

A Calcium Channel $\alpha 2\delta$ Subunit Theory of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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Abstract. Amyotrophic lateral sclerosis (ALS) is a devastating disease whose etiology is currently unknown. This paper presents a novel theory in which ALS is caused by an impairment of the $\alpha 2\delta$ subunit of voltage-gated calcium channels (VGCCs), most likely due to autoimmune targeting. I show how the impairment mechanistically induces chronic calcium, which in turn explains the major clinical, epidemiological, and pathological evidence known about the disease. The same theory explains the major variant of frontotemporal dementia (FTD) that greatly overlaps with ALS.

Keywords. ALS; FTD; high threshold voltage-gated calcium channel; $\alpha 2\delta$ subunit;

1 Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal disease involving the degeneration of motor neurons (MTNs) [1, 2]. Most patients exhibit sporadic ALS (sALS), whose etiology is not known, while 10% of the patients show a familial form (fALS) that involves identified genetic mutations. In both variants, approved treatments only have a marginal benefit, and death occurs a few years after diagnosis. There is substantial overlap between ALS and frontotemporal dementia (FTD), especially its behavioral variant (FTDbv), which is associated with TDP43 [3].

Beyond a few initial hypotheses [4], there is currently no theory that addresses the major facts of sALS, fALS or FTD. Here I present a new theory of ALS and FTDbv that explains their etiology, pathophysiology, and clinical symptoms. The main thesis is that sALS is caused by an impairment of the $\alpha 2\delta$ ($\alpha 2\delta$) subunit of high voltage-activated voltage-gated calcium channels (H-VGCCs), most likely due to autoimmune targeting. This results in chronically higher calcium entry throughout life, which eventually yields toxicity that causes the disease

symptoms, cellular stress and degeneration. The mutations associated with fALS also induce chronic calcium, with similar downstream consequences.

Section 2 provides general background on VGCCs and the $\alpha_2\delta$ subunit, including its role in synapse development, signaling, and maintenance. Section 3 explains the reasons for suspecting $\alpha_2\delta$, and presents an account of the specific calcium and $\alpha_2\delta$ impairment in ALS. Section 4 details evidence supporting the theory, including calcium dysregulation in ALS, the explanatory power of chronic calcium for the facts known about ALS, $\alpha_2\delta$ evidence, and insulin-related evidence. Section 5 discusses autoimmunity in ALS, and Section 6 discusses FTD.

2 Voltage-Gated Calcium Channels (VGCCs)

General background. The family of VGCCs includes high voltage-activated VGCCs (H-VGCC) and the low voltage-activated T-type VGCC, which is not involved in ALS. H-VGCCs are comprised of a pore α_1 (α_1) subunit, an auxiliary beta subunit, and an auxiliary $\alpha_2\delta$ subunit [5, 6, 7, 8]. Each of these has several gene variants and some have alternative splicing variants. H-VGCCs include L-VGCC (L-type, Cav1), P/Q-VGCC (Cav2.1), N-VGCC (Cav2.2), and R-VGCC (Cav2.3). H-VGCCs are activated in relatively depolarized membrane potentials and support transmitter release and sustained execution.

Cav1.1 is expressed in skeletal muscle, where it promotes voltage sensing, calcium outflux from the sarcoplasmic reticulum, and excitation-contraction coupling. Cav1.2 is expressed in neurons, the cardiovascular system, pancreas beta cells, adrenal chromaffin cells [9], and skin [10]. Cav1.3 is expressed in metabolism and valence areas, including the extended amygdala, hypothalamus nuclei, and brainstem [11]. Cav2.1 is expressed in neurons, including MTN axons (presynaptically) and cerebellar Purkinje cells. Cav2.2 is highly expressed throughout the brain during development, with high cortex and hippocampus expression remaining in adulthood [12]. Cav2.3 is expressed in neurons, cardiac and smooth muscle, and beta cells [7]. L-VGCC-like channels are expressed in immune cells, including B cells and T cells [13, 14, 15, 16, 17].

The $\alpha_2\delta$ subunits. The $\alpha_2\delta$ subunit is present in L-VGCC, P/Q-VGCC, and N-VGCC [6]. There are four $\alpha_2\delta$ genes, $\alpha_2\delta_1$ -4. The subunit is generated by a single gene whose product is cleaved into two disulfide-bonded polypeptides. This cleavage is essential for its function [18]. The subunit is extracellular, glycosylated, and GPI-anchored at the membrane [19].

With respect to distribution, $\alpha_2\delta_1$ is widely expressed in the brain, mainly in excitatory neurons, including in the hippocampus, hypothalamus, the insula, olfactory areas, and dorsal root ganglia [20, 21]. It is less strongly expressed in frontal areas. It is expressed in skeletal muscle [22], the heart, smooth muscle [6] and pancreatic beta cells, where it is the main $\alpha_2\delta$ subunit [23]. $\alpha_2\delta_2$ is mainly expressed in inhibitory neurons and in the cerebellum [20]. It is also expressed in immune T cells [17]. $\alpha_2\delta_3$ is expressed in MTNs and caudate-putamen (i.e., the motor system) [20, 21], and also in the retina [24]. $\alpha_2\delta_4$ is limited to the retina [25].

Physiological roles of a2d. Both of the auxiliary H-VGCC subunits, beta and a2d, promote VGCC trafficking to the plasma membrane [26]. A specific role of the a2d subunit is to promote channel anchoring in stable membrane sites, including neural synapses [27]. There is unequivocal evidence that a2d promotes synapse formation, maintenance and neurotransmission [28, 29, 30, 31, 32, 33, 34, 35]. a2d supports high amplitude synaptic calcium entry, both via VGCC activation and via association with NMDA receptors and promotion of their surface trafficking [36, 37, 38]. The subunit supports anchoring of VGCC in lipid rafts [39, 40, 19], and synaptic adhesion [41, 42, 43, 44]. These roles are highly preserved: invertebrate a2d homologs are expressed at the neuromuscular junction (NMJ), where they are involved in synaptogenesis and presynaptic morphology [45, 46].

We particularly emphasize the role of a2d within the larger plasticity picture of adhesion molecules such as neuroligin/neurexin and LRRTM. Such proteins connect pre- and postsynaptic sites to improve synapse stability and signaling [47]. The a2d subunit participates with these proteins in trans-synaptic nanocolumns that are connected to the cytoskeleton and restrict the spatial propagation of neurotransmitters, allowing strong and rapid downstream signaling [47].

3 Proposed Calcium & a2d Impairment in ALS

3.1 Motivation

Calcium evidence. The involvement of high calcium and calcium channels in ALS pathology is well-known and supported by overwhelming evidence given in Section 4. The present paper is the first to propose a causal role for a2d impairment. There are several reasons to focus on a2d as the main culprit in ALS, summarized here.

Suspect by subcellular location. The role of a2d is to support synapses, and synaptic impairment at the NMJ is the major pathological feature in ALS [48, 49, 50, 51, 52], with axon degeneration preceding MTN death.

Suspect by membrane location. a2d is located extracellularly, making it accessible to antibodies, immune cells, and other circulating factors. The beta subunits are intracellular, and the a1 subunits are transmembrane proteins.

Suspect by elimination. The antibody evidence listed in Section 4 clearly demonstrates autoimmune targeting of H-VGCCs in ALS. However, it does not point to any specific channel or protein, with positive results regarding the involvement of L-, P/Q-, and N-VGCCs, which have different a1 subunits. This lack of specificity with respect to a1 seems to point to the target being the a2d and/or the beta subunits (although Section 5 explains why this argument actually does not rule out the L-VGCC a1 subunit). However, the target could also be a region of genetic overlap between several a1 subunits. Naturally, there may also be several disease variants, each targeting a different subunit.

3.2 Calcium part of the theory

My account of calcium in ALS is as follows. Usually starting in early life, most likely as a result of a channelopathy or autoimmune targeting, H-VGCCs calcium metabolism is excessively active in ALS. This high basal activity provides ALS patients with physical and mental strength that are commonly higher than in other people (e.g., ALS is known to be associated with sports).

Calcium can induce toxicity, and this is eventually what it does in ALS. At some point in life, some of the tissues expressing H-VGCC start showing decreased function. There are three major such tissues, MTNs, skeletal muscle (SKM), and insulin-releasing pancreas beta cells (in FTD, it is specific areas of the brain). Cardiorespiration is also damaged, as seen by the most common death cause.

As is the case for many biological agents, healthy calcium signaling involves strong (high amplitude) influx that is rapidly terminated and cleared via negative feedback (negFB) mechanisms [53, 54, 55, 56]. In addition, healthy signaling occurs in restricted nanodomains where the Ca^{2+} signal is boosted [57]. In ALS, due to calcium channel subunit impairment, at disease onset we have the opposite situation, prolonged and weaker signaling. This is what the term **chronic** commonly refers to. Thus, our premise is that ALS involves strong cellular calcium that at some point turns to be chronic and toxic.

3.3 a2d part of the theory

How does a2d impairment cause excessive calcium throughout life, which turns into chronic calcium at disease onset? The main role of a2d is to anchor the channel at stable membrane sites where it supports signaling nanodomains. Without a functioning a2d, the beta subunit still supports channel trafficking, but it is not anchored properly at the membrane. Without nanodomains, more calcium enters the cell.

Moreover, it is reasonable to assume that part of the action of a2d is to exert negative feedback on channel transcription and trafficking, because stable anchoring at the membrane is the last step needed for channel formation. Without a functioning a2d, the number of a1 subunits would be larger, which induces larger calcium entry.

A simple complementary mechanism whereby reduced a2d function can yield chronicity is by increasing the channel's lateral mobility [58], which can decouple it from the synapse and yield similar consequences as reduced negative feedback.

Which a2d? As there are four a2d genes, a theory that points to a2d needs to address their respective roles in the disease. There are at least three possible hypotheses. First, each a2d could give rise to a different disease. a2d3, which is expressed in skeletal muscle and MTNs, would be responsible for ALS; a2d1, with its cortical expression, would be the target in FTD; a2d2, with its cerebellum expression, would be involved in some variants of autoimmune cerebellum ataxia (some of which are known to attack VGCCs) [59]; and a2d4 might be involved in some autoimmune retinal diseases (see below). A problem with this (too) elegant proposal is that beta cell impairment is fundamental to ALS (see Section 4), and beta cells mainly express a2d1.

Another hypothesis is that the main culprit in ALS is a2d1, since it is strongly expressed in the tissues most affected in ALS (the motor system, brain, and beta cells). Yet another possibility is that all a2d subunits can be affected, since they possess considerable genetic overlap.

At present, all of these hypotheses are possible, since there is insufficient evidence to arbitrate between them. The last two seem more likely than the first for ALS, but the role of a2d2 and a2d4 in the first hypothesis may be valid.

In summary, impaired a2d function can induce chronically higher calcium entry into cells throughout life. At some point, this results in calcium toxicity that yields degeneration and death.

4 ALS Evidence

4.1 Calcium

Serum, Ig, CSF evidence. There is overwhelming direct and indirect evidence that patient antibodies, serum, and CSF interact with calcium channels in ALS. A thorough search found 36 papers [60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95].

In 18 of these papers, the interaction directly involved increased calcium. Patient antibodies and sera induce the following effects: (most of these results are MTNs) increased P-VGCC currents (and open time) in cerebellum Purkinje cells [67], extracellular calcium-dependent cell death in MTN-neuroblastoma hybrid cell line (VCS4.1), prevented by N- or P-VGCC (but not L-VGCC) antagonists [68], increased calcium and vesicle number in spinal MTNs [72], increased Ca^{2+} currents in a hybrid MTN VCS4.1 cells [71], increased extracellular calcium entry via VGCCs leading to higher intracellular calcium in hybrid MTN VCS4.1 cells [77], increased Ca^{2+} influx via P/Q-VGCC, N-VGCC (only weakly via L-VGCC) in cortical synaptosomes [81], development of L-VGCC (but not P/Q-VGCC)-induced transmitter release in the neuromuscular junction (NMJ) [84], increased presynaptic Ca^{2+} in MTNs, along with golgi pathology and increased golgi Ca^{2+} [82], faster H-VGCC current activation in MTNs [85], MTN degeneration and significantly increased number of Ca^{2+} -containing golgi, mitochondria, and rough endoplasmic reticulum (ER) [86], potentiation of transmitter release from MTN via influx through N-VGCC [88], necrosis, MTN degeneration, swelling and Ca^{2+} of golgi endoplasmic reticulum, mitochondria [87], increased muscle miniature end-plate potential frequency, NMJ ACh release, which are significantly decreased in P/Q-VGCC (but not N-VGCC) KO [89], intracellular Ca^{2+} release in astrocytes via IP3 (intracellular stores) [91], high rather than oscillating L-VGCC-mediated intracellular Ca^{2+} in mouse islets (in ALS patients with type 2 diabetes) [92], MTN degeneration, inclusion-like IgG accumulation, increased intracellular Ca^{2+} [93], increased number of synaptic and Ca^{2+} elevation in MTN terminals [94], and increased cytoplasmic calcium, cell death in mice MTNs [95] (the last two results are with serum of patients with identified mutations).

In 8 of the 36 papers, the interaction resulted in directly decreased Ca^{2+} . This includes decreased charge movement, peak calcium current, and slower channel inactivation in skeletal muscle L-VGCCs [63], decreased mean skeletal muscle L-VGCC open time with stabilization to a smaller amplitude [65], decreased peak L-VGCC current with increased tail current deactivation rate in mammalian skeletal muscle fibers [66], decreased charge movement and peak L-VGCC currents in isolated muscle fibers [70], depressed inward Ba^{2+} -mediated (high threshold) VGCC currents in rat cerebellum granule culture [69], suppressed intracellular Ca^{2+} increase via P/Q-VGCCs (but not N-, L-VGCC) influx in cultured neurons [74], decreased H-VGCC peak and faster and more hyperpolarized inactivation in mice DRG neurons [76], and inhibition of L-VGCC-mediated dopamine release [79].

In 8 of the 36 papers, the reported results indirectly support increased calcium, including increased NMJ presynaptic ACh release frequency following 4 hour and 4-12 week incubation [60, 61], increased NMJ miniature end-plate potential frequency (resting quantal ACh release) [62], enhanced excitatory transmission in hippocampus culture via presynaptic glutamate release [75], increased CSF glutamate [78], increased NMJ spontaneous transmitter release and quantal content, with similar effects produced by caffeine via Ca^{2+} [80], increased spontaneous NMJ ACh release in 3/4 cases [83] (this paper also reported decreased spontaneous NMJ ACh release in some cases, indirectly supporting decreased calcium), and endoplasmic reticulum stress in spinal MTNs [90] (this result is with sALS CSF). These results only provide indirect support, because although increased neurotransmitter release can stem from increased calcium, it can also be due to other reasons.

The presence of VGCC antibodies was reported in several papers, including antibodies to L-VGCC [64, 70, 83] and P-, Q-, and N-VGCC [73]. Patient IgGs were found to be present in MTN axon terminals [96].

Many of these results were done in the same lab (Appel), but more than half of them were done by unrelated labs, over more than two decades. A technical argument was raised against the initial wave of these results [97], but it has been refuted [87].

It should be emphasized that this evidence is from ALS patients, not from animal models, which are not valid for sALS (according to the present paper).

Direct MTN evidence. There is direct and indirect evidence for calcium involvement in ALS beyond calcium-related antibody/serum effects. Calcium is significantly increased in patient muscle nerve terminals [98].

Drug evidence. Taking VGCC blockers or ACE inhibitors (which decrease intracellular Ca^{2+} [99]) against hypertension is associated with decreased ALS risk [100, 101, 102].

Genetic evidence. MTNs induced from pluripotent stem cells of patients with all major fALS-associated genes (C9orf72, SOD1, TDP43, FUS) show higher Ca^{2+} (basal and transients) [103]. In SOD1 mutants, increased MTN calcium and sodium persistent inward currents is early post-natal change [104].

Other tissue. It can be argued that a general H-VGCC impairment cannot be the core cause

of ALS, because this would cause a much wider impairment than the MTN one seen in ALS. However, ALS patients actually do show wide impairment, which overlaps the distribution of H-VGCCs. In addition to MTNs and skeletal muscle issues, ALS patients show cognitive deficits [105, 106], cardiorespiratory and cardiovascular damage [107, 108, 109, 110, 111, 112], skin neuropathy [113, 114, 115], pain in all disease stages, sometimes before motor symptoms [116], impaired insulin secretion [117, 118], Purkinje cell damage [119], and immune system dysregulation (excessive inflammation coupled with inefficient immune responses) [120]. The affected tissues are precisely those with high H-VGCC expression. In addition, calcium is significantly increased in patient blood lymphocytes [121].

The beta cell evidence is especially relevant. Like the calcium evidence, both increased and decreased insulin release have been reported [117]. The decrease is of early phase secretion [118].

The question why MTNs are more sensitive than the other tissues is discussed below.

In summary, there is overwhelming evidence for calcium dysregulation in ALS, pointing to chronic calcium.

4.2 Explanatory power for ALS facts

Studying the sALS literature, several major pathological phenomena can be identified: muscle use problems, muscle wasting, muscle spasms (fasciculation, twitches) (the three main clinical symptoms); symptom appearance with aging (the main epidemiological datum); degeneration of spinal or bulbar (but not ocular) MTNs (the main pathological datum); aggregation of the TDP43 protein [122], oxidative stress [123, 124, 125, 126, 127, 128, 129], and mitochondria dysfunction [130] (the main cellular data); hypermetabolism in motor areas [131] (the main imaging datum); and toxicity of patient serum/Ig/CSF (much of it related to calcium, as detailed above).

Chronic intracellular calcium can explain all of these data points. It can cause muscle spasms because intracellular Ca^{2+} elevation is what induces muscle contractions [132]. Hypermetabolism is identified using PET via increased glucose uptake capacity. Chronic calcium would yield a chronic activation of calcium pumps (PMCA, SERCA), which consume ATP [133]. Relative ATP deficiency activates AMPK, which increases Glut4 translocation to the cell surface [134] and thus glucose uptake capacity. Increased activated AMPK is indeed present in patient MTNs [135, 136]. Chronically high ATP consumption can also account for muscle wasting (see also insulin discussion below).

Chronic calcium induces oxidative stress (mainly H_2O_2), mitochondria permeability pore opening, and ER stress [137, 138, 139, 140, 141, 142]. Oxidative stress and H_2O_2 induce and are enhanced by the cellular stress responses, including the unfolded protein and heat shock responses, and can lead to apoptosis [143, 144, 145]. TDP43 is a major protein recruited by cellular stress responses [122]. Cellular stress and oxidative stress (specifically H_2O_2) induce TDP43 aggregation, and its localization in stress granules [146, 147, 148, 149, 150, 151]. TDP43 is strongly related to ALS, both due to intracellular aggregates in almost all patients

[152, 153] and to mutations in 10% of fALS patients [122]. In our view, it is not causal in sALS, since the parsimonious account of its aggregation is chronic activation by stress responses. Chronic cellular stress eventually activates the death arms of cellular stress responses [154, 155], explaining MTN degeneration and muscle use problems.

The late onset age of ALS can be explained by the fact that normal aging involves increased calcium currents [156, 157] and reduced calcium buffering [158], making aged cells more susceptible to abnormal Ca^{2+} *

The specific vulnerability of non-ocular MTNs in ALS has been explained by the fact that these MTNs express very low levels of calcium binding proteins [159, 160] (although there are also conflicting results [161]). Eye movements and the cerebellum might be relatively preserved because the level of these proteins in oculomotor neurons and Pukinje cells are 5-6 and 15 times higher, respectively [160, 162]. Moreover, sensitive MTNs (tongue) express 3 fold higher levels of H-VGCC currents than ocular MTNs [163]. In addition, MTNs have long axons and high energy requirements, making them more sensitive to chronic ATP consumption and oxidative stress.

High pre-onset physical activity is strongly associated with increased ALS risk [164, 165, 166, 167, 168, 169, 170] (although there are also neutral results [171, 172]). High calcium increases muscle contraction capacity and performance, explaining this datum.

In summary, chronic calcium is toxic to cells and can account for the salient data of ALS.

4.3 a2d

There is some evidence for the a2d hypothesis. It is small compared to the strong evidence implicating H-VGCCs, but sufficient for lending credibility to the theory.

Patient IgG interacts with a2d, with the predicted effects. There is one direct evidence supporting the hypothesis. In a study with ALS patients comorbid with type 2 diabetes (DB2), in 60% of the patients, serum induced high rather than oscillating L-VGCC-mediated intracellular Ca^{2+} in mouse pancreas beta cells. The ALS-DB2 serum induced 2.4x higher basal insulin release, and somewhat lower glucose-stimulated insulin secretion than DB2 serum. Crucially, this was shown to be mediated by IgG targeting the a2d1 subunit [92]. IgGs from ALS patients not diagnosed with DB2 were not tested.

Thus, patient IgG contains a2d1 antibodies, and these induce effects that are similar to those induced by chronic calcium (increased release with a smaller amplitude).

a2d is decreased in ALS. The a2d1 subunit was shown to be significantly decreased in patient MTNs (8 vs. 3 controls) and in familial iPSCs-derived MTNs (24 vs. 18) [173]. However, the same project reported similar results for a large number of other proteins.

a2d suppression increases risk. Gabapentin and pregabalin are a2d ligands that disrupt chan-

*A more specific mechanism for disease onset is impaired insulin signaling (see below), which also occurs with aging.

nel function and are routinely used for treating epilepsy and pain. Suppression of a2d should enhance the a2d impairment present in ALS and exacerbate the state of ALS patients. Indeed, a regional census study in Italy found that usage of antiepileptic drugs 5-6 years prior to ALS diagnosis was associated with increased ALS risk [174]. In about half of these patients, the drug was gabapentin.

a2d suppression accelerates symptoms. Respiration problems are a hallmark of ALS. In a clinical trial of gabapentin in ALS, it exacerbated patient condition by accelerating respiration (forced vital capacity) decline [175]. Gabapentin therapy can also induce recurrent hypoventilation and respiratory failure in non-ALS patients [176, 177].

a2d suppression is associated with motor diseases. Gabapentin can induce severe myopathy and other muscle problems (myositis, rhabdomyolysis) [178, 179, 180, 181, 182, 183, 184]. It can also induce myoclonus (twitches, spasms, fasciculations), a core feature of ALS [185, 186, 187, 188].

Myasthenia gravis (MG) is an autoimmune disease involving antibodies targeting the NMJ (mainly nicotinic receptors) [189]. There is some evidence that gabapentin may be harmful in MG [190], showing that suppression of a2d can be involved in NMJ damage.

Animal models. Mice with a2d2 mutations display impaired motor performance [191]. Two non-lethal a2d knockout mouse models have been reported, with a2d1 KOs showing no apparent motor deficits [192], and a2d4 KOs being hyperactive [25]. The latter model shows again that a2d impairment can result in increased physical activity, but a2d4 is not expressed in relevant tissue in humans. The former model does not support the a2d hypothesis, but it does not refute it either. Mice do not exhibit the neurodegenerative diseases associated with aging in humans, so the a2d1 model might be a model of pre- but not post-onset human ALS.

4.4 Insulin

The fact that a paper presenting a theory of ALS contains a section on insulin may be surprising, since insulin is not mentioned at all in neurology textbooks and general ALS reviews [193, 1, 2], and is mentioned very little even in focused reviews of metabolism in ALS [194, 195].

In my view, insulin is fundamental to the disease [117]. Not only is insulin function impaired by the causal processes of ALS, it is this impairment that serves as a major trigger of disease symptoms. Here I summarize results from a companion paper focusing on insulin in ALS [117], and add new evidence specific to calcium channels and cholesterol.

Insulin impairment. There is a large body of evidence from glucose tolerance tests showing that insulin secretion and/or signaling are impaired in ALS. However, this is usually not detected: although some ALS patients are diagnosed with diabetes (DB), most of them are not, because patient glucose levels are inside the normal range. My explanation for this is that chronic calcium induces excessive insulin-independent glucose uptake (IIGU) that masks the insulin problem. As detailed above, chronic calcium can explain the ALS hypermetabolism results via

excessive ATP consumption, activated AMPK, and Glut4 membrane translocation, resulting in increased glucose uptake capacity that compensates for reduced insulin. Moreover, intracellular calcium directly induces IIGU, even at amounts below the muscle contraction threshold.

Insulin and ALS processes. Insulin and the detrimental processes taking place in ALS mutually oppose each other. Insulin promotes the growth mode of cellular stress responses, and opposes their death mode. It promotes protein synthesis, opposes degeneration, and reduces excessive H₂O₂ and intracellular calcium. Conversely, the harmful modes of cellular stress responses induce insulin resistance [117].

Insulin and diabetes. Early diabetes (usually type 1 (DB1)) is associated with increased risk for ALS, which we explain in two complementary ways. First, the VGCC impairment that causes ALS also damages beta cells, and this can manifest as early DB. Second, the lifelong impairment in insulin shown in DB1 is difficult to control even with modern medicine, yielding an earlier onset in people with ALS. In the former, the two diseases have the same core cause, while in the latter, insulin deficiency increases risk. Indeed, DB1 patients and mouse models exhibit early MTN loss and impaired muscle function [196, 197, 198].

Late diabetes (type 2 (DB2)) is associated with decreased ALS risk, which seems to contradict our view that insulin impairment is the trigger for disease symptoms. However, DB2 involves elevated levels of blood insulin, and many patients are given insulin-based treatment. The theory here predicts insulin to be protective, and indeed, there is epidemiological evidence showing that taking insulin-based drugs reduces ALS risk [117]. DB2 also involves higher blood glucose, which was recently shown to be associated with lower TDP43 pathology in normal aging [199].

Trajectories. Insulin and body mass index (BMI) trajectories are detailed in the companion paper [117].

Calcium. Insulin's beneficial effect on acute (non-chronic) calcium signaling is detailed in the companion paper [117]. For example, insulin rapidly activates Akt, ERK1/2, and CaMKII [200, 201]. Crucially, L-VGCC or CaMKII inhibitors significantly decrease insulin-stimulated glucose uptake in SKM [202]. As long as insulin release is OK, it manages to cover up for the ALS problem. As it starts deteriorating, disease symptoms erupt.

Cholesterol. Insulin is a major factor in cholesterol synthesis via the Akt/mTOR/SREBP axis [203, 204]. Cholesterol is in turn an essential component of lipid rafts, which are crucial for synaptic receptors, including nicotinic receptors at the NMJ [205, 206], and for cytoskeleton health during both axonal growth and neurite maintenance [207, 208]. Indeed, ALS patients show cholesterol dysregulation [209, 210]. However, cholesterol does not seem to be a major factor in the disease, in light of the quite small effect exerted by statins [211]. Insulin's protective effect occurs via calcium metabolism much more than with lipid metabolism.

5 Autoimmunity

In addition to the calcium-related antibody evidence cited above, it has long been recognized that ALS shows elevated circulating immune complexes [212, 213, 214] and spinal cord infiltration by T cells [215]. More recently, reduced numbers of regulatory T cells were shown to correlate with disease progression [216, 217]. Impaired blood-spinal and blood-brain barriers in ALS were reported [218]. Thus, the immune system is clearly active in ALS [219, 120], and immune T cells express a2d, in T cells [17].

Immune involvement in a neurodegeneration disease is not surprising, since degenerating cells recruit immune (and glia) cells. Here I present the case for autoimmune reactivity being actually causal in ALS.

Calcium-related antibodies. The strongest body of evidence for autoimmunity is the one detailed above with respect to the presence and effect of patient antibodies interacting with calcium channels. While such antibodies can result from degeneration or injury that are due to other reasons (e.g., an inherent born VGCC abnormality, or traumatic brain injury, which is indeed associated with increased ALS risk [220, 166, 167]), autoimmunity trivially explains their existence. Moreover, the physically fit lifetime phenotype of ALS supports an early event rather than a relatively late injury.

Immune complement. The classical pathway of complement activation is by antigen-antibody complexes [221], and the complement terminal pathway induces neurodegeneration and cell death. Among the four IgG subclasses, only IgG1 and IgG3 bind complement effectively [222], and IgG3 is the subclass of 1B50-1, an a2d1 antibody [223]. There is substantial evidence for classical complement activation in ALS [213, 212, 224, 225, 226, 227], including in spinal cord and motor end-plates [228, 229, 230]. Activation in motor end-plates includes ones that are still innervated [230].

Complement provides strong evidence to an active antibody-mediated process that contributes to or is responsible for NMJ deinnervation, and the antibodies are of the type that can target a2d.

Association with autoimmune diseases. General support for ALS being related to autoimmunity (but not specifically to a2d) comes from its disproportional pre-onset association with autoimmune diseases, including in an all-England study [231], an all-Sweden study [232], a large Netherlands study [233], and the FDA adverse event reporting system [234].

Association with myasthenia gravis (MG). As mentioned above, MG is an autoimmune disease that targets nicotinic receptors in the NMJ [189]. ALS is associated with MG beyond chance levels [235, 236, 237]. A possible scenario here is that the NMJ impairment in MG triggers the autoimmune reaction against VGCCs, inducing ALS symptoms.

Muscular autoimmune diseases targeting VGCCs. There are several autoimmune diseases that target VGCCs. The most relevant one is Lambert-Eaton myasthenic syndrome (LEMS), which is a neuromuscular disease. LEMS involves antibodies against the a1, beta or a2d sub-

units of P/Q-VGCC, but not antibodies to a2d alone [238]. Thus, an autoimmune disease that targets a2d can be expected, but has not been found yet.

NMJ exposure. Above, we explained the specific vulnerability of non-ocular MTNs in ALS via reduced calcium buffering and increased VGCC currents. A simpler account is that the NMJ lies outside the blood-brain barrier, exposing it to circulating antibodies and immune cells.

Ocular MTNs. The well known ocular immune privilege [239] can be argued to provide another explanation for the relative protection of ocular MTNs, supporting autoimmunity in ALS. However, this account is not that convincing, given that ocular MTNs are damaged in the autoimmune diseases LEMS and MG [189].

The retina strongly expresses a2d4 [25] and also some a2d3 [24]. It can be speculated that these are the targets in the minority of LEMS and MG patients that do not exhibit the antibodies usually associated with these diseases. However, I am not aware of any evidence supporting this idea.

Adaptive immune trajectories in ALS. If ALS is caused by autoimmunity, both active adaptive immune cells and antibodies should be detected, which is indeed the case. However, there is a factor that complicates the situation: immune cells themselves, including B and T cells, express L-VGCCs or L-VGCC-like channels [13, 15, 240, 241]. In particular, L-VGCCs are crucial for B cell proliferation and their transition into plasma cells, which are the ones secreting antibodies [14, 242, 243, 244]. Plasma cells also express L-VGCCs [241], and a2d2 is essential for the proper function of Th2 T cells [17]. This means that an immune reaction against H-VGCCs could target immune cells themselves, eventually resulting in decreasing antibody and lymphocyte levels.

This observation supports the theory instead of opposing it, since it can explain why serum/Ig-VGCC interaction was not identified in all ALS patients.

There is some evidence supporting this model. IgG levels were reported to be normal at early disease stages, and strongly decrease with disease progression, along with CD4 T cell activation [245]. IgG levels were significantly higher in male patients than in controls, but negatively correlated with disease severity and duration [225]. Serum circulating immune complexes, mainly composed of IgG, were significantly elevated on diagnosis, but decreased to control levels at the 2nd visit (although IgG remained high) [214]. A recent meta-analysis found that lower IgG is associated with early- vs. late-onset patients, indicating that disease severity negatively correlates with IgG levels [226]. In addition, B cells were reported to be completely absent from spinal cord infiltrates [246], and lymphocytes were markedly decreased in early stage ALS [247].

In summary, there is some support for autoimmunity being causal in ALS, but most of the evidence is also consistent with immunity being recruited by other causal factors, e.g., genetic mutations or injury.

6 Frontotemporal Dementia (FTD)

Background. FTD has substantial overlap with ALS. 40% of FTD patients show MTN dysfunction [248], and 50% of ALS patients show FTD symptoms, with many meeting diagnosis criteria [249]. FTD has two main variants, tau-positive and tau-negative, and the tau-negative variant involves pathological TDP43 as in ALS [152, 153]. As in ALS, most TDP43 inclusions colocalize with stress granules [250]. There are also FTD variants distinguished by symptoms (e.g., with certain types of language damage). For simplicity, I will focus on the TDP43 variant (roughly FTDbv) here.

FTD is classified as a dementia because it eventually involves degeneration and memory loss. However, it is very different from other dementias. Symptoms manifest as executive dysfunction, apathy, behavioral disinhibition, hypereating, and lack of empathy, with relative sparing of episodic memory [251, 252].

Theory. Due to the tight link with ALS, our starting point is that the underlying cause is similar, a VGCC impairment. As in ALS, we need to ask whether there are neurons that are particularly vulnerable, and why. It turns out that the von-economo neurons (VENs) in the insula and ACC are specifically vulnerable [253, 254], and that their anatomical and molecular profile [255] is very similar to that of MTNS: they have large axons and low expression of calcium buffer proteins [256]. Thus, **in both ALS and FTD, the main damage is in neurons that are more sensitive to calcium overload.**

To complete the picture, we need to show that VEN impairment can explain the major FTD symptoms. It has been shown that lesions of the anterior insula (but not ACC) impair the capacity of identifying others' pain [257], explaining the lack of empathy and the behavioral disinhibition in FTD. Feeling your own pain is known to be essential for empathy for others' pain [258], and gray matter loss in the right insula and anterior temporal cortex is associated with blunted pain in FTD [259]. Relatedly, the anterior insula is the major site of interoceptive heart inputs to the brain, with blunted heart reactivity to emotional stimuli in FTDbv [260].

As in ALS, there is a link between the appearance of symptoms and low insulin-induced glucose uptake. Regardless of FTD, self control is known to be lowest when brain glucose is low [261]. The symptoms of insulin-induced hypoglycemia are well-known and include rudeness, violence, abnormal sexual behavior, exhilaration, asociality, perseveration, impulsivity, slowness, speech difficulties, and even hallucinations [262]. Hypereating and sweet craving in FTD can simply be explained by the brain sensing a deficiency in insulin-induced glucose uptake. Further supporting metabolism involvement, excessive eating is correlated to hypothalamus atrophy [263, 264].

FTD apathy can be explained via blunted facial EMG responses due to right insula damage [265].

Insula lesions yield swallowing problems, which may directly explain swallowing problems in many ALS patients (in addition to the classical accounts) [266].

Glucose evidence. In addition to the evidence cited above with respect to the damage in FTD fo-

cusing on frontal areas and VENs, there is a large body of evidence showing ‘hypometabolism’ in FTD in these areas [267, 268]. This is generally shown using FDG-PET, where decreased PET ligand binding is interpreted as hypometabolism. However, what decreased PET ligand binding shows is reduced expression of glucose transporters, which implies higher utilization (ligand binding can also show higher occupancy, which has the same effect due to negative feedback). Thus, it is not that the vulnerable areas utilize less glucose, they in fact uptake more glucose. This we explain exactly as in ALS – chronic activation of VGCCs induces excessive insulin-independent glucose uptake, where glucose is not used for protein synthesis and anti-oxidant defenses, promoting degeneration.

Insulin is involved in these results. FTD involves increased fasting insulin, HOMA-IR (indicating insulin resistance), triglycerides, and lower HDL [269]. DB type 2 occurs in 39% vs. 23% in controls [270]. C-peptide, indicating insulin, is increased, with 5y earlier onset with higher levels [271]. Note that higher C-peptide is associated with regional cortical thinning in cognitively healthy people [272].

There are other indications of hypoglycemia, including dysregulation of CRH, ACTH, and cortisol [273]. A coarse trend was found where CSF glucose was lower and with a lower standard deviation than all other groups tested [274].

Calcium evidence. As in ALS, FTD shows calcium pathology. The calcium binding protein calbindinD28 is dramatically decreased in frontal cortex in patients with both ALS and FTD [275]. Patients CSF is toxic to mouse spinal GABA and calbindinD28 neurons [276].

Interestingly, the brain distribution of L-VGCC (Cav1.2) includes valence (amygdala) and motor areas, but not the hippocampus [11], supporting our account of the difference between FTD and dementias involving episodic memory.

Serum calcium binding proteins (9, 10) are decreased in FTD [277]. FTD patients show significantly increased serum calcium [278]. iPSCs from FTD-tau patients also show dysregulated Ca^{2+} elevation after electrical stimulation [279]. There is Ca^{2+} damage with oxidative stress biomarkers [280]. In one case, P/Q-VGCC, and N-VGCC antibodies caused FTD symptoms in a patient with rheumatoid arthritis and thyroid history [281].

Autoimmunity evidence. FTD shows a wide range of antibodies compared to other dementias [282]. In 41/175 (23%) of patients, serum has anti-GluA3 antibodies [283]. FTD shows blood-brain barrier dysfunction, with significantly higher CSF IgGs. This shows more in ALS-FTD than ALS and is associated with worse prognosis [284]. FTD patients had about 25% more autoimmune diseases than controls (lowest in FTD with C9orf72 mutation) [285].

Additional VEN and areal evidence. In FTD patients, loss of VENs and of GABA theta subunit-expressing pyramidal neurons is associated with TDP43 and FUS pathology [286]. Frontoinsular VENs and fork cells show early, disproportionate TDP43 aggregation, correlating with symptom severity and empathy loss [287].

Obviously, VENs are not the only vulnerable neurons. All frontal areas around them show early atrophy, including ACC, mPFC [288], insula, orbital frontal cortex [289], bilateral inferior frontal gyrus, temporal pole, and the rest of the insula [290].

We also note that aging and high BMI involve hypoperfusion specifically in the insula and limbic brainstem, respectively [291]. Diabetes predicts brain hypoperfusion only in the insula, with higher brain hypoperfusion in insula in diabetes vs. obesity [291]. Thus, the insula is specifically vulnerable to glucose metabolism possibly even without calcium impairment. Hypoglycemia events double dementia risk, and vice versa [292].

7 Discussion

In this paper I presented the first complete theory of ALS and FTD, a theory that explains their etiology and pathology. The theory has three components. First, the fundamental problem is in H-VGCCs. Second, the specific subunit that is most likely involved in most (or all) cases is a2d (although we did leave open which of a2d1-a2d4 are more involved). Third, the source of the problem could be genetic or autoimmune-induced.

TDP43. A large part of the research done in ALS focuses on the TDP43 protein. In my view, it is not a core cause of the disease, since TDP43 aggregates simply indicate chronic metabolic cellular stress. I think that it is a mistake to focus so much research priority on TDP43 instead of on more fundamental topics.

Calcium. I think that the centrality of calcium in ALS would be easily accepted by the readers, due to the fact that calcium dysregulation has been so thoroughly demonstrated [293, 294]. The challenge with calcium in ALS has turned out to be the identification of the specific subunit(s) involved, due to contradictory results produced by the early set of papers. The present paper is the first to raise the idea that the a2d subunit is the core one. This makes sense for a large number of reasons detailed above. (Naturally, other subunits can also trigger the detrimental processes described here in various constellations.)

We should remember that the a2d subunit is cleaved, folded, glycosylated, GPI-anchored in lipid rafts, and uses disulfide bonds. It seems that antibodies only bind it in this state, causing null results in experiments with denatured proteins. This is an empirical issue to be resolved, and it trivially explains some negative results.

Autoimmunity. We have presented the case that the a2d impairment in ALS stems from autoimmunity. It should be emphasized that it is possible that it stems from some genetic (or epigenetic) cause. At present, there is no evidence supporting this direction, but given that mutations in many relevant genes are strongly associated with ALS (fALS), this is a possibility that needs to be examined.

ALS cells are stronger than normal throughout life, because they enjoy more calcium. At some point, calcium gets chronic and toxic, and this self-amplifying process rapidly damages cells, eventually killing them. This model holds for MTNs, beta cells, skeletal muscle cells, and possibly immune cells. The turning point commonly (but surely not always) occurs when insulin function is reduced to such an extent that it cannot support the ATP, anti-oxidative stress, and protein synthesis requirements of the affected cells.

These results are consistent with a model in which antibodies target immune cell VGCCs throughout life, without noticeable damage (or maybe even improving their reactivity). At some point cells cannot contain this state, setting in motion a detrimental process that does not depend on antibodies anymore. Chronic calcium is self-amplifying (since it involves the suppression of negFB), and perfectly fits the properties of such a detrimental process.

Insulin. As explained above, the collapse of insulin signaling is a strong candidate for what actually triggers the eruption of the disease. At present, this view is not shared by anybody (in fact, it is actively disputed by many people I have contacted). It is not clear to me why physicians assume that if patient blood glucose levels are at the normal range, this indicates healthy insulin function. About half of cellular glucose uptake is insulin-independent, and it is known to be triggered by calcium. This excessive calcium masks the insulin problem in ALS, and can easily be identified via the initial stages of the OGTT, which can show decreased insulin secretion. The OGTT is a common and easy test and should be administered to all ALS patients.

FTD. FTD is a simple parallel of ALS in which the vulnerable cells are prefrontal VENTs and their neighbors, which have properties similar to MTNs. I have shown how this simple model can naturally explain the various FTD symptoms, including lack of empathy and disinhibition.

Theory predictions. As far as I know, the basic experiment to support or refute the a2d direction has not been conducted. No study has reported testing ALS IgGs against the a2d subunit (any of them) alone. Studies widely report testing against the a1 subunit, but not against the auxiliary subunits.

The most relevant reported result is Shi et al [92]. They studied ALS patients with DB2, showing that in 60% of them IgGs target the a2d1 subunit when it is expressed in cells. It should not be too difficult to extend this result to the general sALS population.

I believe that insulin treatment may be disease-modifying in ALS if started early enough, and this is one of my theory predictions.

Treatment. In addition to insulin, the theory implies that available immune-based therapies might be beneficial in sALS, since they directly target the disease's causal processes. For example, spectacular results have been recently achieved with CAR T therapy targeting B cells (CD19) against lupus [295]. However, it could be that the damage in ALS has already been done and that B cells are already deficient.

It may be thought that gabapentin and pregabalin, L-VGCC blockers, could help. However, gabapentin impairs a2d membrane trafficking [296].

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