# *N*-Acetylaspartylglutamate in CNS Hypomyelination

Authors
M. M. C. Wamelink[1], E. Struys[1], U. Holwerda[1], E. A. Sistermans[2], R. M. L. van Spaendonk[2], D. Halley[3], M. A. A. P. Willemsen[4], C. Jakobs[1], M. S. van der Knaap[5], N. I. Wolf[5]

Affiliations
Affiliation addresses are listed at the end of the article

**Key words**
- *N*-acetylaspartylglutamate
- hypomyelination
- white matter disorder

## Abstract

CSF *N*-acetylaspartylglutamate (NAAG) has been found to be elevated in some hypomyelinating disorders. This study addressed the question whether it could be used as a marker for hypomyelination and as a means to distinguish between hypomyelinating disorders biochemically. We have measured CSF NAAG in a cohort of 28 patients with hypomyelination with known and unknown aetiology. NAAG was found to be elevated in 7 patients, but was normal in the majority, including patients with defined hypomyelinating disorders. CSF NAAG is not a universal marker of hypomyelination, and the mechanism of its elevation remains poorly understood.

## Introduction

Hypomyelinating white matter disorders are characterised by a substantial permanent myelin deficit of the CNS. They comprise different disorders, the prototype being Pelizaeus-Merzbacher disease (PMD), an X-linked disorder due to alterations of *PLP1*, encoding the most abundant myelin protein, proteolipid protein 1. Clinical signs such as hypodontia or cataract have contributed to delineating new entities [13, 15]. MRI pattern recognition also helps to identify different entities in this heterogeneous group [12]. Nevertheless, at least half of all patients with CNS hypomyelination still remain without a definitive diagnosis. The clinical presentation of these patients is variable. Muscle hypotonia, ataxia, spasticity and learning difficulties are common, but can be surprisingly mild, even in adults. Metabolic markers for hypomyelination have long been sought to facilitate diagnosis, to allow identification of new subgroups and also to understand the pathogenesis of these disorders. *N*-Acetylaspartylglutamate (NAAG) was found to be elevated in the CSF of patients with PMD and initially thought to be a valuable marker for hypomyelination, possibly even correlating with clinical severity [3]. Subsequently, high NAAG concentrations were described in patients with other hypomyelinating disorders: in a child with Pelizaeus-Merzbacher-like disease (PMLD) due to mutations in *GJC2* [11], in patients with Salla disease [10] and in 2 girls with severe clinical presentations and still no known genetic diagnosis [14]. Whether NAAG would be also elevated in the CSF of patients with other defined hypomyelinating white matter disorders, has never been investigated. We therefore decided to systematically investigate CSF NAAG in all patients with hypomyelination under our care.

## Patients and Methods

### NAAG determination

10 µL CSF and 20 µL of internal standard (IS, 10 µM [$^2H_3$] NAAG) were diluted with deionised water to a total volume of 120 µL in vials. Vials were mixed vigorously, put in an ultrasonic bath for 3 min and injected onto a liquid chromatography tandem mass spectrometer (LC-MS/MS) system. Liquid chromatography was performed using a 3.9 × 150 mm Symmetry $C_{18}$ HPLC column (bead size 5 µm, Waters Chromatography BV, Etten-Leur, The Netherlands). An isocratic eluent of 30 % ACN/water containing 500 mg/L octylammonium acetate as ion pair was used. The flow rate was set to 1 mL/min and was split after the analytical column in a ratio of 1:4. A volume of 5 µL of sample was injected onto the column and the total run time was 10 min. Detection of NAAG was carried out on an API-3000 tandem mass spectrometer (PE-Sciex) equipped with a turbo ion spray source operating in the negative mode.

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**Correspondence**
***Nicole I. Wolf***
Department of Child Neurology
VU University Medical Center
Postbox 7057
1007 MB Amsterdam
The Netherlands
n.wolf@vumc.nl

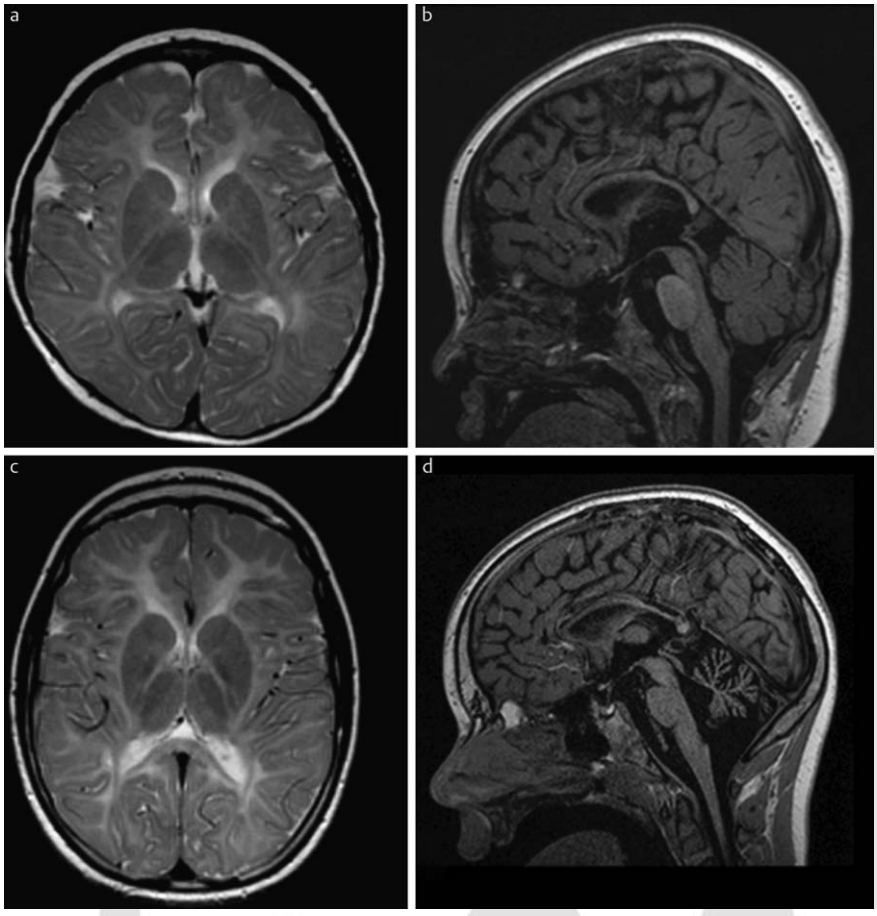


**Fig. 1** Axial $T_2$-weighted images (**a, c**) and sagittal $T_1$-weighted (**b, d**) MR images of 2 patients with hypomyelination. In both patients, the white matter signal on the $T_2$-weighted images is diffusely elevated. **a** and **b** are from a 6-year-old child with Pelizaeus-Merzbacher disease, **c** and **d** from a 10-year-old child with 4H syndrome. Note the cerebellar atrophy in the latter patient (**d**), a typical finding in 4H syndrome.

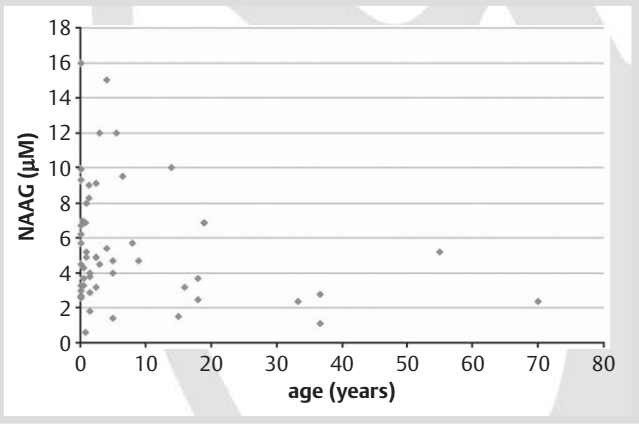


**Fig. 2** Control CSF NAAG concentrations.

Detection of NAAG was performed by multiple reaction monitoring (MRM), measuring the transitions of $m/z$=303/128 for NAAG and $m/z$=306/128 for [$^2H_3$] NAAG.

## Patients

NAAG was measured in the CSF of 28 children with hypomyelination as part of their standard diagnostic work-up. In 8 children, a definitive genetic or clinical diagnosis has been achieved during the diagnostic process; the remaining 20 children still have hypomyelination of unknown origin. Hypomyelination was defined as significant and permanent myelin deficit. the white matter signal on $T_2$-weighted MR images was diffusely elevated (**Fig. 1**). CSF was not examined in children with hypomyelination who had already a definitive clinical or genetic diagnosis.

## Results

### NAAG levels in reference population

In total, 54 CSF samples were available from individuals with normal myelination on MRI scans. The obtained data suggested a moderate continuous age-dependent decrease in the levels of NAAG (**Fig. 2**). According to these values, we have chosen the following age-dependent reference intervals: age 0–8 years: NAAG 0.6–16 µM; age >8 years: NAAG < 12 µM. Non-parametric evaluation of these 2 age ranges revealed a significance of p=0.06. These reference values are in close agreement with those published previously [10, 11]. A p-value of 0.06 reflects a non-significant difference, but it is likely that more control values will decrease the p-value, making the differences between these 2 age groups significant.

### NAAG in patients with hypomyelination

NAAG was elevated in a patient with Salla disease and a patient with PMLD due to a homozygous missense mutation in *GJC2*. In 2 boys with *PLP1* duplication, NAAG was normal (12 and 16 µM). In 2 patients with hypomyelination and congenital cataracts (HCC), 1 child with 4H syndrome (hypomyelination, hypodontia and hypogonadotropic hypogonadism) and 1 child with hypomyelination and atrophy of the basal ganglia and cerebellum (HABC), NAAG was within normal limits (**Table 1**). Regarding the group of 20 patients with hypomyelination of unknown origin, NAAG was normal in 15. In the remaining 5 patients, NAAG was mildly (n=3) or strongly (n=2) elevated (**Table 2**). Mutation analysis of *GJC2*, *PLP1* and *SLC17A5* was negative. MRI and clinical presentations of these 5 patients did not suggest a single underlying disorder.

**Table 1** CSF NAAG in patients with hypomyelination and definitive diagnosis.

| Patient | Age | NAAG (µmol/L) | Diagnosis |
|---|---|---|---|
| HM1 000 | 4y 1 m | 92 | Salla disease |
| HM13 | 12y 11 m | 50 | PMLD (*GJC2*) |
| HM18 | 1y 4 m | 16 | PMD |
| HM3 | 1y 9 m | 12 | PMD |
| HM16 | 13y 3 m | 7.2 | HCC |
| HM1 | 7y 4 m | 4.8 | 4 H syndrome |
| HM1 002 | 2y 10 m | 4.6 | HABC |
| HM14 | 21y 9 m | 1.7 | HCC |

NAAG levels in CSF of patients with hypomyelination of defined origin. PMD = Pelizaeus-Merzbacher disease, HCC = hypomyelination and congenital cataract, HABC = hypomyelination with atrophy of basal ganglia and cerebellum, PMLD = Pelizaeus-Merzbacher-like disease. Reference range: age 0–8 years: NAAG 0.6–16 µM; age >8 years: NAAG <12 µM

**Table 2** CSF NAAG in hypomyelination without definitive diagnosis.

| Patient | Age | NAAG (µmol/L) |
|---|---|---|
| HM27 | 3y 11 m | 89 |
| HM12 | 30y 6 m | 35 |
| HM19 | 7y 4 m | 19 |
| HM7 | 16y 9 m | 18 |
| HM32 | 2y 5 m | 18 |
| HM30 | 5y 4 m | 12 |
| HM39 | 6 m | 11 |
| HM38 | 4y 6 m | 9.0 |
| HM44 | 1y 4 m | 6.0 |
| HM11 | 14y 8 m | 4.5 |
| HM4 | 15y 7 m | 3.9 |
| HM6 | 17y 7 m | 3.3 |
| HM40 | 2y 5 m | 2.6 |
| HM9 | 12 m | 2.3 |
| HM10 | 6y 9 m | 2.3 |
| HM33 | 25y | 1.9 |
| HM22 | 30y 4 m | 1.4 |
| HM23 | 11y 3 m | 1.1 |
| HM25 | 4y 3 m | 1.0 |
| HM24 | 12y 5 m | 0.7 |

NAAG levels in CSF of patients with hypomyelination without definitive diagnosis. Reference range: age 0–8 years: NAAG 0.6–16 µM; age >8 years: NAAG <12 µM

## Discussion

Our data show that NAAG elevation is not a universal marker of hypomyelination. Elevation of this dipeptide has been demonstrated consistently only for 2 disorders: Salla disease [9, 10] and PMLD due to *GJC2* mutations [9, 11]. Our results are in line with these data. Regarding PMD, we found NAAG normal in 2 boys carrying a *PLP1* duplication, in 1 of them at the upper limit of normal. This is somewhat in contradiction with the results of a recently published study stating NAAG would be elevated in patients with PMD due to *PLP1* duplications, but not in patients with missense mutations [9]. In the latter article, however, NAAG concentrations varied widely among the large number of tested patients and correlated neither with age nor with clinical severity. In other hypomyelinating disorders – HCC, 4 H syndrome and HABC – NAAG was not elevated in our study. Regarding the group of patients without a definitive diagnosis, NAAG was elevated in 25 %. The clinical presentation of these patients was not suggestive of a common underlying pathology: on the one side, 1 young adult had only mild ataxia and mild learning difficulties, on the other extreme 1 young child was severely impaired especially in its motor functioning. Also regarding the varying known disorders associated with NAAG elevation, it is likely to expect that the as yet unknown diseases causing high CSF NAAG will be just as heterogeneous. The reason for the considerable variation of NAAG levels in patients with hypomyelination remains unknown.

We can only speculate on the pathomechanism of NAAG elevation. Its metabolism is closely related to that of *N*-acetylaspartate (NAA). Both are elevated in Canavan disease where NAA degradation is hampered by deficient function of aspartoacylase in oligodendrocytes. NAAG and NAA are both synthesised in neurons, NAAG being secreted in the extracellular space, NAA especially being transferred to oligodendrocytes [5]. Astrocytes hydrolyse NAAG to NAA and glutamate and then convert glutamate to glutamine; oligodendrocytes hydrolyse NAA to aspartate and acetate. Aspartate and glutamine are taken up again by neurons. Although NAA is one of the most abundant molecules in the CNS, its biological role remains elusive. Its recycling might be important for the osmotic and energetic regulation of neurons [2]. Another hypothesis about NAA function discusses its role as acetyl coenzyme A storage and transport form specific to the CNS [1]. It has also been implied as a donor of acetyl groups for myelin lipid synthesis [4, 7, 8]. NAAG functions as a non-excitotoxic neurotransmitter targeted at the astrocytic glutamate metabotropic receptor 3. Its effects on oligodendrocytes are less clear. In a cell culture model, NAAG has not proven toxic to oligodendrocytes although it is able to provoke an inward current in these cells [6]. NAAG is elevated in only some children with hypomyelination. This makes a non-specific effect of myelin deficit as the reason for its elevation as improbable as, vice versa, a direct causal relationship of elevated NAAG with hypomyelination via a putative toxic effect on oligodendrocytes. Still, a hypothesis linking myelin lipid synthesis with impairment of the putative NAA-NAAG recycling process and consecutive NAAG elevation in at least some children with hypomyelination remains attractive.

**Affiliations**
[1] Department of Clinical Chemistry, Metabolic Unit, VU University Medical Center, Amsterdam, The Netherlands
[2] Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands
[3] Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands
[4] Department of Child Neurology, Radboud University Medical Center, Nijmegen, The Netherlands
[5] Department of Child Neurology, VU University Medical Center, Amsterdam, The Netherlands

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