Pediatric RESEARCH

SYSTEMATIC REVIEW

Brain proton magnetic resonance spectroscopy and neurodevelopment after preterm birth: a systematic review

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BACKGROUND: Preterm infants are at risk of neurodevelopmental impairments. At present, proton magnetic resonance spectroscopy (^{1}H-MRS) is used to evaluate brain metabolites in asphyxiated term infants. The aim of this review is to assess associations between cerebral ^{1}H-MRS and neurodevelopment after preterm birth.

METHODS: PubMed and Embase were searched to identify studies using ^{1}H-MRS and preterm birth. Eligible studies for this review included ^{1}H-MRS of the brain, gestational age ≤32 weeks, and neurodevelopment assessed at a corrected age (CA) of at least 12 months up to the age of 18 years.

RESULTS: Twenty papers evaluated ^{1}H-MRS in preterm infants at an age between near-term and 18 years and neurodevelopment. ^{1}H-MRS was performed in both white (WM) and gray matter (GM) in 12 of 20 studies. The main regions were frontal and parietal lobe for WM and basal ganglia for GM. *N*-acetylaspartate/choline (NAA/Cho) measured in WM and/or GM is the most common metabolite ratio associated with motor, language, and cognitive outcome at 18–24 months CA.

CONCLUSIONS: NAA/Cho in WM assessed at term-equivalent age was associated with motor, cognitive, and language outcome, and NAA/Cho in deep GM was associated with language outcome at 18–24 months CA.

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IMPACT:

- In preterm born infants, brain metabolism assessed using ^{1}H-MRS at term-equivalent age is associated with motor, cognitive, and language outcomes at 18–24 months.
- ^{1}H-MRS at term-equivalent age in preterm born infants may be used as an early indication of brain development.
- Specific findings relating to NAA were most predictive of outcome.

INTRODUCTION

One in ten infants is born preterm. According to the World Health Organization, the annual number of preterm born infants is assumed to be ~15 million. Around one million children die every year because of prematurity-related morbidities.[1] With advances in neonatal care, mortality after preterm birth has decreased. However, as a result of numerous risk factors preterm survivors, in particular those born before 32 completed weeks of gestation, are faced with a wide range of significant challenges of brain development.[2] Long-term neurodevelopmental impairments (NDIs) like cerebral palsy (CP), neurocognitive, behavioral, and motor impairments affect nearly 25–35% of the preterm infants and increase with a decrease in gestational age (GA).[3,4] Preterm infants have ~12-point lower intelligence quotient (IQ) levels,[5] reduced language and motor abilities,[6,7] attention difficulties and impaired social skills,[8] and academic underachievement[9] later in life. CP in preterm born infants may result from parenchymal brain injury such as periventricular hemorrhagic infarction or cystic periventricular leukomalacia, and can be predicted using term-equivalent age (TEA) magnetic resonance imaging (MRI). However, neurocognitive or behavioral impairments are commonly seen in extremely born preterm infants, and difficult to predict. Prediction of NDIs as a result of prematurity is crucial for proper clinical assessment and parental counseling, and guiding neurodevelopmental follow-up, as well as for the development of future neuroregenerative strategies.

MRI is increasingly used as a diagnostic tool for central nervous system evaluation since the early 1980s in both the neonatal and childhood period.[10,11] Proton magnetic resonance spectroscopy (^{1}H-MRS), a much older technique than MRI, has mainly been used for the prediction of outcome after perinatal asphyxia in term infants.[12]

During brain development, concentrations of metabolites change, with the most rapid alterations occurring within the first 2 years of life.[13–16] It has been suggested that ^{1}H-MRS metabolites are biomarkers for long-term neurodevelopment in preterm infants.

Therefore, the aim of the present review is to assess the association between cerebral ^{1}H-MRS metabolites and neurodevelopmental outcome after preterm birth.

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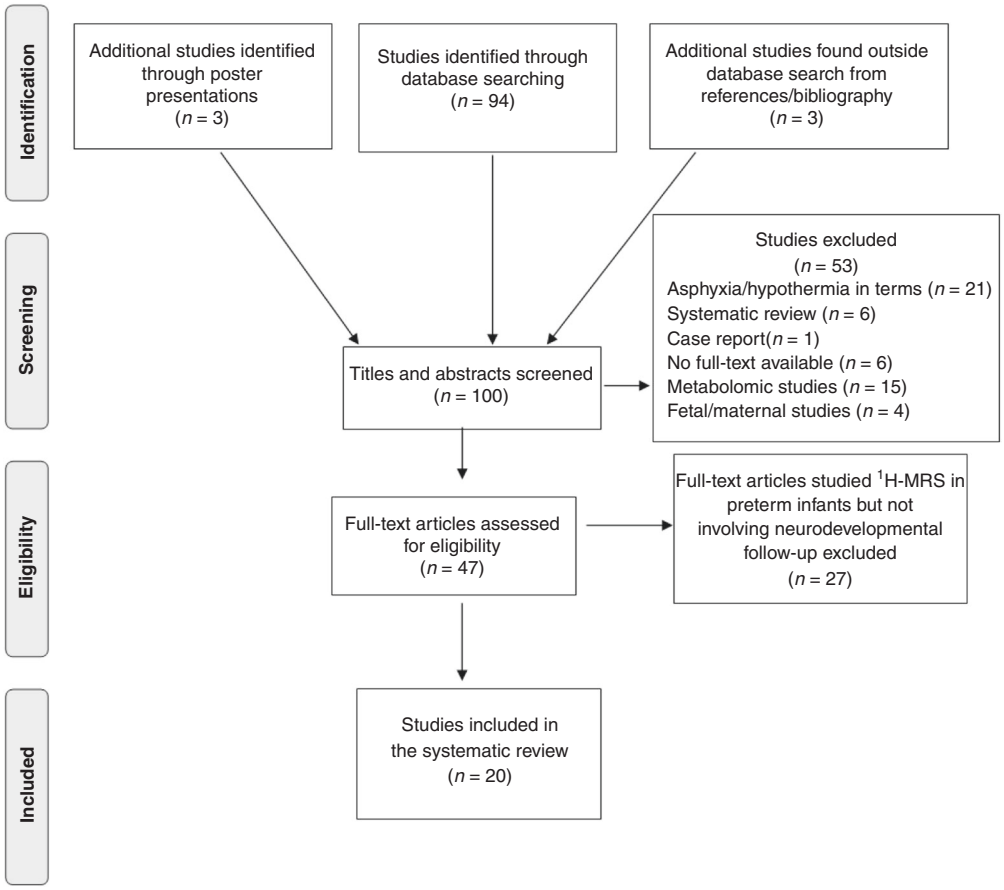

Fig. 1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram of results of electronic database search, title, abstract screening, eligible and included studies.

METHODS

Design

A systematic search strategy was performed following the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement in order to identify eligible studies.[17] In this review, the aim was to identify and discuss all published ^{1}H-MRS studies that predicted any neurodevelopmental outcome in infants (12–24 months), young children (2–6 years), and older children (6–18 years) born preterm ≤32 weeks.

Search strategy and information sources

Identification of studies was performed by an extensive search of electronic database Medline (PubMed, from 1995 to present, and Embase from 1988 to present). The last update of this search was on 31 May 2020. Entry terms were formulated based on the aim of the review, including premature infants, MRI techniques, and neurodevelopmental outcome, and were searched with the Medical Subject Heading search terms. Appendix 1 includes an overview of the entry terms and search strategy. The reference lists and bibliographies of the selected studies for inclusion were also manually reviewed to identify any other additional studies that were not included. The language choice for the published articles was limited to English and papers written in any other language were excluded. All prospective or retrospective human studies were included and no restriction was performed for the type of study to capture all eligible articles. Since the possibility of change in data after published version of studies, conference abstracts were not included in the review. Inclusion criteria were: (1) brain ^{1}H-MRS acquired during the neonatal period and/or later ages, (2) prematurity at or below 32 weeks GA or very-low-birth-weight infants (birth weight <1500 g); (3) neurodevelopmental outcome assessment: CP, cognitive or intellectual impairments, social–emotional problems, and/or behavioral abnormalities diagnosed at a minimum age of 12 months corrected age (CA) up to 18 years.

Study selection

Title and abstract of studies were evaluated first, and if the screened articles met the inclusion criteria, then the full text of eligible articles was assessed. Two independent reviewers (B.C. and F.G.) read the full text of the selected studies. Records of screening and study selection are presented in Fig. 1. When debates occurred regarding the inclusion of studies, it was discussed with a third researcher (A.v.d.H.).

Data extraction

Data were extracted by two independent researchers (B.C. and F. G.) and crosschecked by a third researcher (T.A.). Detailed features of the articles (study design, sample size, ^{1}H-MRS screening protocols, predictor metabolites, and neurodevelopmental tests and outcomes) were extracted to a specifically designed Excel workbook to classify the studies for the systematic review. When a ^{1}H-MRS examination and neurodevelopmental follow-up were performed more than once, they were listed separately in the table (Table 3). When data were missing, the researchers contacted the author to request the data.

Methodological quality and synthesis

Included studies were critically appraised using the McMaster critical review form for quantitative studies[18] and were comprehensively reviewed in terms of methodological quality. The review form consisted of 15 items including the risk of bias, and cut-off

points were set according to the articles from the literature using this critical appraisal tool. Each point was assessed as fulfilled, partially fulfilled, and unfulfilled. Only items scored as fulfilled generated one point. A score of 13–15 was considered high quality, a score of 9–12 was moderate quality, and a score of ≤8 was low quality. If the included studies were heterogeneous, a narrative best-evidence synthesis was applied.

RESULTS

Of 96 reported studies between 1995 and May 2020, 45 articles with results of preterm born infants underwent MRS screening. Among those 45 articles, 17 articles contained both ^{1}H-MRS results and neurodevelopmental outcome and therefore met the inclusion criteria. Three additional articles were found after reviewing references of the initially included articles. Overall, 20 published articles examined brain metabolites using ^{1}H-MRS and performed neurodevelopmental assessments in preterm infants and ten of these studies (four high-quality and six moderate-quality studies) reported significant associations between ^{1}H-MRS metabolites and neurodevelopmental outcome (see PRISMA flowchart in Fig. 1). All characteristics of the studies (design, quality, number and GA of study population, age at MRI, age at follow-up, neurodevelopmental test, and association between ^{1}H-MRS metabolites and neurodevelopmental outcome) are summarized in Table 1. All included studies showed a total score of 10–13 points according to the McMaster critical review form for quantitative studies. Five of these studies were considered as high quality and 15 were moderate quality. After summarizing the included studies, it became apparent that the included studies were heterogeneous, having a diversity of study settings and approaches. To synthesize the methodological quality of the studies and to enable conclusions to be drawn, a narrative best-evidence synthesis was applied.

Overarching outcome and subheadings from the narrative synthesis are described as follows:

1. Magnetic resonance spectroscopic imaging (field strength, echo time (TE), and voxel dimensions).
2. Voxel localization (white matter (WM), gray matter (GM), and cerebellum).
3. ^{1}H-MRS metabolites (*N*-acetylaspartate (NAA), creatine (Cr), choline (Cho), myo-inositol (Ins), lactate (lac), and glutamate/glutamine (Glx)).
4. Neurodevelopmental outcome.

Magnetic resonance spectroscopic imaging

Field strength. Eight recent studies used a 3.0-Tesla scanner,[19–26] the other studies used 1.5 T equipment. ^{1}H-MRS screening protocol and results of included studies are given in Table 2.

Echo time. Among the studies included in the systematic review, Simões et al.[24] used a short TE (30 ms), whereas a long TE (272 ms) was reported in the study of Groenendaal et al.[27] Both short and long TEs were used in two studies.[15,26] Lac was utilized at TE ranging from 135 to 272 ms in four of the studies[27–30] and short TE (of 35 ms) was used in one study to assess Lac.[31]

Voxel dimensions. Single voxel spectroscopy uses one volume of interest. It is the most commonly used acquisition method that can be easily implemented and processed. Two-dimensional (2D) MRS imaging is an extension of the single voxel to a slice, in which the voxels are phase-encoded. The 2D can be further extended to 3D to simultaneous coverage in the z/Feet-Head/caudal–cranial direction. The more extensive coverage of 2D and 3D acquisitions provides more informations, but requires more scanning time. ^{1}H-MRS screening applied single voxel spectroscopy in 17 studies and only Hyodo et al.[22] used 2D and Chau et al.[32] and Xu et al.[33] used 3D spectroscopy.

Voxel localization

White matter: Four studies selected a region of interest in the WM for the prediction of NDI.[19,24,28,29] Of these studies, two were of high quality[19,24] and two were of moderate quality;[28,29] all showed a significant association with neurodevelopmental outcome (motor, cognitive, disabilities, IQ, and memory function). One of the four studies showed an association between low total Cho (tCho)/total creatine (tCr) ratio in preterm born infants and long-term adverse IQ, memory, and attention performance.[19] Two of the studies specifically studied metabolites in the periventricular WM, which is known to be the most vulnerable area as it includes a high proportion of oligodendrocytes.[28,29] In most of the studies, ^{1}H-MRS was performed in both WM and GM with the frontal lobe being the most common region to place the VOI.[20–23,31,33,34] Three of the included studies demonstrated an association between ^{1}H-MRS and developmental outcomes both at 18 months CA and at 3–4 years of age (cognition, language, and motor disabilities).[21,22,31]

Gray matter: Three of the studies with moderate quality performed ^{1}H-MRS only in the GM.[25,35,36] Hippocampus, basal ganglia, and thalamus were the areas most frequently studied. No association between ^{1}H-MRS metabolites and neurodevelopmental outcome was reported in these studies. The VOI was most often placed in the basal ganglia and motor deficit was the prominent NDI in these studies.[22,27,31,32]

The combination of ^{1}H-MRS in both GM and WM was reported in 12 of 20 studies.[20–23,26,27,31–33,36–38] Adverse neurodevelopmental outcomes related to ^{1}H-MRS metabolites were reported in five studies, one study of high quality[21] and four studies of moderate quality.[22,27,31,32] Among these five studies, two studies[21,27] reported a significant association between metabolites only in WM areas and adverse motor and cognitive outcome, whereas two studies[22,31] found significant associations with motor impairment in only GM located metabolites. One study[32] reported the association between metabolites in both WM and GM voxels and NDI (adverse cognitive outcome in WM and GM, and adverse motor outcome in WM).

Cerebellum: Only one study of high quality evaluated the cerebellum for ^{1}H-MRS and reported a significant association between cerebellar metabolites and low cognitive scores at 2 years of age.[26]

^{1}H-MRS metabolites. Main metabolites and factors affecting the alterations in concentrations will be discussed in detail for each metabolite.

N-*acetylaspartate*. NAA, a metabolite present in neurons, is synthesized in the mitochondria and decreases after injury as a result of neuronal integrity loss, but is also present in immature oligodendrocytes.[39,40]

All of the 20 studies evaluated NAA as a metabolite, given as concentration or as the ratio of various metabolites. NAA/Cho ratio was the most commonly used metabolite ratio in WM alone or together with GM areas predicting motor, language, and cognitive outcome,[21,22,26–28,32] and NAA/Cr was the second most commonly used metabolite ratio and was predictive of motor, cognitive, and language scores as well as memory and attention as a long-term outcome measure in the study by Cheong and co-workers.[19,20,24,28,31] Only one study of high quality demonstrated the predictive value of NAA/Ins ratio with the Bayley Scales of Infant Development Third Edition (BSID-III) at 18–22 months CA.[21] NAA concentrations were utilized in two studies (one study of high quality[23] and one study of moderate quality[20]), but no association was found with neurodevelopmental assessment.

Table 1. Clinical data of the included patients.

Author and year of publication	Design	Quality of study	*n*	Control (n)	GA (w)	Age at ^{1}H-MRS scan	Age at follow-up	Neurodevelopmental test	Association between ^{1}H-MRS and neurodevelopmental outcome
Augustine 2008	R	Moderate	36	N/A	28.4	37.9 w	18–24 mo	BSID-III	No significant association between MRS biomarkers and neurodevelopmental outcome
Akasaka 2016	P	Moderate	17	N/A	28	56 w	12 mo	DQ Enjoji Scale score DDST II	No significant association between MRS biomarkers and neurodevelopmental outcome
Bapat 2014	P	High	31	12 term	25.2	38.7 w	18–22 mo	BSID-III	NAA/Cho in SVZ, cortex, and NAA/Ins in SVZ significantly associated with mental and language outcomes
Chau 2013	P	Moderate	177	N/A	27.6	32.1 w 40.3 w	18 mo	BSID-III	NAA/Cho in basal nuclei significantly associated with adverse motor, cognitive, and language outcome NAA/Cho in WM significantly associated with adverse cognitive outcome
Cheong 2016	P	High	150	134 term	25.8	18 y	18 y	WASI IQ Test, Working Memory and Attention Test	High tNAA/tCr in WM correlated with working memory and shifting attention High tCho/tCr in WM associated with better IQ
Durlak 2016	P	Moderate	65	N/A	28	49 mo	3.5–4.5 y	DTVP Leiter Scale	Low Cho/Cr in frontoinsular GM significantly related with moderate/severe DD
Gasparovic 2018	P	Moderate	15	15 preterm 22 term	27	4 y 6 y	4 y 6 y	Full-scale IQ (FSIQ)	No significant association between MRS biomarkers and neurodevelopmental outcome
Groenendaal 1997	P	Moderate	19	N/A	26.7–41.3	1.6-10 w	18–24 mo	Griffiths Mental Development Scale	NAA/Cho in WM significantly lower in preterms with DD
Hart 2014	P	Moderate	67	N/A	31.2	40.1 w	19 mo	BSID-III The Amiel-Tison	Lac doublet in posterior PWM associated with lower fine motor scale
Hyodo 2017	P	Moderate	33	16 term	29.9	40 w	18 mo	Kyoto Scale DQ	NAA/Cho in thalamus significantly lower in preterms with mild DD than pretems with normal development at 18 mo
Kendall 2014	R	Moderate	43	N/A	26.9	40.4 w	12 mo	BSID-III	NAA/Cho in WM significantly associated with motor outcome Cho/Cr in WM significantly associated with both motor and cognitive outcome
Phillips 2011	P	High	16 (18–22 mo) 12 (3–4 y)	7 (18–22 mo) term 8 (3–4 y) term	27.5-28.1	18–22 mo 3–4 y	18–22 mo 3–4 y	BSID-III at 18–22 mo WPPSI (VIQ and PIQ) at 3–4 y	No significant correlation between MRS metabolites and neurodevelopmantal outcome
Podrebarac 2017	P	Moderate	14	163 preterm with no exposure to SSRI	26.9–29.1	32.9 w 41.4 w	18 mo	BSID-III	No correlation reported between MRS metabolites and neurodevelopmental outcomes
Rademaker 2006	P	Moderate	18	19 preterm with no steroid	27.9	8.4 y	8.4 y	15 Words Test, Wechsler Intelligence Scale for Children-Revised	No association between MRS biomarkers and IQ and memory

Table 1. continued

Author and year of publication	Design	Quality of study	*n*	Control (n)	GA (w)	Age at ^{1}H-MRS scan	Age at follow-up	Neurodevelopmental test	Association between ^{1}H-MRS and neurodevelopmental outcome
Roelants-Van Rijn 2004	P	Moderate	14	26 AGA preterm	30.1	32.9 w 41.3 w	24 mo	Griffiths' DQ	No association between MRS biomarkers and DQ in AGA and SGA preterm infants at age of 2 y
Simões 2017	P	High	26	26 term 22 AGA preterm	31.4	16.1 mo	22.5 mo	BSID-III	Low frontal lobe levels of NAA/Cr in 1-y-old preterm infants were significantly associated with poorer neurodevelopmental outcome at 2 y
Tanifuji 2017	P	Moderate	20	N/A	30	37–46 w 64–73 w	64–73 w	DQ Enjoji Scale score and DDST II	No significant association between MRS biomarkers and neurodevelopmental outcome
Taylor 2018	P	Moderate	45	25 term	28.5	10 d 12.8 w 4.2 y	4 y	WPPSI-III, CELF-pre2	No significant association between MRS biomarkers and neurodevelopmental outcome
Van Kooij 2012	P	High	56	N/A	28.4	41.5 w	24 mo	BSID-III	Cerebellar NAA/Cho significantly positive correlated with cognition outcome at 2 y
Xu 2011	P	Moderate	55	N/A	28.4	34.1 w	12 mo	BSID-II	No significant association between MRS biomarkers and neurodevelopmental outcome

^{1}*H-MRS* proton magnetic resonance spectroscopy, *BSID-III* Bayley Scales of Infant Development-III, *CELF-Pre-2* Clinical Evaluation of Language Fundamentals—Preschool, *Cho* total choline-containing compounds, *Cr* combined creatine and phosphocreatine, *Glx* combined glutamate and glutamine, *DD* developmental delay, *DDST II* Denver Developmental Screening Test II, *DTVP* Developmental Test of Visual Perception, *GM* gray matter, *Ins* myo-inositol, *Lac* lactate, *mo* month, *NAA* combined *N*-acetylaspartylglutamate and *N*-acetylaspartate, *PIQ* performance intelligence quotient, *PWM* parietal white matter, *SSRI* selective serotonin reuptake inhibitor, *SVZ* subventricular zone, *TE* echo time, *IQ* intelligence quotient, *VIQ* verbal intelligence quotient, *VLBW* very LBW, *WASI* Wechsler Abbreviated Scale of Intelligence, *WM* white matter, *WPPSI-III* Wechsler Preschool and Primary Scale of Intelligence-III, *y* year.

Table 2. Summary of ^{1}H-MRS and outcome.

Author and year of publication	VOI location	^{1}H-MRS imaging protocol	Predictor metabolites	Results
Augustine 2008	Thalamus Cortex	1.5-T, PRESS, TR:1000 ms, SVS TE: 144 ms, voxel size: 2.25 cm^3 Analysis technique: Functool, GE Healthcare	NAA/Cho, NAA/Cr Cho/Cr	NAA/Cho correlated with PMA in thalamus and basal ganglia Cho/Cr, NAA/Cr, and NAA/Cho not predictive of Bayley MDI and PDI scores at 18–24 mo
Akasaka 2016	Basal ganglia Thalamus Frontal lobe	1.5-T, PRESS, TR: 1500 ms, MVS TE: 35 ms, voxel size: 3.3 cm^3 Analysis technique: LC Model	NAA/Cho, NAA/Cr Cho/Cr Ins/Cho, Ins/Cr	NAA/Cho and NAA/Cr in FL + BG + Th significantly positive correlated with PMA Cho/Cr and Ins/Cr in FL + BG + Th significantly negavite correlated with PMA
Bapat 2014	Subventricular Zone Frontal lobe Hippocampus	3-T, PRESS, TR: 2000 ms, SVS TE: 35 ms, voxel size:1.8 cm^3 Analysis technique: LC Model	NAA/Cho, NAA/Ins	Significantly lower NAA/Cho in the SVZ and cortex in ELBW compared to term infants NAA/Cho in the SVZ and cortex, and NAA/Ins in SVZ significantly associated with Bayley mental and language scores at 18 to 22 mo
Chau 2013	Basal nuclei Superior WM	1.5-T, PRESS TR: 1500 ms, 3D TE: 144 ms, voxel size: 2.5 cm^3 Analysis technique: Siemens	NAA/Cho	In basal nuclei, NAA/Cho significantly lower in preterm infants having adverse motor cognitive and language outcomes In WM NAA/Cho increased significantly less rapidly only with adverse cognitive outcomes
Cheong 2016	Posterior cingulate cortex	3-T, PRESS, TR:3000 ms, SVS TE: 135 ms, voxel size: 3 cm^3 Analysis technique: LC Model	NAA/Cr Cho/Cr	tNAA/tCr and tCho/tCr significantly lower in preterms compared to terms Higher tCho/tCr in GM significantly correlated with better IQ in preterms Higher tNAA/tCr ratios correlated with better scores in working memory and shifting attention in both preterm and term infants
Durlak 2016	Frontoinsular GM Basal nuclei Frontal WM	1.5-T, PRESS, TR: 1500 ms, SVS TE: 35 ms, voxel size: 6 cm^3 Analysis technique: spectroscopy analysis by GE-SAGE	NAA/Cr, Cho/Cr, Ins/Cr, Lip/Cr, Lac/Cr	No difference in NAA/Cr, Cho/Cr and Ins/Cr in frontal WM, basal ganglia, and right frontoinsular higher In left frontoinsular cortex Cho/Cr significantly lower in preterms with moderate/severe DD
Gasparovic 2018	Frontal WM Anterior cingulate gyrus	3-T, PRESS, TR: 1500 ms, SVS TE: 40 ms, voxel size: 3.75 cm^3 Analysis technique: LC Model	NAA, Cr, Cho, Ins, glutamate, concentration	No relationships between ESA, placebo and term groups, and any metabolite levels within 4 and 6 years
Groenendaal 1997	WM, deep GM	1.5-T, PRESS, TR: 2000 ms, SVS TE: 272 ms, voxel size: N/A Analysis technique: Philips	NAA/Cho Lac/NAA	Decreased NAA/Cho in WM significantly related with abnormal motor outcome Absence or presence of Lac not predictive for adverse outcome
Hart 2014	Anterior PWM Posterior PWM	1.5-T, PRESS, TR: 1600 ms, SVS TE: 135 ms, voxel size: 2.25 cm^3 Analysis technique: Philips	NAA/Cho, Cho/NAA NAA/Cr, Cr/NAA NAA/Lac, Lac/NAA Cho/Cr, Lac/Cr, Lac/Cho	Fine motor scores significantly lower in preterms with a visible lactate doublet in the posterior white matter
Hyodo 2017	Frontal WM Thalamus	3-T, PRESS, TR: 1700 ms, 2D TE: 135 ms, voxel size: 1.5 cm^3 Analysis technique: Siemens	NAA/Cho, NAA/Cr Cho/Cr	At 18 months corrected age, preterm infants with a mild developmental delay showed significantly lower NAA/Cho ratios in the thalamus than preterm infants with normal development
Kendall 2014	Posterior periventricular WM	1.5-T, PRESS, TR: 6030 ms, SVS TE: 89 and 200 ms Voxel size: 2 cm^3 Analysis technique: jMRUI	NAA/Cho, NAA/Cr Cho/Cr Lac/NAA, Lac/Cr, Lac/Cho	NAA/Cho significantly positive correlated with gross and composite motor scores Cho/Cr significantly negative correlated with the composite cognitive score and all motor scores
Phillips 2011	Left frontal WM Anterior cingulate gyrus	3-T, PRESS, TR: 1500 ms, SVS TE: 40 ms, voxel size: 3.7 cm^3 Analysis technique: LC Model	NAA/Cho, NAA/Cr Cho/Cr Ins/Cr, Glx/Cr NAA, Cho, Cr, Ins, Glx concentrations	At 18–22 mo, term-born children scored significantly higher than VLBW children on the expressive language subscale of the BSID-III At 3–4 y, term-born children scored significantly higher on the VIQ from the WPPSI-III No correlation between MRS metabolites and neurodevelopmantal outcome at either age

Table 2. continued

Author and year of publication	VOI location	^{1}H-MRS imaging protocol	Predictor metabolites	Results
Podrebarac 2017	Centrum semiovale Basal ganglia	1.5-T, PRESS, TR: 1500 ms, SVS TE: 144 ms, voxel size: 2.5 cm^3 Analysis technique: Siemens	NAA/Cho Lac/Cho	Antenatal-SSRI exposure significantly associated with lower NAA/Cho, higher Lac/Cho in calcarine region relative to non-exposed No statistically significant differences between antenatally exposed to SSRIs on cognitive, language, motor scores compared to non-exposed
Rademaker 2006	Hipocampus	1.5-T, PRESS, TR: N/A, SVS TE: 144 ms, voxel size: 3.75 cm^3 Analysis technique: MRUI-Matlab	NAA/(Cho + Cr)	No significant difference in NAA/(Cho + Cr) between hydrocortisone and non-treated group No association between NAA/(Cho_Cr) and 15-word recall for both groups, nor for the treated group or for the non-treated group No association between NAA/(Cho_Cr) and IQ
Roelants-Van Rijn 2004	PVWM Basal ganglia	1.5-T, PRESS, TR: 2000 ms, SVS TE: 31 and 144 ms, voxel size: 4 cm^3 Analysis technique: MRUI-Matlab	NAA/Cho, Lac/Cho Glx/Cho, Ins/Cho	No significant differences in DQ between AGA and SGA at age of 2 y NAA/Cho, Lac/Cho, Ins/Cho, and Glx/Cho not significantly different between the SGA and AGA preterm groups
Simões 2017	Frontal lobe	3-T, PRESS, TR: 2000 ms, SVS TE: 30 ms, voxel size: N/A Analysis technique: LC Model	NAA/Cho, NAA/Cr Cho/Cr Ins/Cr, Ins/Cho Glx/Cho	NAA/Cr significantly lower in IUGR preterms than AGA preterms, increased in AGA preterms compared to terms Ins/Cr significantly increased in IUGR preterms compared to AGA preterms Low NAA/Cr in preterm infants were significantly associated with poorer BSID-III at 2 y
Tanufiji 2017	Basal ganglia	3-T, PRESS (MEGA-PRESS for GABA), TR:1500 ms, SVS TE: 68 ms, voxel size: 3.4 cm^3 Analysis technique: LC Model	NAA/Cho, NAA/Cr Cho/Cr, Ins/Cho, Ins/Cr, Glx/Cr, Glx/Cho, GABA/Cr, GABA/Cho	GABA/Cr, Cho/Cr, Ins/Cr, and Ins/Cho significantly decreased and NAA/Cr, Glx/Cr, NAA/Cho, and Glx/Cho significantly increased in 64–73 w No significant association between metabolites and DDST II
Taylor 2018	Basal ganglia Thalamus	1.5-T and 3-T, PRESS, TR: 1500 ms TE: 35 and 144 ms, SVS Voxel size: 5.16 and 8 ml Analysis technique: LC Model	NAA/Cho, NAA/Cr Cho/Cr Ins/Cr, Ins/Cho	There were also no differences in the metabolite ratios between preterm and term infants at 4 y No significant effects of metabolites on cognitive outcome each group separately or the two groups combined
Van Kooij 2012	Cerebellum	3-T, PRESS, TR: 2000 ms, SVS TE: 35 and 144 ms, voxel size: 1 cm^3 Analysis technique: Philips	NAA/Cho Lac/Cho, Lac/NAA	Significant positive relation between GA and NAA/Cho, Lac/Cho, and Lac/NAA Cerebellar volume/PMA and cerebellar NAA/Cho ratio acquired with TE 144 ms showed a significantly positive relation with cognition
Xu 2011	Basal ganglia Thalamus Frontal WM Parietal WM Calcarine GM	1.5-T, PRESS/3D, TR:1500 ms, multivoxel 3D TE: 35 ms, voxel size: 1 cm^3 Analysis technique: In-house developed software	NAA/Cho, Lac/Cho, Lac/NAA	NAA/Cho increased significantly with age for all regions Cortical spinal tracts had highest NAA/Cho and temporal visual association tract had the lowest NAA/Cho No association between metabolites and neurodevelopment assessment

^{1}*H-MRS* proton magnetic resonance spectroscopy, *AGA* appropiate for gestational age, *BG* basal ganglia, *BSID-III* Bayley Scales of Infant Development-III, *CA* corrected age, *Cho* total choline-containing compounds, *Cr* combined creatine and phosphocreatine, *DDST II* Denver Developmental Screening Test II, *DTVP* Developmental test of Visual Perception, *ELBW* extremely low birth weight, *ESA* erythropoiesis-stimulating agents, *FL* frontal lobe, *Glx* combined glutamate and glutamine, *GM* gray matter, *Ins* myo-inositol, *IUGR* intrauterine growth retardation, *Lac* lactate, *mo* month, *MRS* magnetic resonance spectroscopy, *MVS* multivoxel spectroscopy, *N/A* not avaliable, *NAA* combined *N*-acetylaspartylglutamate and *N*-acetylaspartate, *PMA* postmenstrual age, *PRESS* Point-Resolved Spectroscopy, *PVWM* periventricular white matter, *PWM* parietal white matter, *SSRI* selective serotonin reuptake inhibitor, *SVS* single voxel spectroscopy, *SVZ* subventricular zone, *T* Tesla, *TE* echo time, *TEA* term-equivalent age, *TR* repetition time, *IQ* intelligence quotient, *VLBW* very low birth weight, *w* week, *VIQ* verbal intelligence quotient, *WM* white matter, *WMI* white matter injury, *WPPSI-III* Wechsler Preschool and Primary Scale of Intelligence-III, *y* year, *2D* two dimensional, *3D* three dimensional.

Table 3. ^{1}H-MRS details of the included studies.

Age at MRI	Author and year of publication	VOI Location	Predictive metabolites	Age at follow-up	Adverse Neurodevelopmental outcome
30–34 weeks	Chau 2013	WM + GM	NAA/Cho	18 mo	Yes (motor, cognitive, and language)
	Podrebarac 2017	WM + GM	NAA/Cho, Lac/Cho	18 mo	No
	Roelants-Van Rijn 2004	WM + GM	NAA/Cho, Lac/Cho, Glx/Cho, Ins/Cho	24 mo	No
	Taylor 2018	GM	NAA/Cho,NAA/Cr, Cho/Cr, Ins/Cr, Ins/Cho	4 y	No
	Xu 2011	WM + GM	NAA/Cho, Lac/Cho,Lac/NAA	12 mo	No
37–44 weeks	Augustine 2008	WM + GM	NAA/Cho, NAA/Cr, Cho/Cr	18–24 mo	No
	Akasaka 2016	WM + GM	NAA/Cho, NAA/Cr, Cho/Cr, Ins/Cho, Ins/Cr	12 mo	No
	Bapat 2014	WM + GM	NAA/Cho, NAA/Ins	18–22 mo	Yes (mental and language)
	Chau 2013	WM + GM	NAA/Cho	18 mo	Yes (motor, cognitive, and language)
	Groenendaal 1997	WM + GM	NAA/Cho	18–24 mo	Yes (motor)
	Hart 2014	WM	NAA/Cho, NAA/Cr, lactate	18 mo	Yes (only Lac doublet in PPWM and fine motor)
	Hyodo 2017	WM + GM	NAA/Cho, NAA/Cr	18 mo	Yes (mild DD)
	Kendall 2014	WM	NAA/Cr, Cho/Cr	12 mo	Yes (motor and cognitive)
	Podrebarac 2017	WM + GM	NAA/Cho, Lac/Cho	18 mo	No
	Roelants-Van Rijn 2004	WM + GM	NAA/Cho, Lac/Cho, Glx/Cho, Ins/Cho	24 mo	No
	Tanifuji 2017	GM	NAA/Cho, NAA/Cr, Cho/Cr, Ins/Cho, Ins/Cr, Glx/Cr, Glx/Cho, GABA/Cr, GABA/Cho	12–16 mo	No
	Taylor 2018	GM	NAA/Cho, NAA/Cr, Cho/Cr, Ins/Cr, Ins/Cho	4 y	No
	Van Kooij 2012	Cerebellum	NAA/Cho	24 mo	Yes (cognition)
1–2 years	Akasaka 2016	WM + GM	NAA/Cho, NAA/Cr, Cho/Cr, Ins/Cho, Ins/Cr	12 mo	No
	Phillips 2011	WM + GM	NAA/Cho, Cho/Cr	18–22 mo 3–4 y	No
	Simoes 2017	WM	NAA/Cr	22.5 mo	Yes (cognitive, language, motor)
	Tanifuji 2017	GM	NAA/Cho, NAA/Cr, Cho/Cr, Ins/Cho, Ins/Cr, Glx/Cr, Glx/Cho, GABA/Cr, GABA/Cho	12–16 mo	No
4–6 years	Durlak 2016	WM + GM	Ins/Cr, Cho/Cr	3.5–4.5 y	Yes (mild, moderate/severe DD)
	Gasparovic 2018	WM + GM	NAA, Cr, Cho, Ins, glutamate, concentration	4 y 6 y	No
	Phillips 2011	WM + GM	NAA/Cho, Cho/Cr	18–22 mo 3–4 y	No
	Taylor 2018	GM	NAA/Cho, NAA/Cr, Cho/Cr, Ins/Cr, Ins/Cho	4 y	No
8 years	Rademaker 2006	GM	NAA/Cho, Lac/Cho, Glx/Cho, Ins/Cho	8.4 y	No
18 years	Cheong 2016	WM	NAA/Cr, Cho/Cr	18 y	Yes (IQ, working memory, shifting attention)

Cho total choline-containing compounds, *Cr* combined creatine and phosphocreatine, *Glx* combined glutamate and glutamine, *GM* gray matter, *Ins* myo-inositol, *Lac* lactate, *mo* month, *NAA* combined *N*-acetylaspartylglutamate and *N*-acetylaspartate, *PIQ* performance intelligence quotient, *IQ* intelligence quotient, *WM* white matter, *y* year.

The relationship between metabolite ratios and neurodevelopmental outcome at different age of MRS scan is given in Table 3.

Creatine. Cr is the primary supply for cellular energy metabolism. It is synthesized in the kidneys and liver and is then carried to the brain to maintain adenosine triphosphate in neurons.[41] Cr peak consists of free Cr and phosphocreatine.[40] Cr is generally utilized as a reference metabolite in ratios since its levels remain constant after the first year of life.[42] Fourteen of 20 included studies used Cr in peak ratios with several metabolites. As a predictor for the neurodevelopmental outcome, NAA/Cr was the most common metabolite ratio in the studies in both WM and GM regions at 18–22 months CA as well as at 4 and 18 years of age.[19,22,24,28,31] One study of high quality and two studies of moderate quality reported an association between Cho/Cr metabolite ratio and motor, expressive language, and memory scores.[19,29,31] Cr concentrations were reported in two studies without any evident relation with outcome.[20,23]

Choline. Cho or tCho is used to address the signals of Cho-containing compounds such as free Cho, phosphocholine (PC), and glycerophosphocholine (GPC), which resonate at almost the same frequency. PC and GPC are found in phospholipids (phosphatidyl Cho and sphingomyelin), which are the main components of cell membranes.[43] Cho is therefore accepted as an indication of structural integrity. Cho is also a precursor of acetylcholine, which is the main neurotransmitter in the brainstem responsible for signaling pathways.[44] Cho levels increased in case of cellular proliferation, membrane turnover, myelination, or inflammation[45–49] and decreased with age as the rapid brain growth in the neonatal period decelerates later in childhood.[33]

Overall, 20 studies involved the Cho peak or concentration to evaluate the adverse developmental outcome. Eight of the studies studied Cho in ratios of either NAA or Cr and reported an association with NDI.[19,21,22,26,27,29,31,32] NAA/Cho ratio in frontal WM and deep GM nuclei was the most common predictor of NDI in one study of high quality[21] and four studies of moderate quality[22,27,31,32] and Cho/Cr ratio was the metabolite ratio to predict adverse developmental outcome including both motor and cognitive problems at childhood and adolescence period.[19,31] Low Cho/Cr level was significantly associated with motor impairments in childhood period[31] and higher tCho/tCr in GM was significantly correlated with higher IQ in preterm born adolescents.[19]

Myo-inositol. Ins is the precursor metabolite of phosphatidylinositol, which is essential for signal transduction, especially in WM.[50] It has a crucial role in the regulation of extracellular osmolality[51,52] and is accepted as a marker of gliosis in the brain.[53] It increases in the early stage of hypoxia–ischemia and has a decreasing trend in the perinatal period.[54] Ins was evaluated in 9 of 20 included studies[20,21,23–26,31,36,37] and an association between metabolites and NDI was noted in two studies of high quality[21,24] and one study of moderate quality.[31] Among these studies, one study of high quality reported NAA/Ins ratio in the WM was significantly associated with BSID-III mental and cognitive scores at 18–22 months CA.[21]

Lactate. Lac is accepted as hypoxic and/or ischemic marker. At a TE of 144 ms, Lac is fully inverted and a TE of 288 ms allows Lac to be seen in a completely upright position and reduces the amount of lipid contamination.[55] Elevated Lac is indicative of brain parenchyma ischemia after underlying nonoxidative glucose consumption. Several studies of term neonates with encephalopathy demonstrated that an increase in Lac concentration and a decrease in NAA concentration is correlated with NDI.[56–62] A meta-analysis about neonatal encephalopathy in (near) term infants reported that Lac/NAA ratio in deep GM is the most precise biomarker currently available for prediction of adverse neurodevelopmental outcome.[63] Elevation of Lac in preterm born infants might be interpreted as normal because of usual alterations in metabolism in the preterm brain.[2,64–66] Lac was evaluated in 8 of 20 included studies,[15,26–31,33] and among these eight studies, five studies reported an association between metabolites and NDI, and only study of Hart et al.[28] with moderate quality identified high Lac doublet to be significantly associated with fine motor scores of BSTID-III.

Glutamate/glutamine. Glutamate, the major component of the Glx peak including γ-aminobutyric acid, glutamine, and glutamate, is the most abundant excitatory neurotransmitter in all brain regions. Increased glutamate concentration after hypoxia causes toxicity to neurons, which ends up in cell injury and/or death.[67] Five of the included studies used Glx either in metabolite ratios or as Glx concentrations.[15,20,23–25] No association was reported between Glx and neurodevelopmental outcome in two studies of high quality and three studies of moderate quality.

Neurodevelopmental outcome. The neurodevelopmental assessment was conducted with the BSID-III in ten of the included studies,[21,23,24,26,28–30,32,33,38] and among these studies, three studies of high quality[21,24,26] and three studies with moderate quality[28,29,32] demonstrated adverse neurodevelopmental outcome relative to neurometabolites. Two studies estimated NDI using other tests. The Wechsler Preschool and Primary Scale of Intelligence-III test was used as a developmental test tool at 3–4 years of age in the study of Phillips et al.[23] and Cheong et al.[19] performed a two-subtest version of the Wechsler Abbreviated Scale of Intelligence test at 18 years. Motor disability was the main reported outcome in six studies[22,24,27,29,31,32] and it was mostly associated with NAA/Cho ratios in WM at 18–24 months CA in one study with high quality[24] and two studies with moderate quality.[22,27] Only one study with moderate evidence assessed developmental delay at 3.5–4.5 years reporting lower Cho/Cr ratios in both WM and GM.[31] NAA/Cho ratios in WM were associated with low cognitive scores at 18–24 months CA in three of the studies.[24,26,32] Low NAA/Cho ratios in both WM and GM were significantly associated with lower language score in two studies in preterm born infants assessed at 18–24 months CA in two studies of high quality.[21,24] Only Cheong et al.[19] reported that low Cho/Cr ratios correlated with lower IQ in preterm born adolescents.

DISCUSSION

This systematic review included 20 studies investigating the predictive role of ^{1}H-MRS metabolites in neurodevelopmental outcome in infants (12–24 months), young children (2–6 years), and older children (6–18 years) in infants born preterm. Ten of the studies reported the association between ^{1}H-MRS metabolites and NDI.

Conventional MRI is not able to define changes in cellular biochemical composition and structure that can be available with ^{1}H-MRS. The number and quantitation of detectable metabolites depend on the pulse sequence and its parameters, besides spectral resolution and signal-to-noise ratio (SNR).[68,69] Metabolites obtained from ^{1}H-MRS are reported most frequently as either peak–area ratios or absolute concentrations. Measurements of absolute concentrations of metabolites have been performed by using external or internal standards, although both methods have some disadvantages. Using external standards is inconvenient and time-consuming,[70,71] and use of water as the internal standard has the disadvantage of the assumption that brain water content is constant, which is not true in different brain diseases such as post hypoxia–ischemia. During brain development, concentrations of several neurochemicals change, resulting in various alterations that occur during the first 2 years of life.[15,16,72] The maturational pattern of preterm infants is different compared to term infants. In preterm infants, NAA, Cr, glutamate increase, and Ins and Lac decrease towards TEA.[73]

Most of the tissue abnormalities seen by MRI in preterm infants are observed in the WM, including small punctate or cystic lesions, diffuse excessive high signal intensity, impaired myelin maturation, parenchymal tissue loss, and corpus callosum abnormalities.[74–76] Disrupted maturation and chronic myelination disturbances are accepted as the main underlying pathologies[77] and CP is the most common cause of long-term NDI in children with severe WM injury.[78–80] Since the largest number of developing oligodendrocytes are localized in the posterior periventricular white matter, this area is one of the most vulnerable regions in the preterm brain.[81]

GM is composed of the cerebral cortex and deep central nuclei (basal ganglia, thalamus), which provide interconnection between cerebral cortex and several other brain areas. Preterm infants with GM damage are at risk for long-term neurocognitive impairments via direct or secondary injury to sensory and motor axons.[2] Some

studies revealed that reduced cortical and deep GM volume in preterm born infants had an association with moderate-to-severe NDI at 12 months CA compared to term infants.[37,82] Additionally, cognitive disabilities including working memory and IQ were found to be correlated with GM damage.[83–87] Any WM injury is also assumed to disturb the development of GM affecting motor, cognitive, and intellectual outcomes.[88–91] The cerebellum provides interconnection with the cerebral hemispheres and processes in higher functioning, such as motor functions, as well as learning, memory, cognitive, and behavioral functions.[92,93] Cerebellar hemorrhage is a serious and not well-recognized complication in preterm born infants related with high mortality and NDI.[94–97]

Our review demonstrates that in preterm infants, ^{1}H-MRS performed in WM areas at TEA is associated with neurodevelopmental outcome at 18–24 months CA. Motor disability at 12–24 months CA is the most commonly reported adverse finding among all other evaluated parameters, and low cognitive score was the second common outcome performed at 18–24 months CA. Most studies evaluated ^{1}H-MRS at TEA and a few studies did a scan at a later age including four studies at 4–6 years of age,[20,23,31,36] one study at 8 years of age,[35] and 1 study at 18 years of age.[19] Several studies had repeated MRI scans at different time points[20,22,23,25,26,30,32,36] and two studies (one study of high and one study of moderate quality) performed more than one neurodevelopmental assessment at different time period.[20,23] Only one study of moderate quality found a relation between an early ^{1}H-MRS age (30–34 weeks) with an adverse motor and cognitive outcome.[32] The region with the highest association between brain metabolites and neurodevelopmental outcome cannot be identified based on data from the present review. More studies measuring various brain areas are necessary to clarify this. There are limited data assessing other cognitive, behavioral, and language scores at a later age so that further studies are needed to evaluate long-term neurodevelopmental outcome.

Studies included in this systematic review were composed of variable populations involving both a small number (range 12–43) and a large number of infants (range 65–177) born at or below 32 gestational weeks. The number of infants that participated in the majority of the studies was <30. More than half of the studies had a control group consisting of term babies to compare with the preterm infants. Only one study of moderate quality reported sensitivity and specificity to define predictive value of metabolites for neurodevelopmental outcome.[29] In addition, only two studies reported the relation between IUGR in preterm infants and ^{1}H-MRS metabolic ratios.[15,24] Therefore, no definite conclusions on the effects of IUGR in preterm infants can be made.

Detection of in vivo biochemical data provides not only a perspective to the metabolic assessment that enlighten brain development, but also may enable a better insight to alterations of metabolites in case of abnormal processes.[13] Several metabolites were measured to predict neurodevelopmental outcome, however, mostly restricted to the first 2 years of life with limited data for later ages. NAA/Cho ratio has the highest predictive value for motor impairments in infants, but still there is a lack of data to predict cognitive impairments because outcome reports of school-aged children were limited. The use of NAA as a biomarker for neurodevelopment carries a high potential given the almost exclusive presence of NAA in neurons and immature oligodendrocytes, cells that have a particular vulnerability in the preterm brain.

Concerning field strength, the older studies have been performed in using a 1.5 T system, the newer studies used 3.0 T equipment, which may improve the identification of metabolites. Studies performed with 3.0 T report tissue levels for metabolites such as Ins, Glx, and gamma-aminobutyric acid (GABA) as quantification of these metabolites is more reliable at 3.0 T because of its increased SNR. Recently, the first study of human infants in a 7 T field was published, which demonstrated increased chemical shift dispersion and less overlap between the different metabolite peaks, which benefits the detection of the overlapping signals of, e.g., glutamate, glutamine, and GABA.[98] For the major signals in (ischemic) brain, such as NAA, Cho, and Lac, the use of 1.5 T or 3.0 T ^{1}H-MRS did not result in different clinical decision-making.[99]

TE is important in the identification of metabolites. ^{1}H-MRS at short TE enables the quantification of a comprehensive biochemical profile including several brain metabolites.[100] More compounds can be detected using shorter TEs (25–35 ms) compared to longer TEs (144 or 288 ms). The disadvantage of the short TE might be the presence of broad signal from high-molecular-weight macromolecules and lipids,[101] which have a relatively short T2. Longer TEs are mainly preferred to discriminate Lac from lipids and overlapping multiplets.[55,102]

There are several limitations of the data used in our review. ^{1}H-MRS techniques varied among the studies including different TEs, magnetic field strengths, preferred regions, and voxel size, which may affect tissue specificity and resulted in regions of interest that include nontargeted tissues. The larger chemical shift dispersion at higher field strengths provides a better discrimination of individual components, and SNR is improved. Ideally, raw data need to be processed using the same processing pipeline to compare ^{1}H-MRS results between different scanners. Recently, recommendations on single voxel MRS data processing have been summarized by the MRS experts' working group.[103] The methods used in the main steps, preprocessing, analysis, and quantification, should be reported along with study results to be able to compare results from different studies. The reporting of data processing methods for most studies in the present review did not meet the new standards as proposed by the MRS expert's working group.[104] Nevertheless, by comparing ratios of metabolite levels and not the absolute concentrations of metabolites, the effect of differences in processing methods on clinically relevant parameters is largely reduced.

There were insufficient data investigating metabolites like glutamate, taurine, lipids, and other macromolecules, which might be associated with neurodevelopment. Neurodevelopmental assessment in the included studies was evaluated with a variety of developmental tests and the results may be affected by several variables as linguistic, socio-economic, and cultural patterns.

Future developments

^{1}H-MRS plays a special role in the assessment of brain development, providing information on the molecular composition of brain tissue. Use of chemical shift imaging preferably at higher magnetic field strength to reduce scanning time will enable clinicians to obtain detailed information on metabolites of several brain structures, including periventricular WM, deep GM, and the cerebellum, which are all known to be at risk in preterm neonates.

With short scanning times, metabolic imaging can be added to the standard imaging protocol even in very preterm and vulnerable patients. Multicenter studies in preterm infants will be needed to compare findings obtained in different scanners, which will facilitate the use of ^{1}H-MRS as a surrogate end point in clinical trials.

CONCLUSION

^{1}H-MRS is a potential surrogate end point for neurodevelopment in preterm infants: NAA/Cho ratio in WM at term equivalent age is associated with motor outcome in preterm infants at 18–24 months' CA. NAA/Cho ratios in the WM were associated with cognitive scores and NAA/Cho ratios in the WM and GM were significantly related to language scores in preterm born infants assessed at 18–24 months' corrected age. There is a need for further studies evaluating the association between neonatal

^{1}H-MRS in preterm infants and long-term neurodevelopmental outcome.

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ADDITIONAL INFORMATION

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