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Brain GABA and glutamate levels across pain conditions: A systematic literature review and meta-analysis of 1H-MRS studies using the MRS-Q quality assessment tool

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ABSTRACT

Background: A proposed mechanism of chronic pain is dysregulation between the main inhibitory (GABA) and excitatory (glutamate) neurometabolites of the central nervous system. The level of these neurometabolites appears to differ in individual studies of people with pain compared to pain-free controls across different pain conditions. However, this has yet to be systematically investigated.

Aims: To establish whether GABA, glutamate, glutamine and Glx levels differ across pain conditions when compared to pain-free controls.

Methods: Five databases were searched. Studies were included if they investigated: 1) A pain condition compared to control. 2) Reported GABA, glutamate, glutamine or glutamate/glutamine level. 3) Used 1H-Magnetic Resonance Spectroscopy (Prospero Project ID CRD42018092170). Data extracted included neurometabolite level, pain diagnosis, and spectroscopy parameters. Meta-analyses were conducted to establish the difference in neurometabolite level between participants with pain and pain-free controls for different pain conditions. The MRS-Q was developed from existing clinical consensus to allow for the assessment of quality in the included studies.

Results: Thirty-five studies were included investigating combinations of migraine (n = 11), musculoskeletal pain (n = 8), chronic pain syndromes (n = 9) and miscellaneous pain (n = 10). Higher GABA levels were found in participants with migraine compared to controls (Hedge's G 0.499, 95%CI: 0.2 to 0.798). In contrast, GABA levels in musculoskeletal pain conditions (Hedge's G −0.189, 95%CI: 0.530 to 0.153) and chronic pain syndromes (Hedge's G 0.077, 95%CI: 1.612 to 1.459) did not differ from controls. Results for other brain neurometabolites revealed significantly higher levels for glutamate in participants with migraine and Glx in chronic pain syndromes compared to controls.

Conclusion: These results support the theory that underlying neurometabolite levels may be unique in different pain conditions and therefore representative of biomarkers for specific pain conditions.

1. Introduction

Two key neurometabolites implicated in the pathophysiology of pain are glutamate and gamma-aminobutyric acid (GABA). Glutamate is the principal excitatory neurometabolite in the central nervous system, and is involved in many metabolic pathways (Rae, 2014; Ramadan et al., 2013; Zhou and Danbolt, 2014). GABA is the most abundant inhibitory neurometabolite in the central nervous system (Enna and McCarson, 2006; Rae, 2014) and is considered an important regulator of the balance between excitation and inhibition in the brain (Petroff, 2002). Both glutamate and GABA are critical for many centrally regulated physiological functions, including pain processing and pain modulation.

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Dysfunctions in glutamate and GABA metabolism, resulting in too much or too little of the neurometabolite, have been implicated in clinical conditions, such as chronic pain (MacDermott, 2001; Zunhammer et al., 2016).

In-vivo quantification of these neurometabolites is possible through proton magnetic resonance spectroscopy (1H-MRS). 1H-MRS is a non-invasive neuroimaging technique that allows for the separation of neurometabolites based on their chemical structure. Separation of spectra is possible through observing the radiofrequency signal detected from hydrogen nuclei spins and their chemical environment when placed in a magnetic field (Puts and Edden, 2012). Accordingly, neurometabolites can be separated along an x-axis dependent on their chemical specific radiofrequency, otherwise known as their chemical shift. The strength of this signal is reflective of the level of neurometabolite. Whilst 1H-MRS has been helpful in quantification of many neurometabolites, the measurement of GABA and glutamate have their own specific challenges.

Quantification of GABA is problematic due to its low concentration (1–2 mM (Govindaraju et al., 2000; Petroff, 2002)), and spectral overlap with other more abundant neurometabolites such as creatine at 3 ppm (Puts and Edden, 2012). To resolve GABA, J-difference editing can be used to selectively edit the signal of interest. J-difference editing of GABA uses frequency selective 'editing' pulses, applied to the 1.9 ppm GABA signal, which in turn, selectively refocused the GABA signal at 3 ppm, but not the creatine signal. The most widely used sequence for measuring GABA is MEGA-PRESS (Mikkelsen et al., 2017; Mullins et al., 2014). Here, editing pulses are applied on-resonance (edit-ON) at 1.9 ppm in half the acquisition, and not in the other half (edit-OFF). The difference spectrum contains only those signals affected by the editing pulses, revealing a quantifiable GABA signal at 3 ppm (for a review, see Puts and Edden, 2012). However, various implementations of the sequence exist, utilizing different radio-frequency pulses and timings (Mikkelsen et al., 2017, 2019; Saleh et al., 2019). One limitation of J-difference editing is macromolecule contamination meaning that studies using this editing report GABA + rather than measures of GABA only. This can be overcome with symmetrical editing or measured macromolecule baselines which reflect a more refined measure of GABA (Edden et al., 2012; Mikkelsen et al., 2018a).

There is little consensus as to the best way to measure glutamate. Glutamate is present at higher concentrations (12 mM (Choi et al., 2006)) than GABA, however, difficulties separating it from glutamine (1–4 mM) and glutathione (2–3 mM (Rae, 2014)) have been highlighted. Whilst some studies estimate glutamate alone (Schubert et al., 2004), others choose to estimate Glx, the combined measure of glutamate and glutamine, although the signal also contains some glutathione. Glx is measured either from edited MRS (Sanaei Nezhad et al., 2017) or from short echo time PRESS (≈30 ms) (Gonzales de la Aleja et al., 2013). Other techniques specific to measuring glutamate (separating it from glutamine), including TE-averaging, also exist (Hurd et al., 2004).

Several 1H-MRS studies to date have demonstrated changes in GABA and glutamate in pain conditions compared to controls. However, the direction of concentration change is inconsistent across pain conditions. For example, Aguila et al. (2015) demonstrated an increase in GABA levels in individuals with migraine compared to controls. In contrast, GABA levels were decreased in people with fibromyalgia (Foerster et al., 2012) and chronic pelvic pain (Harper et al., 2018). Similarly, glutamate levels were higher in people with migraine compared with controls (Gonzales de la Aleja et al., 2013; Zielman et al., 2017), however, lower in people with low back pain (Gussew et al., 2011). The variability in these data suggest that there may be a unique neurometabolite signature for each pain condition. However, to-date these data have not been systematically appraised.

An alternative explanation for the variability of neurometabolite levels between pain conditions and MRS studies could be reflective of the quality of the magnetic resonance (MR) acquisition and analysis. This includes the 1H-MRS sequence utilised. For example Bridge et al. (2015) used an unedited sequence and demonstrated a 10% decrease in GABA level in individuals with migraine compared to controls (Bridge et al., 2015). Conversely, Aguila et al. (2015) used an edited sequence and demonstrated a significant increase in GABA level in people with migraine compared to controls. More importantly, it is well-established that reporting of acquisition parameters is important for allowing of interpretation and reproducing prior studies.

The role of different brain regions in pain processing have been extensively studied using a variety of imaging and in vitro methods in both humans and animals It is therefore possible that the level of neurometabolites differ between brain regions. Differences have been demonstrated in people with fibromyalgia (Foerster et al., 2012), where the same individuals demonstrated an increase in GABA level in the insula but not the anterior cingulate cortex (ACC). Alternative explanations for these differences include; variation in signal to noise ratio dependent on location of brain region being scanned, the composition of the voxel in terms of grey and white matter, and the distribution of GABA and glutamate receptors in that specific brain region. Advances in analysis techniques and the introduction of volume-based correction allows better understanding of these factors, however, they have not been uniformly applied across studies, and therefore their impact must be considered when synthesizing results from studies.

The primary aim of this review therefore was to determine whether GABA, glutamate, glutamine and Glx levels differ across pain conditions compared to pain-free controls. The secondary aim was to report on the quality of the MR data acquired in the literature in this field and then, to determine whether the quality of reporting, or brain region, influences brain neurometabolite levels. Assessing appropriate acquisition parameters necessitated us to develop the MRS-Q tool for systematic review of 1H-MRS acquisitions, as no such tool previously existed. The determinants are based on prior consensus.

2. Methods

2.1. Protocol registration

This review was conducted in adherence with the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement (PRISMA) (Moher et al., 2015) and was registered prospectively on Prospero (CRD42018092170).

2.2. Eligibility criteria

Studies were included if they used 1H-MRS of the brain to report measures of GABA, glutamate, glutamine or the combination measure of glutamate and glutamine i.e. Glx. Among these studies were Spectroscopic Imaging (MRSI) studies. Studies were required to have recruited human participants who had a pain condition that was compared with healthy pain-free controls. Included studies were of primary research design, such as cross-sectional, longitudinal, interventional or case series, and written or translated into English via Google Translate.

Studies were excluded if they used other forms of spectroscopy e.g. phosphorous MRS or examined other tissues, such as the spinal cord. Studies that investigated animals or conditions which were primarily psychological disorders without pain as a predominant feature (e.g. post-traumatic stress) were also excluded, as were literature reviews or conference proceedings.

2.3. Search methods for identification of studies

A comprehensive search strategy was derived and piloted with assistance from the University's librarian. The full search strategy and search terms are attached in Appendix 1. In brief we combined MeSH headings and key words for **magnetic resonance spectroscopy** (for example: magnetic resonance imag*, NMR- Spectroscopy, MEGA-PRESS) AND **neurometabolites** (for example: GABA or glutamate or glutamine or Glx, brain neurochemical*, metabolite*) AND **pain** (for example:

chronic pain (expanded), musculoskeletal pain).

OVID MEDLINE, EMBASE, WEB of SCIENCE, CINAHL and Pubmed were electronically searched without any restrictions to date, study design or language (inception to September 4, 2019). Reference lists of included studies and systematic reviews in this field were searched to ensure key studies had not been missed.

2.4. Study selection

A two stage approach was used to screen studies for inclusion (Furlan et al., 2009). In the first stage, two reviewing teams (AP) and (MA or AL or TR or JW) independently screened titles and abstracts to identify titles for full text retrieval. Where there was uncertainty, the full text was requested. In the second stage, two reviewers (AP) and (MA or AL or TR or JW) independently assessed the full text of all studies to determine their eligibility. Disagreements were discussed and resolved by a third independent reviewer (AL or TR). Reasons for exclusion were documented and duplicates were removed.

2.5. Data extraction

Data were extracted in duplicate by 2 reviewers (AP, JW) using a standardised form (Appendix 2). Authors were contacted for missing data or raw data when data was only presented graphically. When authors failed to respond, graphical results were extracted using on-screen callipers (Screen Callipers Version 4.0). Where non-parametric statistics were reported, means and SD were imputed using methods recommended in the Cochrane handbook (Higgins et al., 2011). Only primary analyses were extracted from the included studies. In the case of longitudinal or interventional studies, only baseline data were extracted. Data from different brain regions of the same individual was interpreted as being independent, and therefore extracted separately for each brain region (Aoki et al., 2012; Schur et al., 2016). A secondary meta-analysis (not shown) averaged across brain regions of the individual.

Data were extracted into 4 tables: 1) spectroscopy parameters, where data extracted included scanner make, acquisition parameters e.g. voxel size and location, TR, TE and post processing details such as software and fitting methods (see Appendix 2); 2) neurometabolite levels, where the primary outcome of interest (mean (SD)) of GABA, glutamate, glutamine or Glx levels for subjects with pain and control subjects; 3) participant characteristics, including age, sex, pain condition, excluded comorbidities, and 4) Bibliometric data, including authors, year of publication, country, funding sources and if prospectively registered (Appendix 2).

2.6. Quality metrics

2.6.1. AXIS

Two quality measures were used. Firstly, the modified Appraisal tool for Cross-sectional studies (AXIS) (Downes et al., 2016) was used to determine the methodological quality of the research design (Appendix 3). The modified AXIS was piloted on 1H-MRS studies prior to inception of the review (AL, AP, JW, NP). Two reviewers (AP, NP or JW) independently assessed the quality of each included paper. Disagreements were subsequently discussed and resolved by a third reviewer (AL).

2.6.2. MRS-Q

Secondly, the quality of the 1H-MRS acquisition method was assessed. Although two recent 1H-MRS white papers suggest that researchers use standardized acquisition and analysis metrics (Mullins et al., 2014; Wilson et al., 2019) to-date there are no published standardized tools to objectively evaluate the quality of spectroscopic acquisition. For assessment, this necessitated the development of a new quality appraisal tool (MRS-Q) for this review, based upon consensus papers and expert opinion on best-practice (Harris et al., 2017; Mikkelsen et al., 2017, 2019; Mullins et al., 2014; Wilson et al., 2019). The MRS-Q evaluates 12 and 13 parameters for unedited and edited studies respectively. The MRS-Q has three parts, Part 1 checks whether appropriate sequences and adequate parameters were used to accurately detect the neurometabolite of interest. The criteria in Part 1 include quality parameters that are considered fundamental to producing good quality spectra. Two of these parameters, *appropriate sequence and adequate parameters,* were also used to determine the quality of acquisition for the purpose of our sensitivity analysis. Part 2 evaluates whether sufficient quality checks were utilised such as reporting full width at half maximum (FWHM) and the visualisation of data. In Part 3, details of study design such as sample size calculations and post processing methods were appropriate and explicitly reported are evaluated. As such, this tool reports on both acquisition and the adequate reporting of this information (e.g. for allowing reproducibility of such studies). Each study was assessed independently by 2 reviewers (AP,NP). The cut-off points and rationale for each of the criteria are displayed in Table 1. Only studies that reported using adequate spectroscopy parameters for the neurometabolite of interest were considered high-quality and used in the sensitivity analysis (see Data synthesis: secondary aims).

2.7. Data synthesis and analysis

2.7.1. Primary aim

In line with the review's primary aim; to determine if brain neurometabolites are different across pain conditions, it was decided a priori to categorise studies into one of four pain categories for analysis. The categories were migraine, musculoskeletal pain, chronic pain syndromes or miscellaneous pain for studies that did not fit into the above categories. The migraine group was inclusive of any form of painful migraine or headache listed in the ICHD 3b. Musculoskeletal pain was defined as any condition diagnosed from a single anatomical site, and likely to be driven by a nociceptive input e.g. low back pain, knee osteoarthritis. Conversely, chronic pain syndromes were defined as any widespread chronic pain condition affecting multiple regions with a non-specific musculoskeletal diagnosis, that is predominately associated with central processing abnormalities (Arnold et al., 2016) e.g. fibromyalgia, complex regional pain syndrome, the remaining studies were considered as miscellaneous pain and encompassed any other non-musculoskeletal pain conditions such as abdominal pain, spinal cord injury with painful neuropathy. From here-on the group name will be used to refer to individuals who experience these particular conditions e.g. people with migraine (migraine).

Data was labelled according to brain region investigated. It was decided a priori that labelling would be regardless of hemisphere investigated, unless a single study contributed both a left and right data set. Nomenclature of brain region was simplified in terms of region, for example the dorsolateral prefrontal cortex was labelled and identified as the prefrontal cortex.

Meta-analysis was performed using Comprehensive Meta-analysis software (Borenstein et al., 2005) on studies pooled by neurometabolite, and sub-grouped by pain condition to allow comparison of neurometabolite level between pain conditions. Because the “miscellaneous” group were a heterogeneous category, these results in this group were not pooled in the meta-analysis. Standardised mean differences and 95% confidence intervals (Hedge's G) were used to compare the pain groups to the painfree controls to allow for data presented in different units (mmol, IU, ratios).

Results were analysed by neurometabolite and sub-grouped by pain type (migraine, musculoskeletal pain, chronic pain syndromes) regardless of brain region studied. Where multiple results were presented for the same neurometabolite preference was given to results of actual concentration or institutional units over ratios. Heterogeneity was assessed using the i^2 test and a random-effects model was implemented to compensate for variation in acquisition parameters, voxel location and the selective use of partial volume correction.

2.7.2. Secondary aims

To investigate the review's secondary aims, firstly summary measures

Table 1
Criteria and rationale of the MRS-Q tool[a].

	Criteria	Setting	Rationale
1. Parameters	Scanner Strength	**Edited for GABA:** Over 3T **Unedited:** 3T preferable	Scanning at 3T and above provides a higher signal-to-noise ratio, with increased spatial and temporal resolution. Reducing spectral overlap of Glu, Gln, and GABA (Di Costanzo et al., 2007; Wilson et al., 2019)
	Appropriate Sequence:	**Edited for GABA:** MEGA-PRESS, MEGA-semi LASER, other editing	To accurately quantify GABA a specific editing sequence must be implemented (Mullins et al., 2014). EDITINGSCHOOL [b], Expert opinion [c]
	***Used to determine quality**	**Unedited:** PRESS or vendor specific PRESS, semi-LASER or STEAM	Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
	Adequate Parameters:	**Edited for GABA:** Averages over 240	This number of averages are required due to the low amplitude of signal due to the typically low concentration of GABA and splitting due to coupling (Mikkelsen et al., 2018b) EDITINGSCHOOL [b]
	***Used to determine quality**		
		TE: GABA+ 68, GABA 80 (68 S)	*See Appropriate TE below
		Voxel Size ~27 ml	The voxel size required to quantify GABA as a compromise between localization and adequate signal to noise (Mullins et al., 2014)
		Unedited: 128 Av, $15 \times 15 \times 15 mm^3$ voxel, 3T; 64 Av, $20 \times 20 \times 20$ mm^3 voxel, 3T; 256 Av, $15 \times 15 \times 15$ mm^3 voxel 1.5T; 128 Av, $20 \times 20 \times 20$ mm^3 voxel, 1.5[33]	In order to produce adequate SNR, the number of averages need to be increased when using lower strength scanners, or smaller sized voxels (Wilson et al., 2019) Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
		MRSI: 3T, 16×16 matrix, voxel 15 mm^3 TR 1500,	
	Data Points	**Edited for GABA:** NA **Unedited:** 1024 complex data points from 2000Hz	– Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
	Appropriate TE	**Edited for GABA:** 68 ms or 80 ms	68 ms is optimal for GABA- due to complete inversion in the ON acquisition. (Rothman et al., 1993) 80 ms for macromolecule editing (Edden et al., 2012) Mullins et al., 2014 [e]. EDITINGSCHOOL [b]
		Unedited: 20/30 ms	Common clinical implementation agreed through consensus opinion (Wilson et al., 2019)
2. Quality Measures	Quality measure	Reported Shim or FWHM (Full Width Half Maximum)	Poor shimming leads to aberrant quantification. Linewidth is known to affect fitting and be an index of data quality (Wilson et al., 2019)
	Quality measure	Fit Error Calculation reported	While the format is less-important, fit error reports on the quality of the spectra and/or appropriate fitting methods. While fit-error cut offs are proposed (e.g. <20% CRLB for LC model analysis (Cavassila et al., 2001)) we did not stipulate specific cut offs here.
	Data visualisation	A visual display of at least one data set	Recommendations are that visual display of spectra (e.g. an example spectrum, all spectra) are reported in a figure (e.g. Zielman et al., 2017)
	Partial volume correction	Partial Volume Corrected- not just for grey matter	For water-referenced data, partial volume can substantially affect data quantification and could be a prominent driver of group differences. In addition, only correcting for grey matter is deemed inappropriate (Gasparovic et al., 2006; Harris et al., 2015; Mikkelsen et al., 2016; Porges et al., 2017) EDITINGSCHOOL [b]
	Scanner drift	Frequency Drift Reported	This has been shown to be of particular importance for edited MRS (Harris et al., 2014; Mikkelsen et al., 2017; Near et al., 2015)
3. Study Design	Power calculation	Report how sample size was determined	Allows demonstration of whether the study is adequately powered to detect between group difference- reducing the chance of type I and II error (Nayak, 2010)
	Frequency/phase corrected	Reported either frequency or phase correction	Frequency and phase correction prior to fitting is strongly recommended, and is key for edited MRS (Mullins et al., 2014; Wilson et al., 2019) EDITINGSCHOOL [b], Expert opinion [d]

[a] Template available for use from http://doi.org/10.17605/OSF.IO/8S7J9.
[b] EDITINGSCHOOL was held in December 2018 and focused on edited MRS. Expert instructors attended (http://www.gabamrs.com/blog/2018/10/12/editingschool-final-schedule).
[c] Co-author NP.
[d] Wilson et al., 2019 Consensus document agreed on by 49 MRS experts.
[e] Mullins et al., 2014 Consensus document written from a meeting of a number of specialist groups in 2011 in the UK documenting current "minimal best practice".

of spectroscopy parameters, and brain region, were tabulated (Tables 2–4) (University of York, 2009) Secondly, a sensitivity analysis was conducted, where the primary meta-analysis was repeated only on the studies that satisfied the use of minimal best practice in terms of appropriate sequence and adequate spectroscopy parameters as determined by the *appropriate sequence and adequate parameters* subsections of the MRS-Q (Table 1, rows 2, 3). To determine the impact of brain region on neurometabolite levels, studies were grouped broadly by brain region. Results were pooled where there were two or more homogeneous studies of a particular brain region within a pain condition. Finally, post-hoc meta-analyses were conducted, where data from multiple brain regions of the same individual were averaged and included within the analysis.

3. Results

3.1. Study selection

An initial search retrieved 8022 studies. Following removal of

Table 2
Study characteristics.

	Strength (scanner)/Sequence/(TR/TE)/Avs/Processing	Region/Voxel size (ml)	Neurometabolites	Participants: Number/Pain/%Female/Age/Duration	Controls: Number/%Female/Age
Aguila et al. (2015)	3T (GE)/MEGA-PRESS/(1800/68)/256/Gannet (GABA), Tarquin (Glu, Gln)	PCG/27 ml	GABA+, Glu, Gln	n = 19/Migraine (ICHD II), 1 attack a month+/70%F/33 IQR (28.2–47.2) yrs/180 months IQR (60–288)	n = 19/70%F/30 IQR (26.5–47.5) yrs
As-Sanie et al. (2016)	3T (GE)/PRESS/(3000/3*)/-/LC model	R ant Ins, R post Ins/ 12 ml	Glx *(NAA)*	**Group 1:** n = 15/Chronic pelvic pain with endometriosis/100%F/26.7 (6.6) yrs/5.5 (3.5–9.5) yrs **Group 2:** n = 6/Chronic pelvic pain without endometriosis/100%F/24.2 (4.6) yrs/3.75 (0.9)yrs	**Matched to Group 1:** n = 14/100%F/26.5 (6.6) yrs **Matched to Group 2:** n = 11/100%F/24.2 (4.0) yrs
Bathel et al. (2018)	3T (P)/MEGA-PRESS/(2000/68)/320/Gannet PRESS/(2000/30)/32/LC model	Occ/27 ml, R Thal/22.5 ml	GABA+ Glx *(NAA, Cr)*	n = 15/Migraine without aura (ICHD-II), >2 a month, pain-free 72 h prior and 48 h after scanning/80%F/35.2 (10.8) yrs/-	n = 15/80%F/33.4 (8.5) yrs
Bednarska et al. (2019)	3T (P)/MEGA-PRESS/(2000/68)/-/LC Model	L ant Ins, R ant Ins/24 ml	GABA+, Glx	n = 39/IBS/100%F/32.1 (18–57) yrs/-	n = 21/100%F/32.1 (20–55) yrs
Bigal et al. (2008)	4T (V)/3D-LASER**/(2000/72)**/-/-	Occ/13.5 ml	GABA	**Group 1**: n = 9/Migraine with aura/84.7%F/34.1 (95% CI 27.9–40.2) yrs/-**Group 2:** n = 10/Migraine without aura/84.7%F/41.4 (95% CI 30.5–52.2) yrs/-	n = 9/84.7%F/26.5 (95% CI 22.4–30.5) yrs
Bridge et al. (2015)	3T (Sie)/SPECIAL/(11.2/4.68)/128/LCModel	Occ/8 ml	GABA, Glu	n = 26/Migraine with aura (IHS)/100%F/33 (8) yrs/-	n = 13/100%F/30 (6) yrs
Chan et al. (2019)	3T (Sie)/MEGA-PRESS/(1500/68)/192/Gannet	Occ/15 ml	GABA, Glx	**Group 1**: n = 9/Migraine with aura/88.9%F/31 (95% CI 21–42) yrs/- **Group 2:** n = 7/Migraine without aura/71.4%F/31.1 (95% CI 20–49) yrs/-	n = 16/50%F/27.1 (95% CI 20–34) yrs
Di Pietro et al. (2018)	3T (P)/MEGA-PRESS/(2000/68)/200/jMRUI, AMARES (GABA), QUEST (Cr)	Thal/8 ml	GABA, *(Cr, Other neurometabolites)*	n = 20/Chronic orofacial neuropathic pain/65%F/50.1 SEM (4.4) yrs/>3yrs	n = 20/65%F/42.2 SEM (2.9) yrs
Fayed et al. (2010)	1.5T (GE)/Probe P/(2000/35)/128/LC model	L SM1, L and R Thal, L and R hippoc, PCG/8 ml	Glx, *(Cr, Cho, mI, NAA)*	n = 10/Fibromyalgia (ACR)/80%F/40 (6.2) yrs/1.6 (0.3) yrs	n = 10/80%F/37.8 (8.7) yrs
Fayed et al. (2012)	1.5T (GE)/Probe P/(2000/35)/128/LC model	PCG, pos Ins, L and R hippoc/8 ml	Glx, Glu, *(NAA, Cr, mI, tChol, tNAA)*	**Group 1:** n = 10/Fibromyalgia (ACR)/90%F/38.94 (5.56) yrs/2.13 (0.52) yrs **Group 2:** n = 10/Somatoform disorder (SCID-I)/80%F/43.93 (9.96) yrs/3.82 (0.76) yrs	n = 10/80%F/39.52 (11.32) yrs
Fayed et al. (2014)	1.5T (GE)/PRESS/(2000/35)/128/LC model	V PCG/8 ml	Glu, Glx *(Cr, mI, NAA, tCho)*	**Group 1:** Migraine/n = 33/63.6%F/45.2 yrs/- **Group 2**: Fibromyalgia/n = 54/90.7%F/45.1 yrs/-**Group 3**: Somatoform disorder/n = 10/80%F/44.1 yrs/-**Group 4**: Trigeminal cervical neuralgia/n = 8/75%F/46.9yrs/-	n = 193/60.6%F/53.2yrs
Feraco et al. (2011)	3T (GE)/PRESS/(2000/35)/128/LC model	Thal/5.8 ml VL PFC/9.2 ml	Glu, Glx *(tNAA, Cho, mI)*	n = 12/Fibromyalgia (ACR)/92%F/43.2 range (30–54) yrs/-	n = 12/92%F/41.3 range (28–56) yrs
Foerster et al. (2012)	3T (GE)/MEGA-PRESS/(1800/68)/256/In house-Matlab program with Gaussian curve fitting (GABA) PRESS/(2000/35)/32/LC model (NAA)	ACC, Occ, R ant and R post Ins/18 ml	GABA *(NAA)*	n = 16/Fibromyalgia (ACR), >1 yr/100%F/37.2 (12.8) yrs/>1yr	n = 17/100%F/36.1 (11.7) yrs/>1yr
Gerstner et al. (2012)	3T (GE)/PRESS/(3000/30)/-/LC model	L and R Ins/12 ml	Glu, Gln, Glx *(NAA, Cho)*	n = 11/Temporomandibular disorder-RDC-1- (ongoing pain >3 tender muscle sites ipsilateral to palpation pain)/91%F/25.8 (2.33) yrs/range 0.5–7 yrs	n = 11/91%F/24.8 (1.20) yrs

(*continued on next page*)

Table 2 (*continued*)

	Strength (scanner)/Sequence/(TR/TE)/Avs/Processing	Region/Voxel size (ml)	Neurometabolites	Participants: Number/Pain/%Female/Age/Duration	Controls: Number/%Female/Age
Gonzales de la Aleja et al. (2013)	3T (GE)/Probe P/(2000/28)/160/LCModel	Occ/27 ml, APC/8 ml	Glu, Gln, *(NAA, Cr, Cho)*	n = 28/Migraine or Migraine with aura (ICHD2) >2 attacks a month in the last 3 months, >3 yr history/100%/31.74 (8) yrs/>3yrs	n = 19/100%F/31.79 (4.5) yrs
Grachev et al. (2000)	1.5T (GE)/STEAM, Probe-S, PSD/1500/30/-/Direct from the scanner	DLPFC/8.1 ml Thal/8/1 ml, CC/8 ml (Ins/8 ml, SM1/7.7 ml OFC/8 ml, Vis cort 8 ml)	Glu, Gln, GABA, *(tmI, Glc, Lac, Cr, NAA, Cho)*	n = 9/Low back pain >1yr/22.2%F/45 (6) yrs/9 (5) yrs	n = 11/18.2%F/44 (3) yrs
Gussew et al. (2011)	3T (Sie)/PRESS/(2500/40)/-/LC model	L ant Ins/3 ml, ACC/3.9 ml, L Thal 3.5 ml	Glu, Gln, *(NAA, Cr, tCho, mI)*	n = 10/Low back pain >1yr/80%F/range (22–52 yrs)/range (1–5 yrs)	n = 10/80%F/range (22–52 yrs)
Gustin et al. (2014)	3T (P)/MEGA-PRESS/(2000/68)/100/jMRUI, AMARES (GABA) -/(2000/29)/-/jMRUI, QUEST	R Thal/8 ml	GABA Glu, Gln, *(NAA, Cr, Asp, mI, GroPCho)*	n = 12/Spinal cord injury (SCI) with neuropathic pain, (IASP-SCI)/33.3%F/57 (4) yrs/182 SEM 42 months	n = 21/38.1%F/31 (2) yrs
Harfeldt et al. (2018)	3T (Sie)/-/(2000/30)/-/LC model	R and L post Ins/2 ml	Glu, Glx *(NAA, tCr, Cho, m)*	**Group 1:** n = 19/Temporomandibular disorder with generalised pain/100%F/43 IQR (40–56) yrs/>3months **Group 2:** n = 17/Temporomandibular disorder with local pain/100%F/40 IQR (30–44) yrs/>3months	n = 10/100%F/36 IQR (26–51)
Harper et al. (2018)	3T (P)/MEGA-PRESS/(1800/68)/256/LC model PRESS/(2000/33)/32/LC model	R ant Ins, R post Ins, Mid ACC, Mid Occ (Control Region)/18 ml	GABA Glx, Glu	n = 18/UCPPS- urological chronic pelvic pain syndrome including interstitial cystitis, and bladder pain/100%F/34.8 yrs SD 11/5.9 (6.5) yrs	n = 20/100%F/34.7 (12.3) yrs
Harris et al. (2009)	3T (GE)/-/(3000/30)/-/LC model	R ant Ins, R post Ins/12 ml	Glu, Glx, *(NAA, MI, Cho, Cr, mI)*	n = 19/Fibromyalgia (ACR), >1 year/100%F/45.2 (15) yrs/>1yr	n = 14/100%F/45.9 (11.1) yrs
Henderson et al. (2013)	3T (P)/MEGA-PRESS/(2000/68)/200/jMRUI, AMARES	Contra Thal, (R-controls)/8 ml	GABA	n = 23/Painful trigeminal neuropathy (Liverpool criteria)/82.6%F/46.6 SEM (2.4)/6.1 SEM (4.6) yrs	n = 43/72%F/49.1 SEM (2.5) yrs
Ito et al. (2017)	3T (GE)/-/(2000/30)/96/LC model	ACC/20 × 20 × 40 16 ml	Glu, Gln, Glx *(tCr, mI, NAA)*	n = 56/Chronic pain: neuropathic pain-narrowing of the spinal canal, trigeminal neuralgia, intercostal neuralgia, postoperative neuropathy, radiculopathy, plexus injury, peripheral nerve injury, reflex sympathetic dystrophy, diabetic neuropathy, non-neuropathic-fibromyalgia, cephalgia, somatoform, unidentified general or partial pain/67.9% F/58 range (45–67) yrs/36.5 range (13.5–74.5) months	n = 60/63.3%F/40 range (28–48) yrs
Janetzki et al. (2016)	3T (Sie)/MEGA-PRESS/(2000/68)/-/- PRESS/(1800/30)/-/-	ACC, L Ins/-	GABA Glx *(NAA, mI, tCr, tCho)*	n = 19/Low back pain >3 months/68.4% F/55.3 yrs/42.1% > 5 years, 52.6% < 5 years, 5.3% unknown	n = 19/68.4%F/53.8yrs
Kameda et al. (2018)	3T (GE)/PRESS/(2000/30)/96/LC model	ACC/16 ml	Glu, Glx *(NAA, Cr, mI)*	n = 60/Low back pain >6 months/61.7% F/58.8 (16) yrs/-	n = 56/62.5%F/39.5 (12.8) yrs
Niddam et al. (2011)	3T (Sie)/PRESS/(2000/30)/128/LC model	L and R Hippoc/3 ml	Glu, Glx *(Cho, Cr, mI, NAA)*	n = 15/Irritable bowel syndrome (ROME III), 53%F/36.6 (11.6)/7.2 (6.8) yrs	n = 15/66.7%F/33 (9) yrs
Niddam et al. (2018)	3T (Sie)/MRSI- Proton echo planar spectroscopic imagine sequence/(1500/30)/-/LC model	Whole Brain (ACC, Thal, Occ/8 × 8)	Glx *(Cho, Cr, mI, NAA)*	**Group 1:** n = 24/Episodic migraine (ICHD-II)/80%F/37 (7) yrs/17.2 (9.1) yrs **Group 2:** n = 25/Chronic migraine (ICHD-II)/70.8%F/33.8 (10) yrs/13.3 (9.1) yrs	n = 25/75%F/32.6 (8.3) yrs
Prescot et al. (2009)	4T (V)/2DJ resolved/(2000/30–260)/16 per TE/LC model	ACC, L Ins/8 ml	GABA, Glu, Gln *(Full basis set)*	n = 12/Acute episodic migraine/43 (11) yrs/70%F/23 yrs	n = 8/70%F/41 (9) yrs

(*continued on next page*)

Table 2 (*continued*)

	Strength (scanner)/Sequence/ (TR/TE)/Avs/Processing	Region/Voxel size (ml)	Neurometabolites	Participants: Number/Pain/%Female/ Age/Duration	Controls: Number/%Female/Age
Reckziegel et al. (2016)	3T (GE)/PRESS/(2500/105)/ 128/LC model	ACC/8–12 ml	GABA, Glu, Glx, *(NAA, tNAA, mI, tCho)*	n = 20/Knee osteoarthritis on X-ray, pain mostly constant in the last month/45%F/ 67 (9) yrs/7.7 (4.9) yrs	n = 19/42.1%F/59 (9) yrs
Sharma et al. (2011)	3T (Sie)/MRSI- PRESS/(1500/ 30)/-/LC model	L, R and Mid SSC/matrix size 16 × 16; FOV = 160 mm^2	Glx *(NAA, Cho)*	n = 11/Chronic low back pain over 4/10/-%F/33.6 (10.6) yrs/>3months	n = 11/-%F/31.4 (13.9) yrs
Siniatchkin et al. (2012)	3T (P)/PRESS/(2000/37)/128/-	Primary and Secondary Vis Cort/ 8 ml	Glx, GABA (GABA values not reported) *(NAA, Cr)*	n = 10/Migraine with aura (ICHD-II)/60% F/19.3 (3.4) yrs/4.2 (4.1) yrs	n = 10/60%F/20.3 (3.2) yrs
Valdes et al. (2010)	1.5T (GE)/PRESS/(1500/35)/-/ LC model	L and R Amyg/3.37 ml L and R Thal/2.25 ml, L and R PFC/3.37 ml	Glu, Gln *(NAA, Cho, Cr, mI)*	n = 30/Fibromyalgia (AMR)/100%F/ 42.62 (8.76) yrs/151 (120) months	n = 30/100%F/43.86 (10.60) yrs
Widerstrom-Noga et al. (2013)	3T (Sie)/PRESS/(2000/30)/256/ LC model	ACC/8.75 ml	Glx *(NAA, tCr, Cho, mI)*	**Group 1:** n = 31/SCI with low neuropathic pain/16.12%F/37.5 (13.4) yrs/10.6 (9.07) yrs **Group 2**: n = 19/SCI with high neuropathic pain/26.3%F/40.4 (11.8) yrs/ 12 (9.85) yrs	n = 24/20.8%F/34.4 (8.6) yrs
Widerstrom-Noga et al. (2015)	3T (Sie)/2D chemical shift imaging using PRESS/(2000/30)/ 4/LC model	L and R Thal/matrix size 8 × 8; FOV 160 mm	Glx *(NAA, Cho, mI)*	**Group 1:** n = 35/SCI with low neuropathic pain/20%F/35.7 (12.4) yrs/13.1 (9.7) yrs **Group 2**: n = 19/SCI with high neuropathic pain/15.8%F/43 (12.5) yrs/ 12 (9.66) yrs	n = 24/20.8%F/34.4 (8.6) yrs
Zielman et al. (2017)	7T (P)/Semi-LASER/(5000/30)/ 32/LC model	Vis Cor (Occ)/12 ml	Glu, Gln, Glx *(tNAA, tCr, Ins, tCho, PE, Asp)*	**Group 1:** Migraine without aura (ICHD-3b)/n = 27/51.9%F/35.1 (8.2) yrs/20.9yrs **Group 2:** Migraine with aura (ICHD-3b)/ n = 23/47.8%F/35 (9.3) yrs/20.6yrs	n = 24/50%F/34.8 (8.7) yrs

P- Phillips, Sie- Siemens, GE- General Electric, V- Varian, *Likely Typo, **Not stated-taken from reference, - Not stated, L- Left, R- Right, Ant- Anterior, Post- Posterior, Mid- Midline, V- Ventral, PCG- Posterior Cingulate Gyrus, Ins- Insula, Occ- Occipital, Thal- Thalamus, APC- Anterior paracingulate cortex, Hippoc- Hippocampus, VL PFC- Ventrolateral Prefrontal Cortex, DLPFC- Dorsolateral Prefrontal Cortex, SM1- Primary sensorimotor cortex, CC- Cingulate Cortex, OFC- Orbital frontal cortex, Vis cort- Visual Cortex (Occipital Lobe), Amyg- Amygdala, SSC- Somatosensory cortex, GABA-gamma-aminobutyric acid, Glu-glutamate, Gln-glutamine, NAA- N-acetylaspartate, Cr- Creatine, mI- myoinositol, Cho- Choline, Glx-combined glutamate and glutamine, mI- myoinositol, tCho-total choline, tNAA-total N-acetylaspartate and N-acetylaspartyl glutamate, Glc-glucose, tmI- total myo-and scyllo-inositol, Lac- Lactate, Asp- Aspartate, GroPCho- Glycerophosphocholine, tCr-total creatine and phosphocreatine, phosphorylethanolamine, SCI- spinal cord injury, ICHD- The international classification of headache disorders 3 beta, IHS- International headache society, ACR- American college of Rheumatology, ROME III- Diagnostic criteria for irritable bowel syndrome.

Table 3
Assessment of spectroscopy quality using the MRS-Q tool: un-edited studies.

	Parameters					Utilisation of quality checks			Study design/Post processing				QUALITY
	>3T	Sequenceϕ	Data points	TE	ParametersΔ	Shim or FWHM	Fit error	Data visualised	Power calc	Frequency drift	Partial vol correction˅	Frequency/ Phase corrected	
As Sanie et al. (2016)	Y	Y	<i>	?	<i>	<i>	N	Y	N	N	Y	N	**UNSURE**
Bathel et al. (2018)	Y	Y	Y	Y	Y (Thal #)	<i>	Y	Y	N	N	NA	Y	**HIGH (Thal #)**
Bigal et al. (2008)	Y	<i>	<i>	Y*	<i>	<i>	<i>	N	N	N	N	<i>	**UNSURE**
Bridge et al. (2015)	Y	N	<i>	Y	Y	Y	Y	Y	N	Y	Y	Y	**LOW**
Di Pietro et al. (2018)	Y	Y	Y	Y	<i>	<i>	N	Y	N	N	NA	N	**UNSURE**
Fayed et al. (2010)	N	Y	<i>	Y	Y	<i>	N	Y	N	N	N	N	**HIGH**
Fayed et al. (2012)	N	Y	<i>	Y	Y	<i>	Y	Y	N	N	N	N	**HIGH**
Fayed et al. (2014)	N	Y	<i>	Y	Y	<i>	N	Y	N	N	N	N	**HIGH**
Feraco et al. (2011)	Y	Y	<i>	Y	N	Y	N	Y	N	N	NA	N	**LOW**
Forester et al. (2012)	Y	Y	<i>	Y	Y	<i>	Y	Y	N	N	Y	N	**HIGH**
Gerstner et al. (2012)	Y	Y	<i>	Y	<i>	<i>	N	Y	N	N	N	N	**UNSURE**
Gonzales de la Aleja et al. (2013)	Y	Y	<i>	Y	Y	<i>	Y	Y	N	N	Y	<i>	**HIGH**
Grachev et al. (2000)	N	Y	<i>	Y	<i>	<i>	N	Y	N	N	NA	N	**UNSURE**
Gussew et al. (2011)	Y	Y	Y	Y	<i>	Y	Y	Y	N	Y	Y	Y	**UNSURE**
Gustin et al. (2014)	Y	Y	Y	Y	<i>	Y	N	Y	N	N	NA	Y	**UNSURE**
Harfeldt et al. (2018)	Y	<i>	<i>	Y	<i>	<i>	Y	N	N	N	N	N	**UNSURE**
Harper et al. (2018)	Y	Y	N	Y	Y	<i>	Y	Y	N	N	Y	N	**HIGH**
Harris et al. (2009)	Y	Y	<i>	Y	<i>	<i>	N	Y	N	N	Y	N	**UNSURE**
Ito et al. (2017)	Y	Y	<i>	Y	Y	<i>	N	Y	N	N	NA	<i>	**HIGH**
Janetzki et al. (2016)	Y	Y	<i>	Y	<i>	<i>	N	Y	N	N	N	<i>	**UNSURE**
Kameda et al. (2018)	Y	Y	<i>	Y	Y	Y	Y	N	N	N	N	N	**HIGH**
Niddam et al. (2011)	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	N	**HIGH**
Niddam et al. (2018)	Y	Y	<i>	<i>	N**	Y	Y	Y	N	Y	Y	N	**UNSURE**
Prescot et al. (2009)	Y	Y~	Y	Y	Y	Y	Y	Y	N	N	Y	Y	**HIGH**
Reckziegal et al. (2016)	Y	N (Not for GABA)	N	<i>	N (Not GABA)	N	Y	Y	Y	Y	Y	N	**LOW**
Sharma et al. (2011)	Y	Y	<i>	Y	Y**	Y	Y	N	N	Y	Y	N	**HIGH**
Siniatchkin et al. (2012)	Y	Y	<i>	N	Y	<i>	N	Y	N	N	NA	Y	**HIGH**
Valdes et al. (2010)	N	Y	<i>	Y	<i>	<i>	Y	Y	N	N	Y	Y	**UNSURE**
Widerstrom et al. (2013)	Y	Y	N	Y	Y	<i>	N	N	N	N	NA	Y	**HIGH**
Widerstrom et al. (2015)	Y	Y	N	Y	N**	<i>	N	Y	N	N	NA	N	**LOW**
Zielman et al. (2017)	Y	Y	Y	Y	Y	<i>	Y	Y	Y	N	Y	Y	**HIGH**
% Yes	**83.3**	**87**	**23**	**85**	**52**	**29**	**52**	**81**	**6**	**19**	**41**	**33**	

ϕ = MEGA-PRESS, MEGA-semi-LASER, other GABA editing; **Data points** 1024 complex data points from 2000Hz. **Short TE** = ~20/30 ms, ***Δ*** = ***Adequate parameters*** *128 Av, 15 × 15 × 15 voxel, 3T; 64 Av, 20 × 20 × 20 voxel, 3T; 256 Av, 15 × 15 × 15 voxel 1.5T; 128 Av, 20 × 20 × 20 voxel, 1.5*[33]. ***Partial volume correction*** ˅ not just grey matter correction, not required for data presented as ratios (NA), *Y = Yes, N = No, <i> insufficient information, * = extracted from cited paper, ** = MRSI, ~ = PRESS based 2DJ, ? = likely error in reporting, # = criteria not fully met.*

duplicates, 5505 titles and abstracts were screened for eligibility, with 162 studies deemed eligible for full text screening. Following full text screening, 127 studies were excluded leaving 35 studies to be included in the analysis (Fig. 1). Two of which were translated from German and Japanese prior to inclusion. The 35 studies contributed a total of 140 data sets for inclusion within the study.

3.2. Study characteristics

3.2.1. Spectroscopy

Twenty-eight studies used 3-T scanners, six studies used 1.5 T, and two single studies used 4T and 7T respectively. Some studies used both editing and non-editing: A PRESS sequence or vendor specific variation was used in 30 analyses including, three of which were implemented using 2D MRSI (Niddam et al., 2018; Sharma et al., 2011; Widerstrom-Noga et al., 2015), whilst MEGA-PRESS was used in ten analyses (Aguila et al., 2015; Bathel et al., 2018; Bednarska et al., 2019; Chan et al., 2019; Di Pietro et al., 2018; Foerster et al., 2012; Gustin et al., 2014; Harper et al., 2018; Henderson et al., 2013; Janetzki et al., 2016). Individual studies used 2DJ resolved (Prescot et al., 2009), semi-LASER (Zielman et al., 2017), STEAM (Grachev et al., 2000), SPECIAL (Bridge et al., 2015), and 3D LASER. (Bigal et al., 2008) (Table 2).

3.2.2. Neurometabolites

GABA was reported in 14 studies (Aguila et al., 2015; Bednarska et al., 2019; Bigal et al., 2008; Bridge et al., 2015; Chan et al., 2019; Di Pietro et al., 2018; Foerster et al., 2012; Grachev et al., 2000; Gustin et al., 2014; Harper et al., 2018; Henderson et al., 2013; Janetzki et al., 2016; Prescot et al., 2009; Reckziegel et al., 2016), glutamate in 16 (Bridge et al., 2015; Fayed et al., 2012, 2014; Feraco et al., 2011; Gerstner et al., 2012; Gonzales de la Aleja et al., 2013; Grachev et al., 2000; Gussew et al., 2011; Harfeldt et al., 2018; Harper et al., 2018; Harris et al., 2009; Ito et al., 2017; Kameda et al., 2018; Niddam et al., 2011; Prescot et al., 2009; Zielman et al., 2017), glutamine in eight (Gerstner et al., 2012; Gonzales de la Aleja et al., 2013; Grachev et al., 2000; Gussew et al., 2011; Harris et al., 2009; Harper et al., 2018; Prescot et al., 2009; Zielman et al., 2017) and Glx in 21 (As-Sanie et al., 2016; Bathel et al., 2018; Bednarska et al., 2019; Chan et al., 2019; Fayed et al., 2010, 2012, 2014; Feraco et al., 2011; Gerstner et al., 2012; Gussew et al., 2011; Harper et al., 2018; Harris et al., 2009; Ito et al., 2017; Janetzki et al., 2016; Kameda et al., 2018; Niddam et al., 2011, 2018; Reckziegel et al., 2016; Sharma et al., 2011; Siniatchkin et al., 2012; Valdes et al., 2010; Widerstrom-Noga et al., 2013, 2015; Zielman et al., 2017). None of the included studies used macromolecular suppression and therefore are more likely to reflect GABA+, however for the purpose of this study we refer to this as GABA. The included studies reported level of neurometabolites as either Institutional units, absolute concentration (e.g. mmol/l), ratios relative to Cr, or ratios relative to NAA. (Tables 3 and 4). Raw data was not presented for four studies (Bridge et al., 2015; Kameda et al., 2018; Niddam et al., 2018; Widerstrom-Noga et al., 2015) and therefore required callipers for extraction from graphical representations.

3.2.3. Pain conditions

Migraine was compared to control participants in 11 studies, (migraine sub-classifications studied included two acute episodic migraine (Niddam et al., 2018; Prescot et al., 2009), one chronic migraine (Niddam et al., 2018), four migraine without aura (Aguila et al., 2015; Bathel et al., 2018; Bigal et al., 2008; Zielman et al., 2017), four with aura (Bigal et al., 2008; Bridge et al., 2015; Siniatchkin et al., 2012; Zielman et al., 2017) and three mixed (Chan et al., 2019; Fayed et al., 2014; Gonzales de la Aleja et al., 2013). Musculoskeletal pain (five chronic low back pain (Grachev et al., 2000; Gussew et al., 2011; Janetzki et al., 2016; Kameda et al., 2018; Sharma et al., 2011), one knee osteoarthritis (Reckziegel et al., 2016), two temporomandibular joint pain (Gerstner et al., 2012; Harfeldt et al., 2018)) was compared to control participants in eight studies. Chronic pain syndromes (seven fibromyalgia (Fayed et al., 2010; Fayed et al., 2012; Feraco et al., 2011; Foerster et al., 2012; Harfeldt et al., 2018; Harris et al., 2009; Valdes et al., 2010), two somatoform disorder (Fayed et al., 2012, 2014), one chronic widespread pain (Ito et al., 2017)) were compared to control participants in nine studies and the remaining miscellaneous studies (three spinal cord injury with neuropathic pain (Gustin et al., 2014; Widerstrom-Noga et al., 2013; Widerstrom-Noga et al., 2015), one pelvic pain with and without endometriosis (As-Sanie et al., 2016), one urological chronic pain (Harper et al., 2018), three with facial neuropathic pain (Di Pietro et al., 2018; Fayed et al., 2014; Henderson et al., 2013), two painful irritable bowel syndrome (Bednarska et al., 2019; Niddam et al., 2011) were compared to control participants in nine studies.

3.2.4. Brain regions

Neurometabolites were investigated across 12 brain regions including; amygdala, anterior cingulate cortex (ACC), anterior frontal cortex, cingulate cortex, hippocampus, insula, occipital lobe (including visual cortex), prefrontal gyrus, posterior cingulate gyrus (PCG), sensorimotor-cortex, somatosensory cortex, thalamus (Fig. 2). Thirteen studies reported data from more than one brain region for the review's primary analysis.

3.3. Quality assessment

3.3.1. AXIS

The quality varied from seven studies (Aguila et al., 2015; As-Sanie et al., 2016; Bathel et al., 2018; Di Pietro et al., 2018; Niddam et al., 2011; Valdes et al., 2010; Zielman et al., 2017) satisfying over 80% of the criteria to four studies (Bridge et al., 2015; Fayed et al., 2014; Harper et al., 2018; Prescot et al., 2009) satisfying only 50% of criteria. Quality metrics reported by all studies were the measure used to determine statistical significance, clear aims, and ethical approval or consent. In contrast, few studies justified sample size (5/35, 14.28%) or categorised non-responders (5/35, 14.3%) (Fig. 3). Furthermore, the control of confounding variables such as limiting the inclusion of participants with other comorbidities (25/35, 71.4%), controlling for medications (21/35, 60%), and controlling other confounders (15/35, 42.9%) e.g. smoking, time of day or menstrual cycle were inconsistently addressed across the studies.

3.3.2. Quality assessment: spectroscopy (MRS-Q)

Most sequences used in the studies (n = 21/41 from 35 studies, 51.2%) did not report using adequate spectroscopy parameters. For example, adequate parameters were used in 20% (n = 2/10) of edited, and 52% (n = 16/31) of unedited studies. Of these, 12% (edited) and 35.5% (unedited) studies did not record sufficient details to determine the overall quality of spectroscopy and allow for reproducing these studies. Details not reported included averages, voxel size and scanner strength. Of the 22/41 sequences in studies that did report the parameters used, two (Bridge et al., 2015; Reckziegel et al., 2016) did not use an appropriate sequence to detect all reported neurometabolites of interest. Of the studies using sequences edited specifically for GABA (n = 8/39), 50% (n = 4/8) used the recommended number of averages and 25% (n = 2/8) used an appropriately sized voxel for all regions (Tables 3 and 4).

3.4. Results: primary aim: neurometabolites between pain conditions

3.4.1. GABA level across pain conditions

The level of GABA in migraine was significantly increased compared with controls (Hedge's G 0.394, 95%CI: 0.095 to 0.0.693, $i^2 = 0$). In contrast the level of GABA was significantly decreased in three of the six miscellaneous studies investigating pelvic pain, trigeminal neuralgia and painful spinal cord injury compared to controls. GABA level was not significantly different in musculoskeletal pain (Hedge's G −0.15, 95%CI

−0.44 to 0.15, $i^2 = 0$), or chronic pain syndromes (Hedge's G −0.08, 95% CI −1.61 to 1.46, $i^2 = 89.479$) compared to controls (Fig. 4).

3.4.2. Glutamate level across pain conditions

The level of glutamate in migraine demonstrated a significant increase compared with controls (Hedges G: 0.45, 95% CI 0.17 to 0.73, $i^2 = 56.79$). In contrast glutamate level was significantly decreased in musculoskeletal conditions compared with controls (Hedge's G −0.262, 95%CI −0.481 to −0.043, $i^2 = 0$). There was no significant difference between glutamate level in either chronic pain syndromes or any individual study in the miscellaneous pain category compared with controls (Fig. 5).

3.4.3. Glutamine level across pain conditions

The level of glutamine was not significantly different between any pain condition and controls. Data compared with controls were migraine (Hedge's G: 0.309, 95%CI −0.027 to 0.646, $i^2 = 57.45$) musculoskeletal pain (Hedge's G: −0.124, 95%CI −0.627 to 0.379, $i^2 = 58.87$), chronic pain syndromes (Hedge's G: 0.255, 95%CI −0.035 to 0.857 $i^2 = 36.25$) or the single study in the miscellaneous pain category (Fig. 6).

3.4.4. Glx level across pain conditions

The level of Glx was significantly increased in chronic pain syndromes compared with controls (Hedge's G 0.552, 95%CI: 0.332 to 0.773, $i^2 = 56.97$). This was not evident in any other pain group compared with controls. Data compared with controls were migraine (Hedge's G 0.14, 95%CI: −0.16 to 0.43, $i^2 = 79.14$) musculoskeletal pain (Hedge's G 0.346, 95%CI: −0.169 to 0.861, $i^2 = 79.8$) and studies of miscellaneous pain that had a wide spread of results including a significant decrease of Glx in four studies (two of spinal cord injury, and two of irritable bowel syndrome), and a significant increase in three studies (two studies of pelvic pain with and without endometriosis and one of trigeminal neuralgia (Fig. 7).

3.5. Secondary aims

3.5.1. Does spectroscopy quality influence brain neurometabolite levels

Secondary analysis was performed using 64/137 (47%) data sets from 19/33 (57.6%) studies that reported using adequate spectroscopy parameters (Tables 3 and 4). The analysis using only high-quality studies, demonstrated that GABA remained significantly increased in migraine (Hedge's G 0.394, 95%CI: 0.050 to 0.739, $i^2 = 6.048$) as per the original analysis. Similarly, as demonstrated in the original analysis, there was no difference in GABA levels in people with chronic pain syndromes compared to controls. There were no high-quality spectroscopy studies that investigated GABA levels for musculoskeletal pain.

When only high-quality studies were analysed, glutamate levels remained significantly increased in people with migraine (Hedge's G 0.443, 95%CI: 0.154 to 0.732, $i^2 = 56.79$), and decreased in a single study of musculoskeletal pain (Hedge's G −0.387, 95%CI: −0.752 to −0.022) compared with controls. There remained no differences in glutamate levels in chronic pain syndromes compared with controls in the high-quality studies. Glutamine continued to show no significant level changes in migraine and there were no high-quality studies for musculoskeletal pain, and chronic pain syndromes.

Glx was the only neurometabolite to demonstrate a difference when only high-quality studies were used in the meta-analysis. Whilst the original analysis demonstrated a non-significant trend towards an increase in Glx levels in people with migraine (Hedge's G 0.135, 95%CI: −0.161 to 0.432, $i^2 = 79.14$), the high-quality studies demonstrated a significant increase (Hedge's G 0.657, 95%CI: 0.417 to 0.898, $i^2 = 12.01$). The increase in Glx level in chronic pain syndromes compared to control remained significant when only high-quality studies were considered (Hedge's G 0.508, 95%CI: 0.292 to 0.723, $i^2 = 6.1$). Glx levels in musculoskeletal pain, were not different to the controls in the high-quality studies in line with the original analysis.

Table 4
Assessment of spectroscopy quality using the MRS-Q tool: edited studies.

	Parameters						Utilisation of quality checks			Study design/Post processing					QUALITY
	>3T	Sequence ϕ	Avs	TE	Voxel size	Parameters Δ	Shim or FWHM	Fit error	Data visualised	Power calc	Frequency drift	Partial vol correction˅	Frequency/ Phase corrected		
Aguila et al. (2015)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N (GM)	Y		**HIGH**
Bathel et al. (2018)	Y	Y	Y	Y	Y (#Occ)	Y (#Occ)	<i>	Y	Y	N	N	NA	Y		**HIGH (#Occ)**
Bednarska et al. (2019)	Y	Y	<i>	Y	Y	<i>	<i>	N	Y	N	N	N	Y		**UNSURE**
Chan et al. (2019)	Y	Y*	N	Y	Y	N	<i>	Y	Y	Y	N	Y	Y		**LOW**
Di Pietro et al. (2018)	Y	Y	N	Y	N	N	<i>	N	Y	N	N	NA	N		**LOW**
Foerester et al. (2012)	Y	Y	Y	Y	Y	Y	<i>	Y	Y	N	N	Y	N		**HIGH**
Gustin et al. (2014)	Y	Y	N	Y	N	N	Y	N	Y	N	N	NA	Y		**LOW**
Harper et al. (2018)	Y	Y	Y	Y	N	N	<i>	Y	Y	N	N	Y	N		**LOW**
Henderson et al. (2013)	Y	Y	<i>	Y	N	N	Y	N	Y	N	N	NA	Y		**LOW**
Janetzki et al. (2016)	Y	Y	<i>	Y	<i>	<i>	<i>	N	Y	N	N	NA	<i>		**UNSURE**
% YES	**100**	**100**	**40**	**100**	**50**	**30**	**20**	**50**	**100**	**20**	**10**	**30**	**56**		

ϕ = PRESS/semi-LASER (or vendor specific) or STEAM; Δ = Averages over 240, TE GABA+ 68, GABA 80 (Siemens 68) voxel size around 27 ml.
Y = Yes, N = No, <i> insufficient information, (Cr) = Creatine ratio used instead of partial volume, GM = only corrected for grey matter, # = criteria not fully met, * typo-study reported using point resolved spectroscopy but was later apparent that it was MEGA-PRESS.

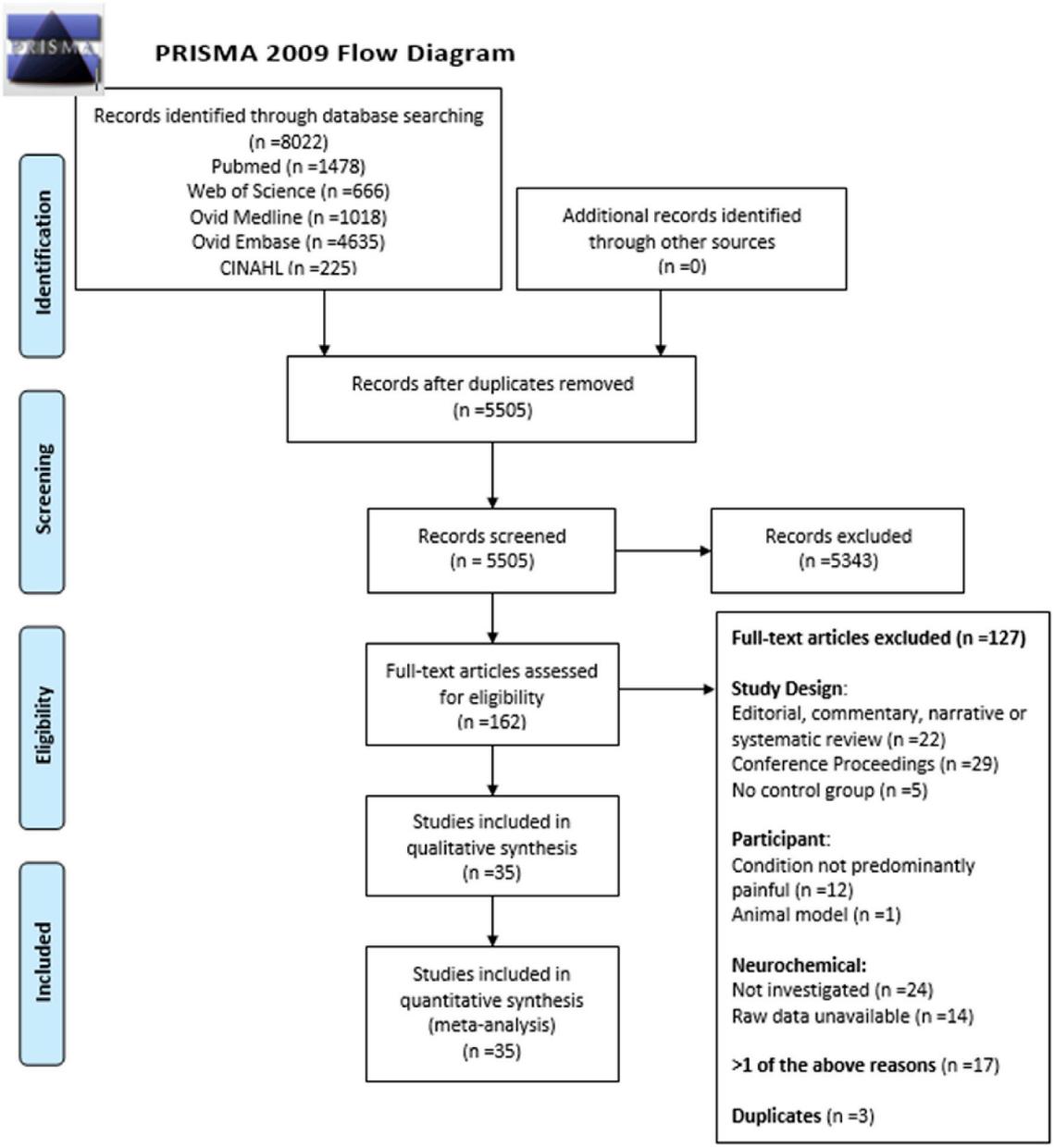

Fig. 1. PRISMA flow diagram (Moher et al., 2015).

3.5.2. Does brain region influence brain neurometabolite levels

There was insufficient data from the majority of brain regions to answer the question; are brain neurometabolite changes influenced by brain region. Across all neurometabolites and pain conditions, six brain regions demonstrated significant differences in neurometabolite level between pain group and control (ACC, PCG, occipital lobe, thalamus, hippocampus, insula). The number of data sets contributing to these results varied from one single data set to 11, with the occipital lobe providing the most comparisons. Pooled data from 11 data sets investigating the occipital lobe demonstrated a significant increase in level of Glx (Hedge's G 0.452, 95%CI: 0.184 to 0.721, $i^2 = 53.12$) and glutamate (Hedge's G 0.572, 95%CI: 0.230–0.904, $i^2 = 46.56$) in people with migraine compared with control. However, there were insufficient data to compare occipital region with other regions in the brain and the occipital region was not studied in any other pain condition other than migraine.

The ACC was the only region to be studied across all neurometabolites and pain conditions. Single studies demonstrated a significant increase in glutamine level in the ACC in migraine (Hedge's G 1.148, 95%CI: 0.214 to 2.083) and conversely a decrease in glutamine level in the ACC in musculoskeletal pain (Hedge's G −1.102, 95%CI: 2.008 to −0.196) compared with controls. Glx levels in the ACC were significantly increased in chronic pain syndromes (Hedge's G 0.308, 95%CI: 0.308 to 1.053) compared with controls. All other neurometabolites in other pain conditions were insignificant. There were insufficient data to compare levels of neurometabolites between the ACC and other brain region.

When brain neurometabolite levels were averaged across brain regions, there was no significant change except glutamine in migraine, which remained increased compared to control but reached statistical significance (Hedge's G 0.350, 95%CI: 0.021 to 0.680).

4. Discussion

The meta-analyses presented here demonstrate that different pain conditions appear to have unique neurometabolite signatures. Individuals with migraine appeared to have generally increased levels of

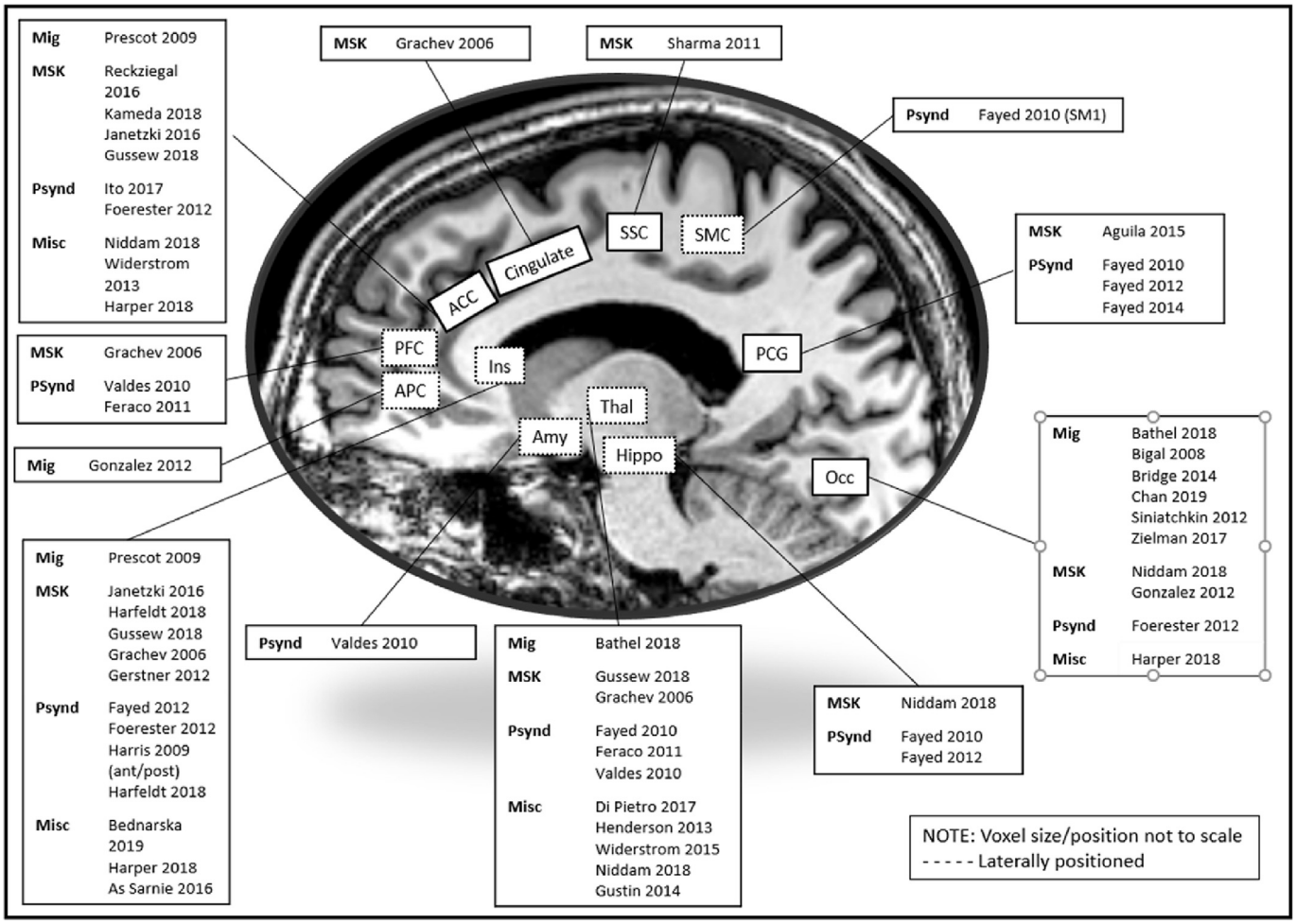

Fig. 2. Brain Regions examined in included studies.

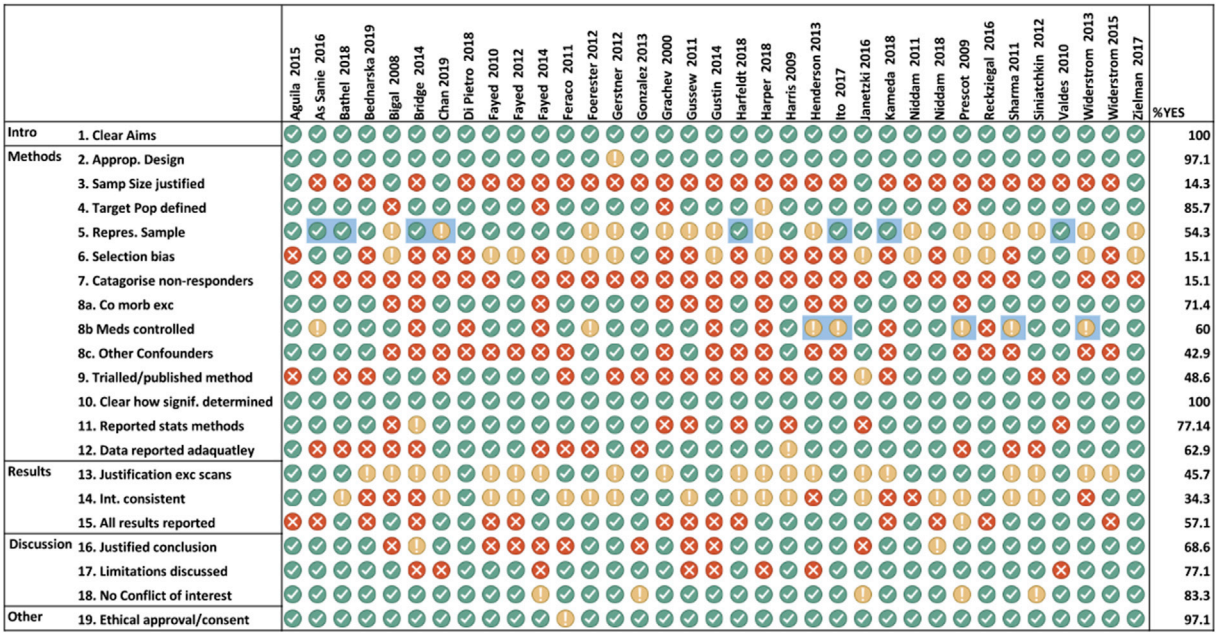

Fig. 3. AXIS methodological quality.

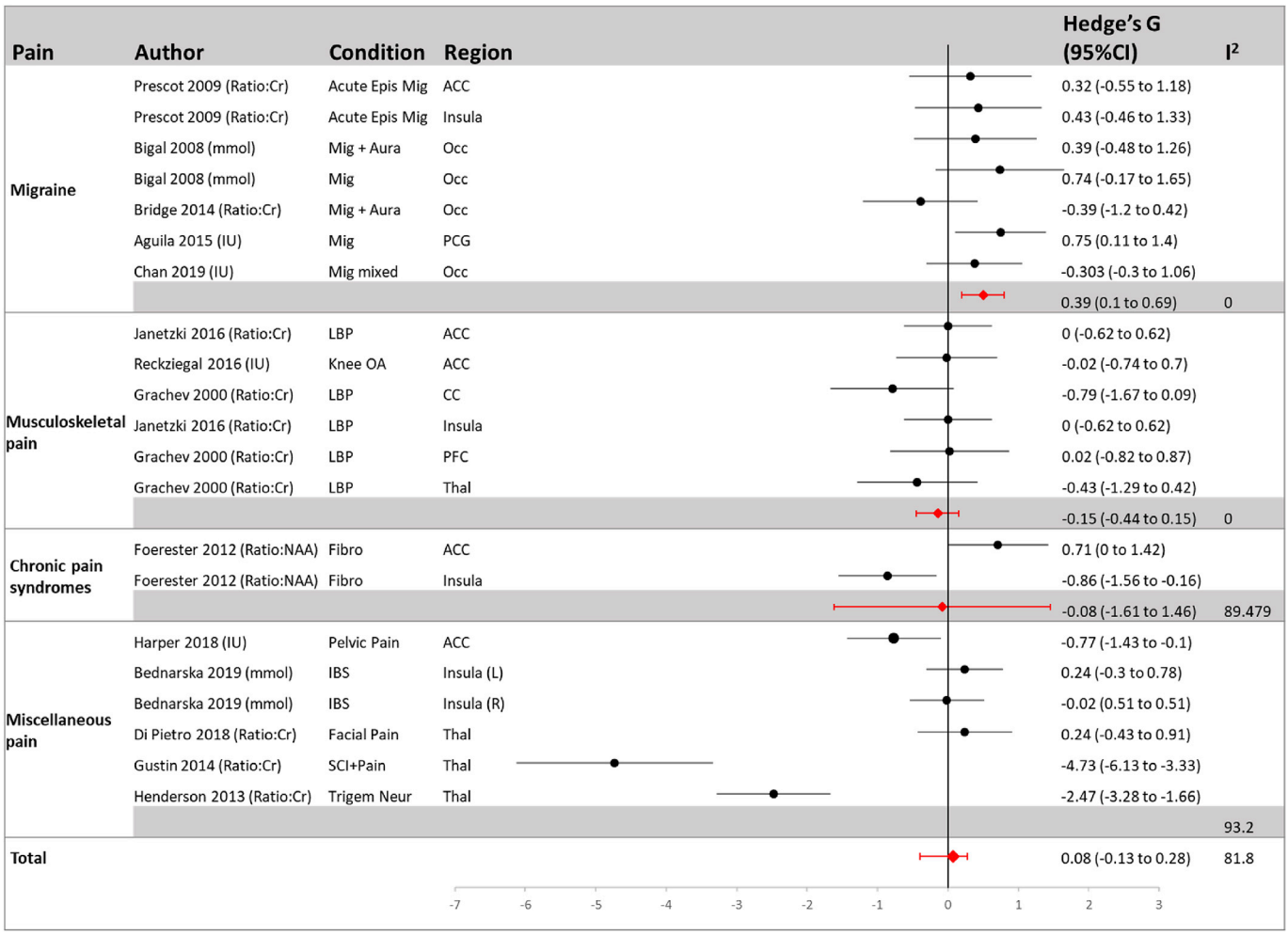

Fig. 4. GABA: analysed by pain conditions.

brain neurometabolites (GABA, Glu. Glx), whilst those with the other pain conditions studied varied in their neurometabolite profile. Four unique neurometabolite signatures were observed across the different pain conditions. Some of these observations are consistent with current theories in chronic pain, others are divergent from them. Hypotheses for these different observations are discussed below. We also discuss how results may be influenced by factors such as the quality of reporting and brain region investigated. This review also highlights that the quality of reporting 1H-MRS acquisition and methods is generally poor and calls for the introduction of a standardized reporting tool.

The neurometabolite signature observed in people with migraine appears to be unique, people with migraine demonstrated increased levels of glutamate and GABA compared to control participants, which was not seen in other conditions. One plausible explanation for higher glutamate levels occurring in migraine and not in other pain conditions could be cortical spreading depression, a process uniquely associated with transient neurological disorders such as migraine and epilepsy (Cozzolino et al., 2018). Cortical spreading depression is characterized as a wave of excitation, followed by inhibition which spreads across the brain. High levels of glutamate have been hypothesized to initiate this process (Charles and Baca, 2013; Cozzolino et al., 2018). The observed increase in inhibitory GABA however is more difficult to explain (Aguila et al., 2015; Bigal et al., 2008). Proposed hypotheses include that GABA has a protective role in suppressing headaches (Bigal et al., 2008), or that increased GABA levels reflect a homeostatic response to the increased glutamate through the GABA metabolic pathway (Pearl et al., 2006). Alternatively, increased GABA may reflect a pathophysiological mechanism of migraine which has yet to be fully explained. For example GABA may have a role in the regulation of vasodilation (Kocharyan et al., 2008), or with neurogenic inflammation seen in migraine (Palmer et al., 1994).

It remains unclear exactly what mechanisms underlie the findings of increased GABA and Glu in migraine. The downside of MRS is that there is no specificity as to what pool of GABA is being measured. MRS measures the presynaptic pool of GABA as a neurotransmitter, and studies have shown that the GABA measured with MRS is most related to GAD1, the gene encoding for GAD67 which is predominantly present in the soma (Marenco et al., 2010). Therefore, GABA is generally thought to reflect 'inhibitory tone' (Rae, 2014). Increased GABA may be a response to increased excitation and indeed, several studies (Diener et al., 2015) suggest drugs targeting GABAA or GABAB-receptor function may be promising as treatment for pain disorders, including migraine. Endogenous increases in GABA could reflect a similar mechanism to increased Glu. However, it is possible that dysfunctional GABA signaling through GABA receptors plays a key role in the emergence of migraine; Studies have implicated polymorphisms in genes encoding for GABA receptor subunits in the migraine (Garcia-Martin et al., 2018). Reduced GABA-receptor function could lead to hyperexcitability of both inhibitory and excitatory neurons and thus, increased neurotransmitter levels.

In contrast, people with chronic pain syndromes (e.g. fibromyalgia) demonstrated an imbalance between the level of the inhibitory and excitatory neurometabolites. An imbalance in neurometabolites have been frequently hypothesized as a mechanism underlying chronic pain (Chang et al., 2013; Sanaei Nezhad et al., 2017). People with chronic

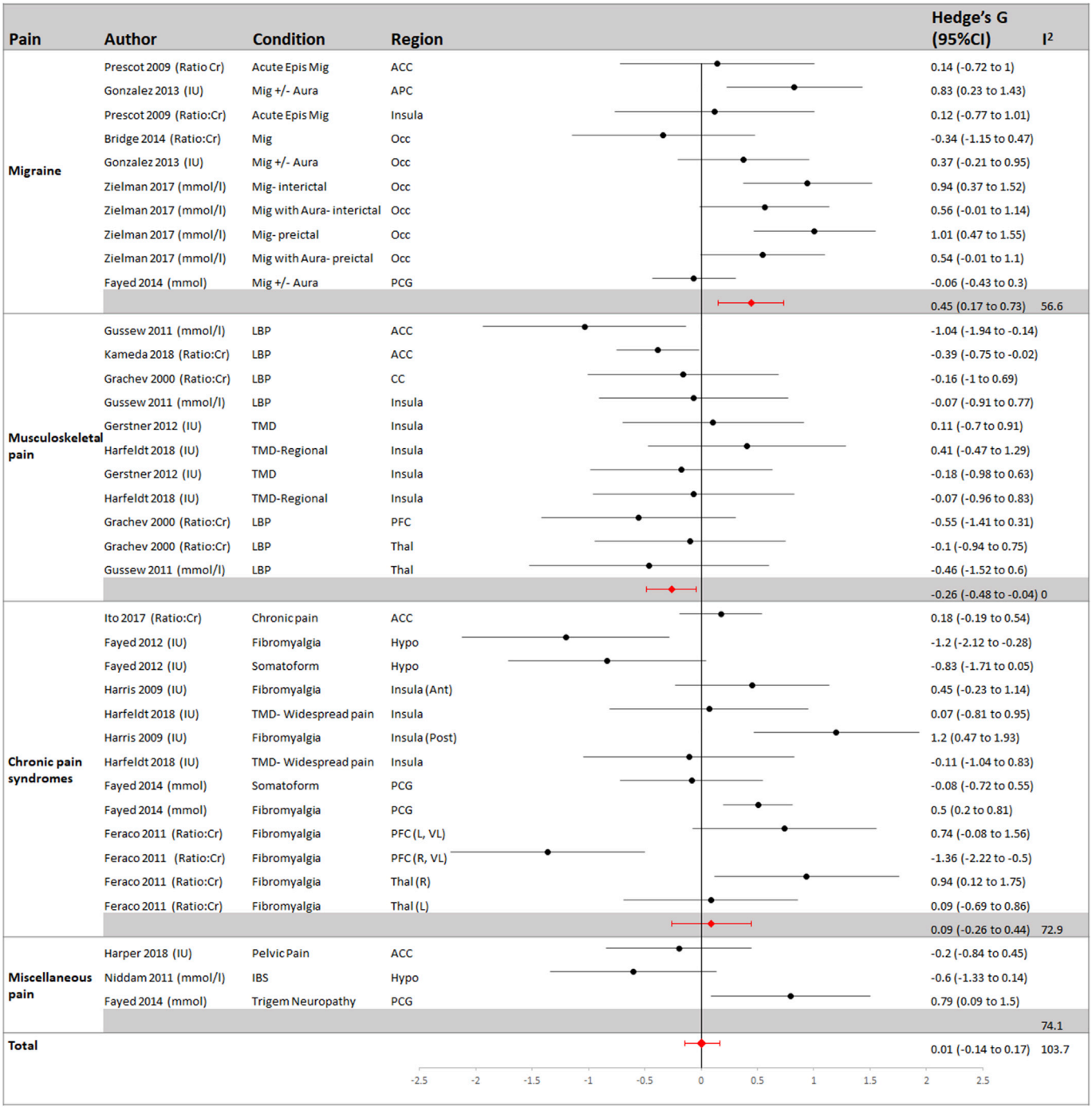

Fig. 5. Glutamate: analysed by pain conditions.

pain syndromes demonstrated an increase in excitatory Glx with no difference in inhibitory GABA. This neurometabolite pattern has been associated with increased pain catastrophizing (Fayed et al., 2012), suggesting that increased Glx in conditions such as fibromyalgia could reflect the psychological aspects of living with a widespread chronic pain syndrome. It has been suggested that the balance of excitatory and inhibitory tone and its relationship with pain could be explored through ratios such as GABA to Glutamate. This was not investigated within this review, but may be considered in future studies, to better understand the relationship between excitation and inhibition in pain conditions.

Musculoskeletal conditions also demonstrated a unique neurometabolite signature, with a significant decrease in glutamate. However, only one of the eleven studies used sufficient acquisition parameters such that this result requires further confirmation. In summary, our observations together with known observations in the literature suggest there are distinct neurometabolite signatures for different pain conditions, which potentially allows for specific disease biomarkers.

Glutamine did not demonstrate significant changes across any of the pain conditions in the primary analysis. Difficulties in quantifying Glutamine have been reported, and therefore it is often not reported alone, except in cases of significant elevation, such as hepatic encephalopathy (Rama Rao et al., 2012). Glutamine's contribution to the Glx signal is not fully appreciated and can be problematic in conditions, where the Glu and Gln levels change in opposite directions (Sanaei Nezhad et al., 2017). To overcome this issue study of the Glu/Gln ratio has been recommended. Whilst this was not within the scope of this

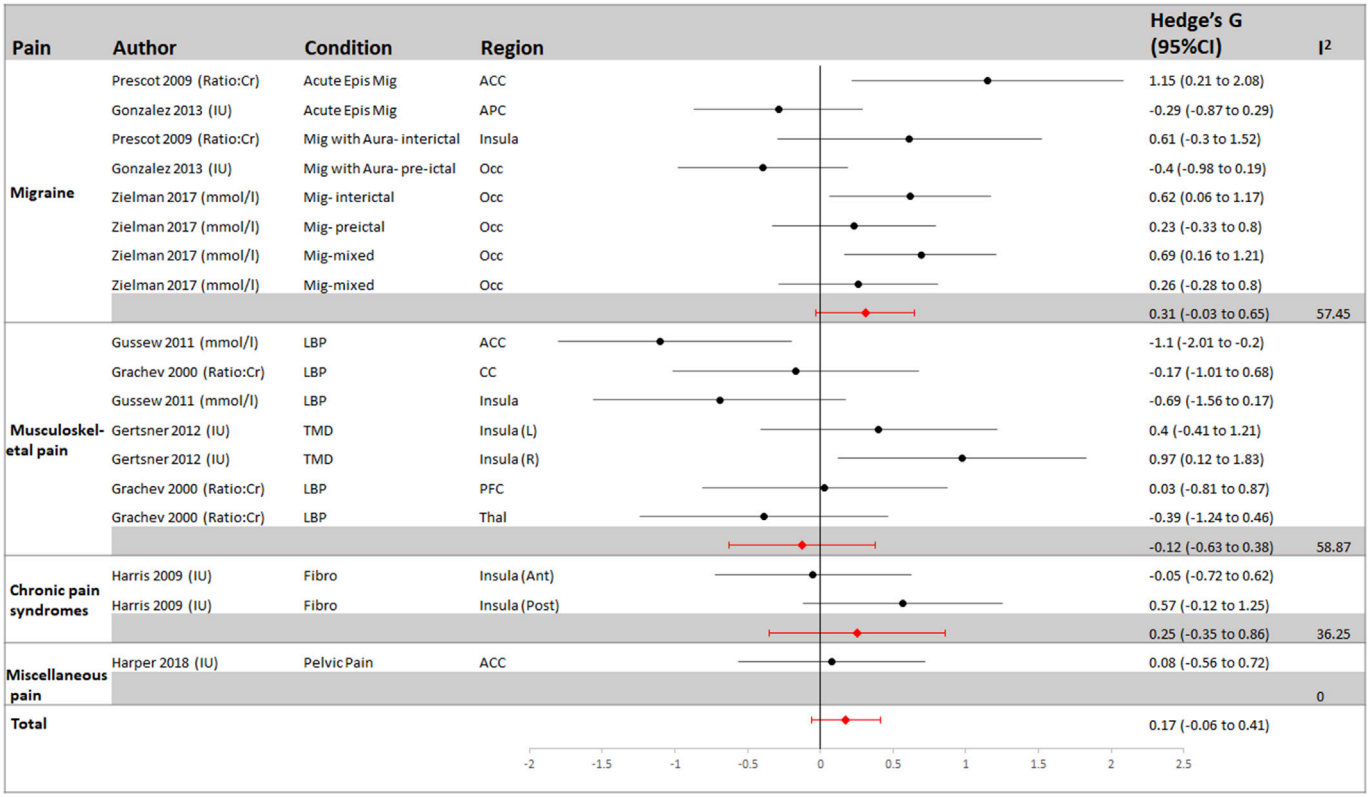

Fig. 6. Glutamine: analysed by pain conditions.

review, future studies may consider this approach to gain a better insight into the nature of this relationship in pain conditions.

Our MRS quality appraisal undertaken in this systematic review suggests that the reporting of MR spectroscopy parameters could be improved. One-third of all studies did not report key MRS parameters including the use of an adequately sized voxel, scanner strength and number of acquisitions. Reporting in studies of GABA in musculoskeletal pain, would particularly benefit from improvement, where none of the included studies documented these three key parameters required to reproduce or evaluate the study. A common methodological limitation in the spectroscopy studies was not controlling or reporting potential confounders such as medication use (Cai et al., 2012; Kuzniecky et al., 2002; Monteleone et al., 1990), smoking status or substance use (Schulte et al., 2017), menstrual phase (De Bondt et al., 2015; Epperson et al., 2002, 2005) or alcohol intake (Meyerhoff et al., 2018). The lack of detail makes it difficult to pool data in meta-analysis such as these and to be certain about accuracy of reported results in individual studies.

Despite the paucity of reporting, our sensitivity analysis suggests that adequate spectroscopy parameters were likely used in the majority of studies. This notion is supported given that results were mostly unchanged in the sensitivity analysis compared with the original analysis. A call to improve reporting has been made in other research designs and imaging modalities. This has led to the successful introduction of checklists such as PRISMA (Moher et al., 2015) in systematic reviews, and the CONSORT (Schulz et al., 2010) in randomized controlled trials and more specifically in functional MRI (Poldrack et al., 2008). Whilst there have been three white papers recommending the optimal spectroscopy parameters for use in MEGA-PRESS (Mullins et al., 2014), PRESS (Wilson et al., 2019) and Universal (Saleh et al., 2019) this has yet to be translated into a standardized methodological reporting tool. We believe the MRS-Q, introduced and developed in this study is an important first step. Both our finding (only 46% of studies reporting using adequate parameters) and the call to improve reporting in other fields suggests the need for the field of MRS to develop a standardized reporting tool. We propose the MRS-Q could be further validated for this purpose.

There was insufficient data to establish whether brain region influenced differences in neurometabolite levels. The results presented here demonstrate that there were inconsistencies in voxel naming, shaping and positioning. An example is in the ACC where several studies positioned a long rectangular voxel dorsally along the corpus collosum (Gussew et al., 2011; Widerstrom-Noga et al., 2013), yet others used a shorter voxel positioned rostrally (Harper et al., 2018; Prescot et al., 2009; Reckziegel et al., 2016), without adjusting the nomenclature accordingly. While we aimed to pool data based on brain region within pain groups, there were insufficient data to do so. The most frequently studied brain region was the occipital lobe in people with migraine. Pooled results for the occipital lobe demonstrated a significant increase in level of Glx and glutamate in migraine compared to controls. The occipital lobe has been frequently studied in both headache and mental health studies partially owing to the high-quality spectra that can be obtained compared with other brain regions (Puts and Edden, 2012). Hence, the significant findings found in people with migraine may be due to the more homogenous field allowing more consistent findings, resulting in narrower confidence intervals, rather than the region being clinically different from other regions. Nonetheless, for people with migraine, the occipital lobe may be relevant to study, due to its's role in migraine with aura (Charles and Brennan, 2010; Hadjikhani et al., 2001). Despite these observations, comparison of brain neurometabolites between brain regions requires further primary studies.

There are several limitations that need to be considered when interpreting the findings of this review. Our meta-analyses pooled results from studies that reported neurometabolite levels using absolute concentrations, institutional units and ratios. This firstly assumes these measures are reflecting the same variable, and in the case of ratios and institutional units assumes the creatine and water remain stable. Whilst there is some evidence that the denominator neurometabolite, most commonly creatine, is indeed stable across various conditions including pain (Chang

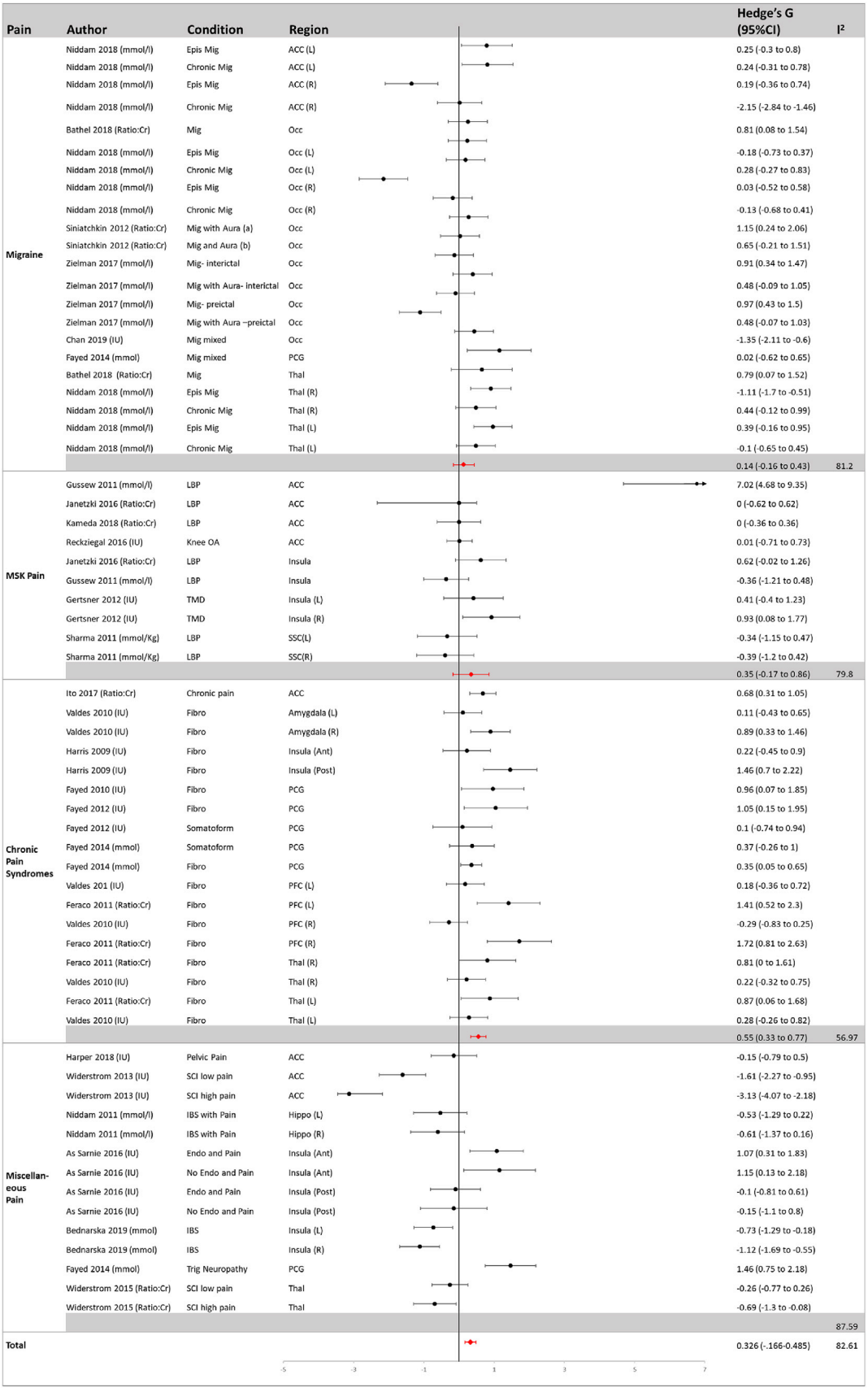

Fig. 7. Glx: Analysed by pain conditions.

et al., 2013; Govindaraju et al., 2000; Gussew et al., 2011) there still remains some uncertainty (Rae, 2014). Steen et al. (2005) were able to demonstrate equivalence between studies measuring both ratios and absolute values, further supporting their inclusion in the meta-analysis. Secondly our primary analysis assumed independence of brain regions and included data from different brain regions of the same participants, which may cause over inflation of results. Therefore, we conducted a post-hoc meta-analysis (not shown) akin to Schur et al. (2016) and Luykx et al. (2012) and demonstrated that averaging brain neurometabolite concentration across all brain regions had minimal effect to the overall results with the exception of glutamine in migraine. Finally, our primary analysis included studies regardless of quality, the sensitivity analysis used only studies that reported using acquisition parameters that satisfied minimal best practice as determined by published clinical consensus (Mullins et al., 2014; Wilson et al., 2019).

The accuracy of quantification of GABA and Glutamate is continually developing, and we can expect to see considerable advances in the field with improved methods of macromolecule suppression, better analysis techniques, and further insight into the application of partial volume correction. Whilst we acknowledge these aspects can create heterogeneity and variation in outcome measures the synthesis of information remains important to help inform future directions in biomarker and pain research.

In conclusion this meta-analysis serves to catalog what is known in the field of excitatory and inhibitory neurometabolites in pain conditions. Furthermore, it provides evidence that unique neurometabolite signatures may exist in different pain conditions. The main limitation in the field of spectroscopy is failure to adequately report acquisition parameters and calls for the development and integration of a standardized reporting tool for magnetic resonance spectroscopy research, allowing for improved reproducibility and validation of prior work.

Declaration of competing interest

Researchers Trudy Rebbeck, Maria Eliza Aguila, and Andrew Leaver were authors of a paper included in the review (Aguila et al., 2015). These three authors had no role in the data extraction or quality assessment of any of the included papers. No other competing interests to disclose.

Author contribution

AP, TR, AL conceived the study idea and designed the study protocol; AP carried out the searches; AP, TR, JW, MA, AL screened studies for inclusion; AP, JW, NP completed data extraction; AP, NP designed and developed the MRS-Q; AP, NP, JW assessed quality of studies using AXIS and MRS-Q; AP conducted meta-analysis; AP, TR, NP, AL wrote and edited manuscript.

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Julia Watson Queensland University of Technology - Translational Research Institute (TRI) Radiographer Research Scholarship, Australia.

Appendix 1. Search strategy from OVID

Mesh	Keyword
gamma-aminobutyric acid	gaba.mp
glutamic acid	glutamate.mp
glutamine	Glx
brain chemistry	brain adj 3 chemistry.mp
neurotransmitter agents	neurotransmitter*
	neurochemical*
	metabolite*
	brain metabolite*
	neurometabolite*
AND	
spectrum analysis	spectroscop*.mp.
magnetic resonance imaging	(magnetic resonance and (imag* or spectroscop*)).mp.
magnetic resonance spectroscopy	magnetic resonance spectroscopy.mp
proton magnetic resonancy spectroscopy	proton magnetic resonancy spectroscopy.mp
	mega press
	1 hmrs
	1h-mrs
	in-vivo mrs
	Mr
	Mrs
	mr-specto*
	nmr
	in-vivo nmr
AND	
pain- exp	pain
migraine disorders	migraine.mp
back pain exp	back pain
low back pain	low back pain
fibromyalgia	fibromyalgia.mp
chronic pain	chronic pain
headache	headache
headache disorders primary exp	

(*continued on next column*)

(*continued*)

Mesh	Keyword
headache disorders, secondary exp	
migraine with aura	migraine with aura
migraine without aura	migraine without aura
musculoskeletal pain exp	musculoskeletal pain
whiplash injuries	whiplash
cancer pain exp	cancer pain.mp
wounds and injuries exp	
peripheral nervous system exp	
trauma nervous system	

Appendix 2. Data extraction sheets

Data extraction sheet 1: Bibliometric data, and clinical characteristics.

Study #	Study	Country	Funding Source	Prospectively Registered	Mean Age	Age: if other measure used HC (Healthy control):Mean, SD; Patient group: Mean, SD	Female (%)	Pain Category	Description of Pain Condition	Subjects recruited From	Duration of Illness (months)	Excluded Comorbidities	Co-morb Comments	Meds Stopped?	Meds Comments	Pain Score	Other Outcomes	Sx

Data extraction sheet 2: Spectroscopy parameters.

Study	Scanner Make	Scanner Strength	Head Coil	Sequance	Brain Region	Voxel size	TR/TE (ms)	Averages	Number of Points	Spectral Width	Shim (Hz)	Quantification	Voxel based morphometry	Analysis Software	Metabolites

Data extraction sheet 3: Results.

Study	Pain Condition	Metabolite	Region	Pain									Control								Measure	Std diff means	p-value	Comments
				N	Mean	SD	SEM	Median	IQR-Low	IQR-High	Range	Lower 95% CI	Upper 95% CI	N	Mean	SD	SEM	Median	IQR-Lower	IQR-Higher	Range	Lower 95% CI	Upper 95% CI	Unit	

Appendix 3. Modified AXIS marking sheet-adapted from Downes et al., 2016

Questions				
Introduction		Yes	No	Don't Know/Comment
1	Were the aims/objectives of the study clear?			
Methods				
2	Was the study design appropriate for the stated aim(s)?			
3	Was the sample size justified?			
4	Was the target/reference population clearly defined?			
5	Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?			
6	Was the selection process likely to select subject/participants that were representative of the target/reference population under investigation?			
7	Were measures undertaken to address and categorise non-responders?			
8	Co-morbidities Excluded Were meds stopped/restricted/or adjusted for Were other confounders accounted for			
9				

(*continued on next column*)

(*continued*)

Questions	
	Were the risk factors and outcome variables measured correctly using instruments/ measurements that had been trialled, piloted or published previously
10	Is it clear what was used to determine statistical significance and/or precision estimates? (e.g. p-values, confidence intervals)
11	Were the methods (including statistical methods) sufficiently described to enable them to be repeated?
Results	
12	Were the basic data adequately described?
13	Was there a full justification of any scans excluded from the analysis
14	Were the results internally consistent?
15	Were the results presented for all the analyses described in the methods?
Discussion	
16	Were the authors discussions and conclusions justified by the results?
17	Were the limitations of the study discussed?
Other	
18	Were there any funding sources or conflicts of interest that may affect the author's interpretation of the results?
19	Was ethical approval or consent of participants attained?

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