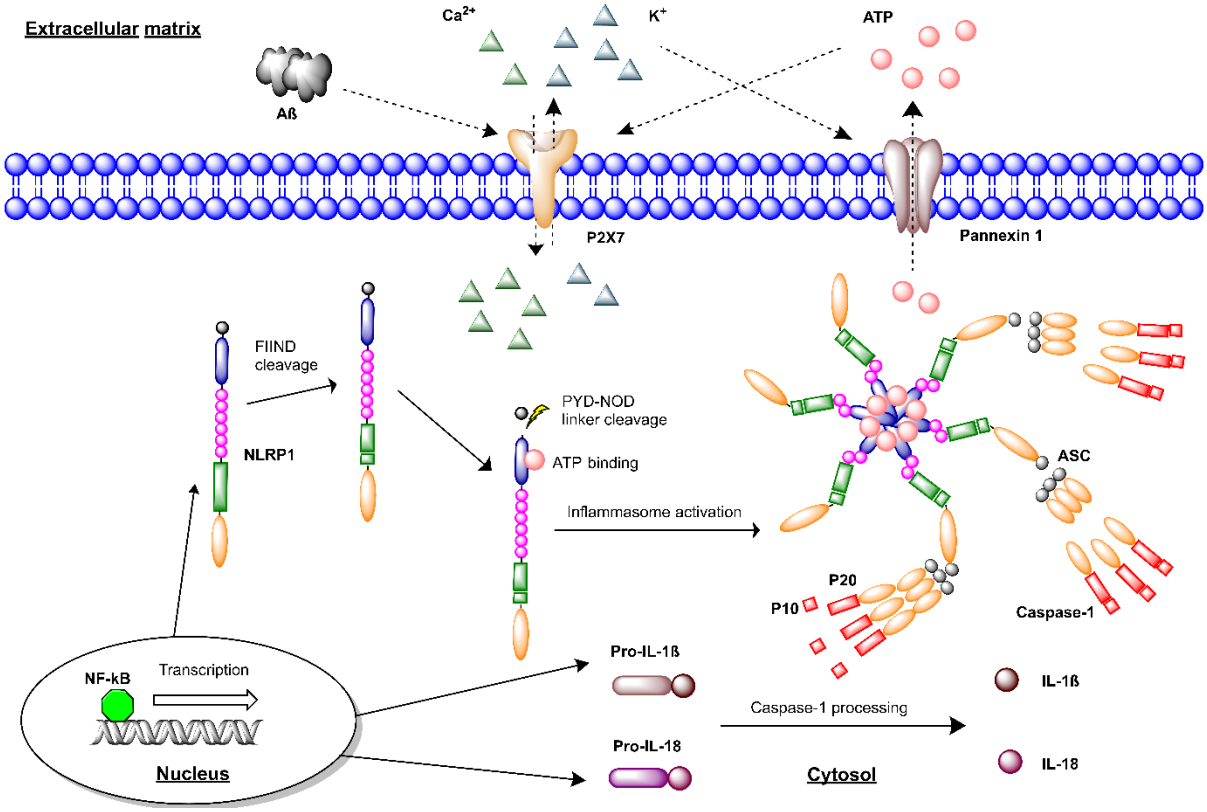

Cytosol
Ubiquitin
N-terminal cleavage
ATP binding
ZU5-CARD
ASC
FIIND
cleavage
NLRP1
N-terminal
proteasomal degradation
Caspase-1
NF-kB
Transcription
Nucleus
Pro-IL-1ß
Pro-IL-18
Caspase-1 processing
IL-1ß
IL-18

Fig 4.


Extracellular matrix
Ca2+
K+
ATP
Aß
P2X7
Pannexin 1
FIIND cleavage
NLRP1
PYD-NOD linker cleavage
ATP binding
Inflammasome activation
ASC
P20
P10
Caspase-1
NF-kB
Transcription
Nucleus
Pro-IL-1ß
Pro-IL-18
Caspase-1 processing
Cytosol
IL-1ß
IL-18