Editorial

Brain N-acetylaspartate accumulation in Canavan disease is not neurotoxic *per se*: the implications of the first gene replacement therapy study to demonstrate successful post-symptomatic treatment in mice

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Canavan disease (CD) is identified as a fatal spongiform leukodystrophy caused by missense mutations in the *Aspa* gene (1,2). The latter encodes aspartoacylase (ASPA; EC 3.5.1.15), an enzyme that is highly abundant in oligodendrocytes, and that under physiological conditions hydrolyses N-acetylaspartate (NAA; N-acetyl-L-aspartic acid) to L-aspartate and acetate (*Figure 1A*). In CD, ASPA cannot degrade NAA and, as a result, NAA builds-up in the central nervous system (CNS) (4) and is also present in very high amounts in the urine of these patients (5). Over the last decades, three major hypotheses have been set forward in an attempt to link the lack of ASPA activity to the neuropathological features (namely, diffuse spongiform white matter degeneration, dysmyelination and intramyelinic oedema) of CD (6,7): (I) the "acetate-lipid-myelin" hypothesis (where the lack of NAA-derived acetate is believed to hinder myelin lipid synthesis) (5,8); (II) the "osmotic-hydrostatic" hypothesis (where NAA accumulation in the CNS is suggested to act as a local water molecule trafficker, leading to the formation of intramyelinic oedema) (9), and (III) the more recent "oxidative stress" hypothesis (where NAA catabolism disruption is believed to be a source of oxidative stress that affects optimal myelination) (10). However, to date, none of these, admittedly non-mutually-exclusive, hypotheses has inspired a clinically effective non-genetic approach to the treatment of CD (6); a fact that has highlighted the need for an efficient gene therapy for this rare and devastating disease.

In what appears to be a major study in the field, von Jonquieres *et al.* (3) have recently shown that although increased CNS NAA levels predict pathological severity in CD mice, these same high levels are not neurotoxic *per se*. In a series of well-designed experiments using transgenic mice, the authors have managed to show: (I) that the overexpression of the neuronal NAT8L enzyme (N-acetyltransferase-8-like; *Figure 1A*) that results in high CNS NAA levels, is not linked to CD-simulating neurological deficits; (II) that the targeted, oligodendrocyte-specific elimination of the ASPA enzyme (termed as "conditional *ASPA* deletion") is enough to provoke a CD-like pathology on its own, and (III) that the ASPA activity outside the CNS is useful in lowering CNS NAA levels, ameliorating the CD-like pathology and delaying the CD-linked symptomatology onset in transgenic mice with a conditional (oligodendrocyte-specific) *Aspa* deletion (3). *Figure 1B* provides a visual synopsis of some of the major findings of four of the *in vivo* experiments performed by von Jonquieres *et al.* (3).

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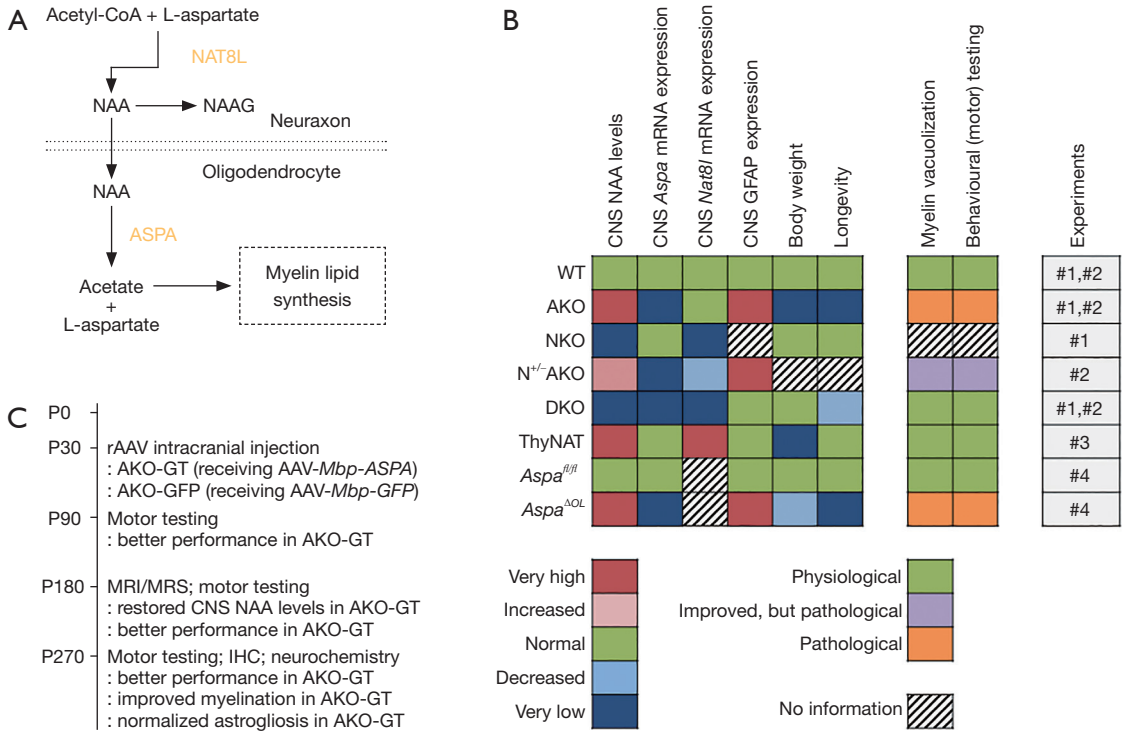

Figure 1 Synopsis of the main NAA-associated metabolic pathway in the CNS and of major experiments and findings in the study of von Jonquieres *et al.* (3). (A) Schematic representation of the main metabolic pathway of NAA in the CNS, where the NAA-synthesizing (NAT8L) and the NAA-degrading (ASPA) enzymes are highlighted in orange colour; (B) overview of the main findings of four *in vivo* experiments performed by von Jonquieres *et al.* (3) on CD-mimicking knockout mice. In the first two experiments, the double-knockout mice for *Aspa* and *Nat8l* (DKO) did not produce a phenotype mimicking that of CD, while *Aspa*-knockout mice with one deleted *Nat8l* allele ($N^{+/-}$AKO) and increased CNS NAA levels could do so to an extent; these experiments proved that in the absence of ASPA, the murine CNS NAA levels correlate to CD-mimicking severity in terms of both the neuropathological and the behavioural aspects of it. In a third experiment in which *Nat8l* was selectively overexpressed in neurons (ThyNAT), the increased CNS NAA levels in these ThyNAT mice did not provoke the appearance of a CD-mimicking neuropathology or symptomatology, indicating that NAA accumulation is not neurotoxic *per se*. Finally, in a fourth experiment, the conditional oligodendrocyte-restricted *Aspa*-knockout mice ($Aspa^{\Delta OL}$) produced a phenotype matching that of AKO mice, suggesting that the depletion of functional ASPA from oligodendrocytes alone is enough on its own to provoke a CD-mimicking neuropathology and symptomatology in these mice; (C) timeline of a fifth experiment performed by von Jonquieres *et al.* (3), in which the intracranial AAV-*Mbp-ASPA* gene therapy specifically targeted oligodendrocytes in post-symptomatic AKO mice (AKO-GT). AAV-*Mbp-ASPA*, adeno-associated virus that contains a plasmid in which the cDNA encoding human ASPA has been introduced after the mouse myelin basic protein (*Mbp*) promoter; AAV-*Mbp-GFP*, the control to AAV-*Mbp-ASPA*; AKO, *Aspa*-knockout mice; AKO-GFP, *Aspa*-knockout mice that received intracranial AAV-*Mbp-GFP* treatment (control); AKO-GT, *Aspa*-knockout mice that received intracranial AAV-*Mbp-ASPA* treatment (gene therapy); ASPA, aspartoacylase; *Aspa*, ASPA gene; $Aspa^{fl/fl}$, the control to the $Aspa^{\Delta OL}$ mice; $Aspa^{\Delta OL}$, conditional oligodendrocyte-specific *Aspa*-knockout mice; CD, Canavan disease; CNS, central nervous system; CoA, coenzyme A; DKO, double-knockout mice for *Aspa* and *Nat8l*; GFAP, glial fibrillary acidic protein; IHC, immunohistochemistry; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; $N^{+/-}$AKO, *Aspa*-knockout mice with one deleted *Nat8l* allele; NAA, N-acetyl-L-aspartic acid; NAAG, N-acetyl-aspartylglutamic acid; NAT8L, N-acetyltransferase-8-like; *Nat8l*, NAT8L gene; NKO, *Nat8l*-knockout mice; Px, postnatal day x (where x is a number; 0, 30, 90, 180, 270); rAAV, recombinant AAV; ThyNAT, Thy-*Nat8l* transgenic mice; WT, wild-type mice.

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In a fifth experiment, von Jonquieres *et al.* (3) have also shown that 5 and 9 months after the delivery of intracranial myelin basic protein (*Mbp*)-*ASPA* gene therapy through an adeno-associated virus (AAV) vector, the *Aspa*-knockout mice exhibit a post-symptomatic, near-complete regression of their CD-like pathology (*Figure 1C*). This regression seems to be accompanied by an improved performance in behavioural (motor) tests in the gene therapy-receiving mice as compared to their respective controls; a finding that signifies the potential of this post-symptomatic treatment approach.

The AAV-mediated ASPA delivery in experimental approaches to CD using animals is not a new concept; in fact, several promising attempts have taken place over the last 20 years to produce ASPA in *Aspa*-knockout or *Aspa*-mutated rodents (11-14). On this particular occasion, von Jonquieres *et al.* (3) had a complementary DNA (cDNA) encoding human ASPA been introduced between the mouse *Mbp* promoter and the woodchuck post-transcriptional regulatory element, followed by a bovine growth hormone poly(A) in an AAV2 plasmid; the latter was packaged in AAV vectors of serotype cy5. This approach was ingenious in that: (I) it allowed for a targeted, oligodendrocyte-specific, AAV-mediated gene therapy based on recent studies that managed to achieve this (15,16), and (II) it embraced a gene therapy approach that has already shown promising results in clinical studies (11,17). However, the recent study of von Jonquieres *et al.* (3) is not only important for the rationale behind the choice of the employed gene therapy methodology, but also for its translational value, as the gene therapy presented in it is post-symptomatic. The *Aspa*-knockout mice received their *Mbp-ASPA* gene therapy at the age of postnatal day 30 (P30; *Figure 1C*), which is a rather late time-point for murine neurodevelopmental standards, at which these mice already seem to exhibit aspects of a CD-mimicking symptomatology; see supplementary material of (3). In this view, this targeted, post-symptomatic and seemingly effective gene therapy approach signifies a big step forward not only for CD, but also for a number of other rare and devastating leukodystrophies that could benefit from such a cell-type-restricted transgene expression-focused therapeutic approach.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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