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New frontiers in neuroimaging applications to inborn errors of metabolism

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Abstract

Most inborn errors of metabolism (IEMs) are associated with potential for injury to the developing central nervous system resulting in chronic encephalopathy, though the etiopathophysiology of neurological injury have not been fully established in many disorders. Shared mechanisms can be envisioned such as oxidative injury due to over-activation of N-Methyl-D-Aspartate (NMDA) receptors with subsequent glutamatergic damage, but other causes such as energy depletion or inflammation are possible. Neuroimaging has emerged as a powerful clinical and research tool for studying the brain in a noninvasive manner. Several platforms exist to study neural networks underlying cognitive processes, white matter/myelin microstructure, and cerebral metabolism *in vivo*. The scope and limitations of these methods will be discussed in the context of valuable information they provide in the study and management of selected inborn errors of metabolism. This review is not meant to be an exhaustive coverage of diagnostic findings on MRI in multiple IEMs, but rather to illustrate how neuroimaging modalities beyond T1 and T2 images, can add depth to an understanding of the underlying brain changes evoked by the selected IEMs. Emphasis will be placed on techniques that are available in the clinical setting. Though technically complex, many of these modalities have moved – or soon will – to the clinical arena.

Keywords

Brain injury; Diffusion tensor imaging (DTI); Functional MRI (fMRI); Inborn error of metabolism (IEM); Magnetic resonance imaging neuroimaging; Magnetic resonance spectroscopy (MRS)

1. Introduction

Many inborn errors of metabolism (IEMs) are associated with irreversible brain injury [1–6]. It is unclear how metabolite intoxication or substrate depletion accounts for the specific cognitive and neurologic findings observed in IEM patients. IEM-associated brain injury patterns are often characterized by whether the process primarily involves gray matter, white matter or both, and beyond that, whether subcortical or cortical gray matter nuclei are involved. Although expected to be global insults, many IEMs result in selective injury to deep gray matter nuclei or white matter, such as the putamen (and to a lesser degree, the globus pallidi) in glutaric aciduria type 1 [7], putamen in certain mitochondrial cytopathies [8], or the globus pallidus in methylmalonic acidemia [9]. Selectivity for particular brain

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regions or even cell types based on morphology or neurotransmitter systems (astrocytes/neuron: glutamatergic, GABA-ergic) is poorly understood. The presenting neurological features may suggest gray or white matter involvement. Patients with cortical gray matter involvement may present with seizures, encephalopathy or dementia, whereas deep gray matter injury typically manifests with extrapyramidal findings of dystonia, chorea, athetosis, or other involuntary movement disorders when the basal ganglia are involved. White matter disorders feature pyramidal signs (spasticity, hyperreflexia) and visual findings. Involvement of the cerebellum or its connecting tracts may lead to ataxia.

Neurological injury may be attributable to either a substrate intoxication or substrate depletion model of injury. Metabolic disorders caused by substrate intoxication include amino acidopathies such as phenylketonuria (PKU) and maple syrup urine disease (MSUD) and organic acidurias such as urea cycle disorders, PA (propionic aciduria), methylmalonic aciduria (MMA) and glutaric aciduria type 1 (GA-1). Substrate depletion disorders include the creatine deficiencies (due to guanidinoacetate methyltransferase and L-arginine:glycine amidinotransferase deficiency (GAMT and AGAT) or due to mutations in the creatine transporter (*SLC6A8*)).

Patterns of injury depend on whether damage affects the gray matter (neurons), white matter (astrocytes), basal ganglia (deep gray matter) or a combination of brain regions. For example, many amino acidopathies and urea cycle disorders affect the white matter, although with early onset and longstanding disease, gray matter damage becomes apparent [10]. Patients with IEMs may develop severe clinical symptoms at any age, however, in some cases onset of symptoms and resulting brain injury occurs before a particular age, suggesting that vulnerability is restricted to a limited period of brain development [11]. Recognition of markers of brain injury vulnerability is needed to for effective clinical intervention that prevents, attenuates or ameliorates injury.

2. Neuroimaging and IEMs

Neuroimaging has manifold potential for the investigation and management of IEMs, allowing clinicians and researchers to non-invasively study the timing, extent, reversibility, and mechanisms of neural injury [12]. Indeed, the term “neuroimaging” has grown in recent years to encompass a wide array of investigative modalities, each with unique strengths that can be combined to gain complementary information regarding the brain’s structural, functional and metabolic dimensions, and how these may be altered in pathologic states (Table 1). For example, structural magnetic resonance imaging (MRI), which encompasses T1- and T2-weighted MRI, fluid attenuation inversion recovery (FLAIR), and voxel-based morphometry (VBM), reveals the brain’s macroscopic structure, functional magnetic resonance imaging (fMRI) is used to study the neural nodes and networks underlying cognitive operations [13], diffusion weighted and diffusion tensor imaging (DWI and DTI) are used to study microstructural variance in white matter fiber tracts [14,15], and magnetic resonance spectroscopy (MRS) is used to measure brain metabolism in static and dynamic models [16]. Using these various methods, one can probe focal, regional and global neuropathological sequelae, and monitor disease progression and response to therapies. Here, we offer a brief introduction to the principles and applications of each of these neuroimaging modalities, and go on to review how they have added to our understanding of neurologic manifestations in an array of IEMs.

3. Neuroimaging modalities: a brief introduction

3.1. Magnetic resonance imaging (MRI)

MRI technology forms the basis of many of our most powerful tools for interrogating the intracranial environment. Fundamentally, MRI exploits differences in water proton spins between tissue types upon exposure to radiofrequency (RF) perturbations in a strong magnetic field. When a tissue is placed in a high-field magnet, water protons align along the direction of the magnetic field (commonly referred to as B_0). A computer-generated RF pulse is then administered perpendicular to the plane of the magnetic field in order to perturb protons from their axes. Following the termination of the RF pulse, the magnitude and rate of energy release that occurs with return to baseline alignment (T1 relaxation) and with the wobbling (precession) of protons during the process (T2 relaxation) are recorded as spatially localized signal intensities by a receiver coil. The coil serves as an antenna, converting electromagnetic waves into an electrical current, which is used to reconstruct three-dimensional images of the tissue under study. The return of the excited nuclei from a high to low energy state is associated with the loss of energy to the surrounding nuclei. T1 relaxation (spin-lattice) is characterized by the longitudinal return of the net magnetization to its maximum length in the direction of the magnetic field (B_0). T2 relaxation (spin-spin relaxation) occurs when the spins in the high and low energy state exchange energy but do not lose energy to the surrounding lattice. Macroscopically, this results in a loss of RF-induced transverse magnetization.

MRI provides multiple channels to observe the same anatomy. The relative signal intensity (brightness) of various tissues (gray matter, white matter, CSF) in MR images is determined by multiple factors, including the radiofrequency pulse and gradient waveforms used to obtain the image. An invaluable clinical tool, MRI allows anatomic characterization of gray matter and white matter and macrostructural features, and can be used to detect and classify pathological processes in disease.

Despite its far-reaching utility as both a research and clinical tool, routine MRI detection is limited to macroscopic alterations in brain structure. It lacks the spatial resolution to provide information regarding microstructural neuropathology, and does not capture dynamic processes in space and time related to brain function and metabolism. Additionally, many macrostructural neuropathologic phenotypes lag behind the presentation of associated clinical manifestations.

MRI is a versatile imaging modality, and holds particular advantages relative to computed tomography (CT) or positron-emission tomography (PET), which require the manipulation of contrast. In both of these methods, image contrast is often achieved via exogenous contrast agents or tracers.

In MRI, three different magnetic field gradients (i.e. static, circular polarized, (linear) orthogonal gradients) interact and several data acquisition parameters need to be adjusted in order to obtain reliable data. The advantages of MRI over CT are manifold including lack of ionizing radiation, ability to image in three orthogonal planes and the ability to visualize deep structures (brainstem) and cerebellum with better resolution. MRI is superior to CT in evaluation of white matter pathologies.

MRI has several logistical limitations including longer imaging sequence times which often mean sedation in the pediatric setting, movement which degrades image quality and it is not often available in the urgent setting. Furthermore, MRI procedures are significantly more costly than CT scanning, and may be subject to further restrictions from third party payers.

While field strength is important, the wavelength within the human body dictates ultimate information content. At lower field strength, RF waves in tissue are longer.

3.2. Anatomical FLAIR imaging

Fluid-attenuated inversion recovery (FLAIR) imaging is an MRI-based methodology that is sensitive to increases in interstitial water content in CNS diseases [17]. It plays a role in delineating white matter integrity in brain tumors, multiple sclerosis, cerebral infarcts, and metabolic white matter diseases. FLAIR works by nulling signals from fluids with long longitudinal (T1) relaxation times. Lesions adjacent to CSF are often more clearly identified on FLAIR. Fig. 1b shows a FLAIR image of white matter injury in patients with Urea cycle disorders (UCD), an easily overlooked finding in traditional MRI scans. FLAIR is useful in disorders of myelination (hypomyelinating and demyelination), and as a tool for classifying normal baseline characteristics of myelin in the subcortical U fibers (the arcuate fibers at the cortical-white matter junction), deep white matter, and periventricular white matter. Myelination is incomplete at birth, and, in healthy children, develops in a predictable, age-specific pattern. Because many IEMs cause white matter injuries in the developing brain, FLAIR is an effective means of comparing neural development in the IEM disease state to established baseline phenotypes from healthy individuals.

3.3. Functional magnetic resonance imaging (fMRI)

Over the past two decades, functional MRI (fMRI) has emerged as an invaluable tool for imaging the time course of activity associated with neurocognitive processes in the brain. This technology has wide-ranging applications for both basic research into brain function, and for clinical research into the neurophysiology of neurologic and psychiatric illness. fMRI investigation of IEMs offers potential insight into the psychosocial challenges experienced by IEM patients, and may help guide the development of educational support structures.

First described by Roy and Sherrington in 1890 [18], neuronal metabolism and cerebral blood flow are intimately linked by neurovascular coupling. fMRI exploits this phenomenon in order to generate a blood oxygenation level-dependent (BOLD) signal in activated regions of the brain. Through the energy-expensive process of neurotransmission, active neurons consume glucose and oxygen, which are restored by local dilation of neural vasculature and subsequent increases in the flow of oxygenated hemoglobin. MRI pulse sequences are sensitive to the magnetic contrast between oxygenated and deoxygenated hemoglobin, and can be used to map the hemodynamic response of local brain regions to the stimuli of a neurocognitive task presented to the subject in the scanner (for a review of fMRI methods and applications, see Logothetis, 2008 [19]).

The signals received by the MRI's receiver coil are digitally reconstructed into a 3-dimensional matrix. Each 3D cell within the matrix is referred to as a voxel. Voxel sizes for fMRI data commonly exist on the order of 2–3 mm³. Following multiple statistical processing steps, each voxel contains a numerical index of BOLD signal intensity. Activation maps are created for a given task condition (e.g. a memory cue in a working memory task), and these maps are then averaged across multiple subjects to generate a group-wise image of the activation elicited by the task paradigm. Statistical analyses are performed to assess BOLD signal differences between experimental conditions (for example, between working memory and non-working memory trials), between different subject populations (for example, between healthy individuals and patients with a neurologic disorder), or both.

Functional MRI allows brain function to be imaged and quantified non-invasively, and data can be collected over relatively short experimental sessions. The principal drawback of fMRI, however, is that inter-subject variability in the spatial extent and precise location of neurocognitive functions within the brain require relatively large numbers of patients in order to achieve an acceptable signal-to-noise ratio, which is often impractical in rare disorders. Additionally, activation differences between disease groups and healthy controls are often subtle, and may represent BOLD signal changes that exist on the order of 1–2%. As such, it is difficult to characterize neurocognitive phenotypes at the single subject level using fMRI, making it a useful tool for exploring alterations in brain function at the group level, but limiting its diagnostic applications in individual patients.

3.4. Voxel-based morphometry (VBM)

Like fMRI, voxel-based morphometry (VBM) is a useful tool for the comparison of brain parameters in a clinical population relative to a group of healthy controls. Whereas fMRI allows the investigator to assess differences in brain function, VBM provides a platform for imaging volumetric differences in brain structures. As in fMRI, VBM relies on the representation of the brain as a 3D matrix of voxels, but rather than indexing BOLD signal, each voxel in the VBM matrix provides an index of local brain volume.

In order to compare brain size (and the size of particular structures within the brain) across groups, each participant's imaging data must be registered to a common 3D template to account for gross variability in brain morphology, allowing subtle differences in brain structure to be appreciated. VBM templates are commonly an average image comprised of scans from large numbers of healthy subjects. The template image provides morphological landmarks that allow the brain to be segmented into gray matter and white matter structures. Analysis of VBM data across patient and control groups allows the investigator to assess not only global differences in whole brain, white matter and gray matter volume throughout the brain, but also fine differences within particular regions of interest at the voxel level. For a detailed review of VBM methods, see Ashburner and Friston [20].

As with fMRI, VBM's utility primarily lies in the comparison of groups of subjects to quantify population-level differences in brain structure within a clinical group, although single-subject data has been reported in IEMs (see "Ornithine Transcarbamylase Deficiency (OTCD) and Urea Cycle Disorders (UCD)"). In order to generate definitive group-level results, sample size requirements often exceed the number of IEM patients that can practically be recruited, owing to the rarity of many of these disorders, and clinicians may prefer traditional MRI, FLAIR or diffusion imaging to assess structural abnormalities at the single-subject level.

3.5. Diffusion-weighted and diffusion tensor MR

Diffusion MRI encompasses diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI), both voxel-based modalities which provide indices of axonal integrity *in vivo* by measuring water movement in white matter. DWI measures contrast between brain regions varying with respect to water diffusivity. Diffusion of water within a magnetic field gradient reduces the MR signal, and as a result, regions in which diffusion is restricted evince lower signal loss and brighter appearance on DWI images, relative to regions of high diffusivity. This signal is called the apparent diffusion coefficient (ADC) and is used to identify brain regions with abnormally high (high ADC) or low (low ADC) diffusivity, providing a means of identifying pathologic abnormalities in the brain's white matter microstructure. Diffusion tensor imaging (DTI) is also sensitive to the ordered movement of water in the brain, but whereas DWI scales each voxel to indicate the degree of water diffusion, DTI indicates the direction of water diffusion in three-dimensional space.

Acquisition of multiple pulse sequences with varying 3D orientation generates a tensor, which indicates the orientation of water diffusion. Ordered water diffusion is said to be anisotropic, due to its lack of randomness, and DTI images render values of fractional anisotropy (FA) to indicate the direction of water diffusion. Decreased FA in the white matter is generally interpreted as a signal of compromised axonal integrity. Taken together, these measures provide a powerful and sensitive basis for inferences about white matter micro-structure in healthy and disease states [15].

Pathologic variations in these measures serve as indices of white matter injury, although the complexity of white matter microarchitecture underscores the need to interpret DTI findings in the context of other disease-specific parameters, including additional imaging modalities and relevant clinical parameters. Depending on the nature of the pathology, either ADC or FA values may be increased, decreased, or show a mixed pattern.

Over the past decade, DTI has emerged as a valuable means of characterizing white matter structure and its alterations in an array of clinical contexts. Its sensitivity to microstructural abnormalities not visible with traditional MRI, and its ability to predict clinical correlates of white matter pathology underscore the value of this imaging platform for multimodal imaging studies of disorders affecting the central nervous system.

While DTI offers a powerful tool to study and visualize white matter, it suffers from artifacts and other technical limitations. Namely, partial volume effect and the inability of the model to accommodate non-Gaussian diffusion are its two major limitations. Eddy currents induced when strong gradient pulses are switched on and off rapidly are another source of artifact. When the diffusion gradient pulses are switched on and off, the time-varying magnetic field of the gradients results in current induction (eddy currents) in the various conducting surfaces of the rest of the MRI scanner. These eddy currents set up magnetic field gradients that may persist after the primary gradients are turned off. Eddy currents generate magnetic field gradients that combine vectorially with the imaging gradient pulses such that the actual gradients experienced by spins in the imaged objects are not exactly the same as those that were programmed to produce and reconstruct the image. Newer acquisition protocols for DTI employ eddy current compensations or corrections [21].

3.6. Proton MR spectroscopy (^1H MRS)

Magnetic resonance spectroscopy (MRS) is a powerful clinical tool for interrogating the neurochemical environment and characterizing the metabolic features of neurologic disease. While imaging coils have been tailored for sensitivity to a variety of isotopes such as ^{13}C and ^{31}P , proton (^1H) spectroscopy is the most commonly used form of MRS, and can be administered on standard MRI hardware at 1.5 T and above. ^1H MRS produces a spectrum of peaks that contains information about biochemical concentrations in a region of interest (typically 1–10 cm^3) within the brain. These spectra contain information about brain metabolite concentrations relevant to a variety of neurological conditions including brain tumors, traumatic brain injury, white matter disorders, epilepsy and metabolic disorders.

MRS exploits the property of proton spin and the manner in which protons absorb electromagnetic radiation when stimulated by a radiofrequency (RF) pulse. When the RF matches the proton spin frequency, known as the Larmor frequency, the proton enters an excited quantum state. As it relaxes back to its unexcited state, the proton emits electromagnetic radiation, which is detected by the MRI head coil and computationally transformed into a 2-dimensional spectrum. MRS spectra contain information about the identity (indexed on the X-axis by the resonant frequency, given in parts per million) and

relative abundance (indexed on the Y-axis by the relative height of peaks within the spectrum) of metabolites within a given neural region of interest.

3.7. What does MRS tell us about in vivo chemistry?

For an element to be studied by MRS it should lend itself to excitation by magnetic resonance, it should be present in a detectable concentration on the order of mg and should produce an acceptable signal-to-noise ratio. These conditions are met by ^{31}P , ^1H , ^{19}F , ^{13}C , ^7Li , and ^{23}Na .

3.8. Types of MRS used in IEM

^{31}P MRS has been the most widely applied technique because of the 100% of all phosphorus nuclei in the body so no labeling is required and the sharp NMR signals for a variety of important phosphorus-containing compounds, however, it is cumbersome and most clinical scanners do not possess a phosphorus head coil nor the expertise to perform these studies. Instead, application of ^1H MRS has been amenable to clinical care. One limitation of ^1H MRS is that the range of visible chemical shifts is much narrower (10 ppm) than that of ^{31}P MRS (40 ppm). Thus, detection of individual resonances is more difficult. Stronger magnet fields will improve the resolution of ^1H spectra and better resolve those compounds with multiple, overlapping peaks.

The ability to differentiate chemical compounds on the MRS spectrum depends on the differences between compounds in the chemical configuration of protons within the molecule, and how these configurations differentially affect the electromagnetic microenvironment in the region being sampled. Commonly assayed metabolites, such as N-acetyl aspartate (NAA), creatine, choline, myoinositol and lactate, to name a few, have unique spectral signatures by virtue of their unique chemical configurations, which allow them to be independently identified on an MRS spectrum. The relative abundance of metabolites of interest can be determined by comparison of the peak heights between two compounds. Successful use of clinical MRS spectra requires a radiologist trained to recognize the patterns in which neurochemical abnormalities elicit deviations from normal MRS spectra, and clinical correlation to determine how these abnormalities may contribute to the diagnosis, course and management of disease.

To-date there are no clear guidelines for the use of MRS in childhood neurological diseases. Additionally, it has been difficult to acquire reimbursement from third party payers for MRS studies, despite its additive contribution to diagnosis and management [22]. A recent, comprehensive review by Panigrahy et al. is recommended [23]. Furthermore, MRS includes the requirement for stronger and more homogeneous magnetic fields than other modalities of MRI and also needs “shimming” for each measurement, making it more expensive in time and cost.

3.9. ^{13}C MRS

While ^1H MRS is a sensitive tool to detect brain biochemical abnormalities in individual patients, the specificity suffers from the complex peak pattern due to J-coupling and signals from different compounds co-resonating at similar chemical shifts. *In vivo* ^{13}C MRS can reliably quantitate distinct signals from Glu and Gln. In contrast to conventional proton MRS, which non-invasively assays metabolites at their steady-state concentrations, ^{13}C MRS depends upon the absence of natural abundance signal in normal brain, and the highly specific chemical shift of MR signal that arises from the stable isotope when introduced via the blood stream into the normal brain metabolite pools [24].

While ^{13}C MRS enjoys the marked advantages of chemical specificity and lack of background signal, its major disadvantage is its inherently low sensitivity and the need to administer exogenous ^{13}C -labeled glucose. Additionally, spatial and temporal resolution is insufficient to be routinely applicable in the clinic.

3.10. SPECT and PET imaging in IEMs

SPECT and PET are conventional imaging techniques well-suited to the study of neurochemistry and neurotransmission [25,26]. Using dynamics related to cerebral metabolism and blood flow, these modalities make it possible to detect abnormal tissue function and neurotransmitter synthesis. Each, however, requires a team of specialists and specific equipment, making their use somewhat complicated and expensive.

PET also requires peripheral injection of radiotracers. When PET tracers degrade, they emit positrons, which collide with electrons, and emit two photons that travel in opposite directions (180° apart). These photons are detected by rotating cameras positioned outside the head. The images rendered are of higher resolution in PET than in SPECT. The majority of PET imaging studies use labeled glucose (18-FDG) or oxygen (O_2) in the water form. These compounds must be produced in a nearby cyclotron, adding significant cost and limiting practical clinical utility as not all medical centers have a cyclotron.

SPECT requires peripheral injection of a radiotracer which settles into neurons and glia within 2–5 min, diffusing with varying concentration throughout the brain depending on cerebral blood flow. Specific tracers target particular receptors, revealing their location and relative distribution. As they decay, these radiotracers emit gamma rays that are detected by rotating cameras to create a three dimensional image. The most commonly used tracers include HMPAO and those tagged to specific receptor ligands such as D1 and D2 dopamine receptors.

Like PET, SPECT also can be used to differentiate different kinds of disease processes and has been applied mainly to dementia and epilepsy clinical and research work. There has been little done in the field of IEMS.

Uptake of SPECT agent is nearly 100% complete within 30–60 s, reflecting cerebral blood flow (CBF) at the time of injection. These properties of SPECT make it particularly useful for mapping epileptic foci in epilepsy imaging. A significant limitation of SPECT is its poor resolution as compared to MRI.

4. Does field strength matter?

Of the techniques covered in this review, imaging techniques originally developed at 1.5 T and applicable at 7 T include high-resolution anatomical MRI, and functional MRI. Some ^1H MRS has been performed on a 7 T scanner on a research basis. There is a gain from increased image signal to noise ratio (SNR). Anatomy and fMRI have benefitted from increased in field strength.

If one compares, for example, 7 T versus 3 T or 3 T versus 1.5 T the major advantages of the high field are an increased signal-to-noise (S/N) which allows a higher spatial resolution as well as reduced scanning times, which is important in pediatrics where the majority of the patients require sedation.

Higher field also affords a significantly increased sensitivity to differences in tissue magnetic susceptibility at the microscopic scale, introducing a new contrast mechanism. High field is favorable for ^1H MRS for increasing the spectral resolution for localized MRS. As many improvements that high field permits, there are significant challenges as well. For

example there is the problem of greater signal inhomogeneity within the patient due to the interaction of high frequency (298 MHz) electromagnetic energy with the body. One concern in moving to high field is the higher energy deposition to the patient, as measured by the term specific absorption ratio (SAR), in the patient with the potential of localized “hotspots” or areas of local tissue heating. There is also greater artifact from macroscopic static field (B_0) inhomogeneities at tissue/air and tissue/bone boundaries. In addition, tissue T1 relaxation times are longer at higher field and T2 values decrease, which reduces some of the improvements in S/N from the high field. Whereas higher spatial resolution at higher field would be expected to be beneficial to diffusion tensor imaging (DTI), a major problem is the increased macroscopic susceptibility effect.

^1H MRS resolution can substantially gain from the high field. Non-proton techniques, such as ^{23}Na and ^{31}P , gain even more since they are very insensitive at low fields. Better SNR afforded by higher field leads to increased spectral dispersion (line splitting), enabling improved quantification of metabolites particularly those with J-coupling at lower fields. Acquisition times per voxel will decrease and the time required to perform multivoxel MRS likewise will decrease.

5. Neuroimaging: implications for understanding pathophysiology in IEMs

A systematic approach across these imaging modalities, based on pattern recognition of abnormal brain phenotypes, is useful in the analysis of brain MRI scans in patients with IEMS [27,28]. The clinician must first determine whether the disorder affects the gray matter, white matter or both, and subsequently must decide which additional imaging modalities may be useful to request. A recent review addresses pattern approach in IEMs, and is beyond the scope of this review [29]. Here we review an array of IEMs, and the report use of neuroimaging in the clinical management of these conditions. We note that these disorders vary considerably in the extent to which they have been investigated by neuroimaging. Further, while some, such as OTCD and PKU, have benefited from more study than others, understanding of the neuroimaging phenotypes in all of these disorders is in its early stages and will benefit from further study and larger sample sizes.

5.1. Ornithine transcarbamylase deficiency (OTCD) and urea cycle disorders (UCD)

Ornithine transcarbamylase deficiency (OTCD), is an X-linked inborn error of metabolism and the most common of the urea cycle disorders (UCD). Caused by mutations affecting the ornithine transcarbamylase enzyme, OTCD patients are unable to complete the conversion of carbamoyl phosphate and ornithine to citrulline, and are consequently vulnerable to neurotoxic episodes of hyperammonemia. OTCD is associated with marked neurocognitive deficits in patients, as well as in asymptomatic carriers of the disease-causing allele [30]. Affected cognitive domains include non-verbal learning, fine motor processing, reaction time, visual memory, attention, executive function and math skills, and deficits in these capacities may be seen in symptomatic patients, as well as asymptomatic carriers with normal IQ [31].

Numerous studies have suggested that brain structure is affected in OTCD, and that these effects may manifest along a continuum of severity. Kurihara and colleagues [32] reported the presence of round subcortical white matter lesions on T1- and T2-weighted MRI in two patients with advanced late-onset OTCD. Similar lesions were not found in earlier onset cases within this series, suggesting that macroscopic alterations may not appear on traditional MRI in the early stages of this disorder. Recently, DTI has been used to identify structural correlates of cognitive impairment and disease severity in OTCD [33]. DTI analysis conducted on a cohort of 19 OTCD patients revealed reduced FA in frontal white matter in OTCD patients relative to controls, with the degree of FA reduction predicting

disease severity among patients. This finding lends potential support to the prior findings of neurocognitive deficits on prefrontal cortex-based tasks, and suggests that subtle microstructural disturbances not detectable on conventional MRI may underlie the cognitive manifestations of both symptomatic and asymptomatic OTCD.

Case reports using VBM and DTI have also offered suggestions regarding underlying pathology in other UCDs. Majoie and colleagues [34] reported a case of neonatal type I citrullinemia, a UCD caused by mutations affecting the argininosuccinate synthetase enzyme. Resulting disruptions in ammonia metabolism cause lethargy, vomiting, respiratory distress and hyperammonemic coma within the first days of life. Early postnatal DWI revealed decreased ADC throughout the brain, suggestive of cytotoxic edema caused by increased ammonium osmolarity in astrocytes. Follow-up DTI at 4 months revealed decreased FA throughout the brain relative to age, indicating systemic myelin destruction and loss of white matter integrity. In a single case of adult-onset type II citrullinemia (CTLN2) [35], VBM was performed following liver transplantation in order to investigate recurrent seizures, revealing reduced left hippocampal volume and suggesting hippocampal sclerosis as the likely cause of seizures in this patient. A recent case report [36] has also used DTI to demonstrate white matter structural correlates of spastic diplegia in arginase deficiency, which is caused by mutations affecting arginase, the final enzyme in the urea cycle. Arginase deficiency is the least common of the urea cycle disorders, and unlike other UCD, neural injury is believed to be unrelated to hyperammonemia. DTI analysis revealed reduced FA in the corticospinal tracts (Fig. 2) and in OTCD, there are differences in the corpus callosum relative to age-matched healthy controls (Fig. 3). Taken together, all of these findings point to heterogeneity in patterns of white matter pathology among the UCD.

Investigations of neurometabolic abnormalities in OTCD using ^1H MRS have revealed a set of useful biomarkers that can help guide diagnosis and treatment of this disorder. In a cohort of six late-onset OTCD patients, Takanashi and colleagues [37] reported increased glutamine and decreased myoinositol as common manifestations of OTCD, with normal levels NAA and creatine. These authors also reported that elevated glutamine levels scaled with disease severity, and that choline reductions were found in two severely affected patients, indicating a potential marker of disease progression. Comparison of ^1H MRS findings in two sisters, one symptomatic and the other asymptomatic for OTCD, relative to a healthy control, revealed decreased myoinositol and increased glutamine signals in both OTCD patients [38]. These ^1H MRS abnormalities were more pronounced in the symptomatic sister, suggesting that asymptomatic OTCD may have a detectable neurochemical signature. This hypothesis was further supported in a cohort of 25 OTCD patients [39], in which myoinositol reductions and glutamine increases significantly predicted disease severity throughout an array of brain regions.

Currently, there are no published fMRI data available for OTCD or any of the other UCDs.

5.2. Phenylketonuria (PKU)

Phenylketonuria (PKU) is caused by genetic mutations in phenylalanine hydroxylase, which catalyzes the hydroxylation of phenylalanine (Phe) to tyrosine (Tyr). Disruptions in this pathway result in hyperphenylalanemia and deficits of other large neutral amino acids in the central nervous system, leading to IQ reductions in untreated individuals, and subtle executive deficits in treated patients with normal IQ [40].

An early case PKU series published by Thompson and colleagues [41] described a pattern of periventricular white matter abnormalities seen in all six patients imaged with T2-weighted MRI. These signal abnormalities were seen to intensify with clinical deterioration in one patient with serial scans, and subsequently improved with resumption of dietary Phe

restriction. A subsequent study imaged 74 PKU patients [42] with the aim of investigating correlations between dietary Phe control and MRI signal abnormalities. The authors found that white matter signal abnormalities were present in all but one patient, and that in addition to diffusely increased signal in the posterior and anterior periventricular regions, 16 patients exhibited discrete punctate lesions within the parenchymal white matter. Additionally, the severity of signal abnormalities was higher in older patients off of dietary restriction than in younger patients still on diet, and a significant positive correlation existed between Phe levels at the time of scanning and signal abnormalities on T2-weighted MRI. Elevated T2 signal is believed to reflect intramyelinic edema in PKU patients resulting from the osmotic pressure exerted in increase cerebral Phe levels. See Fig. 4 for a depiction of white matter lesions in a PKU patient.

Preliminary fMRI studies of PKU have been inconclusive, and demonstrate the novelty of functional neuroimaging investigations into IEMs, as well as the need for further work in this area. An analysis of working memory-related BOLD activation in six PKU subjects [43], suggested possible alterations in prefrontal cortex activation and in the connectivity between prefrontal cortex and other nodes in the brain's working memory network. A recent study [44] of BOLD activation during a STROOP task, a paradigm designed to elicit conflicting cognitive color-word representation, found no differences in neural activation between 17 patients with PKU and 15 control subjects. More studies with larger sample sizes are needed to more fully characterize the functional brain abnormalities underlying PKU, and how they relate to the neurocognitive manifestations of this disease.

Since 2001, numerous studies have sought to characterize white matter structural pathology in PKU using both DWI and DTI. These studies have consistently reported significantly reduced ADC values in PKU patients, with preserved FA [45–53], although one study has also reported increased ADC [49], and another has reported normal ADC with reduced FA [54]. Evidence also suggests an inverse correlation between ADC and phenylalanine levels in PKU [55,56], with one study failing to identify pathologic signals on either T2- or diffusion-weighted MRI in individuals whose Phe levels were maintained below 8.5 mg/dl [47]. These findings underscore the vulnerability of white matter to phenylalanemia and demonstrate the importance of dietary control in PKU management. Additionally, studies suggest an early DWI study of PKU in three patients found with lower ADC in adult patients relative to a pediatric patient [49], and a recent study reported an inverse correlation between age and ADC levels in the anterior aspects of the corpus callosum in 34 early- and continuously-treated PKU patients [57]. These findings suggest that, in addition to Phe levels, age may also predict the degree of water restriction in PKU.

Ding and colleagues [45] analyzed eight PKU patients using both DTI and proton density (PD) imaging, another MR-based modality which minimizes T1- and T2-weighted signal to render images sensitive to proton concentrations in tissue. These authors found increased ADC and increased proton density within affected tissue, with normal FA. Taken together, the diffusion MRI findings in PKU strongly indicate reduced water diffusion with increased cell packing and preserved cytoarchitectural geometry. Despite the strong consistency of reduced ADC findings in PKU, however, the mechanisms underlying restricted water diffusion remain a focus of inquiry. It has been proposed that white matter vacuolization may underlie water restriction, with numerous small vacuoles generating signals of low diffusivity [49]. It has also been proposed that increased myelin turnover resulting from damaged white matter may be responsible for reducing ADC values, as white matter remodeling processes cause reductions in the extracellular compartments of axon tracts [49]. Alternatively, some authors have suggested that these abnormalities may result from an edematous manifestation, due either to increased concentrations of intracellular Phe, or Phe-mediated inhibition of Na^+/K^+ pumps, disrupting cellular osmolarity and/or electrolyte

homeostasis. While future studies are needed to resolve these questions, the potential for diffusion MRI to advance understanding of neurologic sequelae in IEMs is clearly demonstrated in the PKU literature, particularly given the increased sensitivity of DTI to white matter pathology relative to traditional MR approaches.

Finally, MRS has also been used to investigate the neurochemical manifestations of PKU. In 2000, Moller and colleagues [58] reported elevated Phe on MRS in the parieto-occipital periventricular white matter in six PKU patients, and revealed limited transport of Phe across the blood-brain barrier, with brain Phe levels significantly lower than serum levels. These authors isolated a population of “atypical” patients with blood Phe elevations, brain Phe reductions and the normal mental status, supporting the importance of brain Phe levels as predictors of PKU-associated mental retardation [58]. Further, the ceiling effect of brain Phe concentration, despite increases in serum levels, suggests LAT1 transporter saturation. MRS has been used to determine whether intake of excess LNAAAs could change brain or plasma levels of Phe through competitive inhibition at the LAT1 transporter [59], resulting in brain Phe reductions in patients on unrestricted diet, and lending further support to the LAT1 saturation hypothesis.

A concern when using MRS is the reproducibility of metabolite content. This has been determined in a number of studies for the most abundant metabolites in the upfield part of the spectrum (0–4 ppm) [60,61]. Depending on the metabolite of interest and details of data acquisition, processing, and fitting, it has been found to vary from a few percentage points to 20% and more, which normally translates to 0.5 mmol/kg of equivalent protons at best. When studying low-concentration metabolites, like phenylalanine (Phe), this problem is more salient as the tissue content of interest can be below 100 $\mu\text{mol/kg}$. Kreis and colleagues [62] have recently shown that with optimized methodology it is possible to determine brain Phe content by ^1H MRS in PKU subjects with a variation in independent sessions of a mere 7 $\mu\text{mol/kg}$, corresponding to a Correlation of Variance of 3%. Thus, careful attention to acquisition parameters is critical in ^1H MRS measures, especially in longitudinal studies that will address changes after therapeutic interventions.

Given the metabolic pathways affected in PKU reduced concentrations of the main dopamine metabolite, homovanillic acid, and the serotonin metabolite, 5-hydroxy-indoleacetic have been reported in the cerebrospinal fluid of PKU patients, suggesting a central impairment of monoamine neurotransmission [63]. This raises the possibility that certain aspects of neurological impairment in PKU patients may be mediated in part by impaired dopamine transmission, affecting the dorsolateral prefrontal cortex, which serves as the seat of executive function performance.

PET is well suited to study this question. Landvoigt and colleagues [64] tested this hypothesis by using PET to measure the tissue utilization of 6- ^{18}F fluoro-L-dopamine (FDOPA) in the brain of adult patients suffering from PKU and in healthy controls. The results showed a qualitatively impaired influx and distribution of FDOPA throughout the brain of adult patients suffering from PKU, which was attributed to competitive inhibition of the LNAA transporter by plasma Phe. Further studies will help refine this hypothesis and allow assessment of therapeutic interventions.

Recently, a pilot study to investigate whether ^{18}F -deoxyglucose PET is an effective tool to study cerebral glucose metabolism in early treated PKU [65]. Patients with PKU in comparison to controls had decreased regional glucose metabolic rates in the prefrontal cortex as would be expected due to executive function deficits in this condition, but were also found to have similarly decreased metabolic rates in the somatosensory, and visual cortices. In contrast this group reported increased activity in subcortical regions including

the striatum and limbic system. Correlation between blood phenylalanine levels on the day of the scan correlated with abnormal activity in subcortical structures. There was an age effect with older age being associated with decreased activity in the prefrontal and visual cortices.

5.3. Maple syrup urine disease (MSUD)

Maple syrup urine disease (MSUD) is caused by genetic defects in the mitochondrial branched chain α -keto acid dehydrogenase complex, blocking the catabolism of branched chain amino acids (BCAA). Untreated patients usually die within the first week of life, while those who receive peritoneal dialysis and BCAA-restricted diet may develop with minimal neurologic complications.

Diffusion MRI has been applied to the study of this disorder, with improved sensitivity to white matter changes relative to traditional MRI methods. Case studies reporting diffusion MRI findings in MSUD suggest that two forms of cerebral edema may exist in MSUD. Decreased ADC suggestive of cytotoxic edema has been described for all cases reported in the literature [66–73], with several reports highlighting coexistent ADC signals in unmyelinated regions [66,69,70]. Increased diffusion is believed to reflect a vasogenic edematous process, in which increased water volumes collect in extracellular compartments. Sakai and colleagues [72] report on a child imaged initially at 8 days of age with follow-up imaging at 18 months. They demonstrate a mutable edematous pattern over this developmental period, with pervasive vasogenic edema on early scanning giving way to a more pronounced pattern of cytotoxic edema on later scanning, appearing to reflect developmental changes in myelination. Additionally, DTI findings demonstrate that reduced FA is also seen in regions of low ADC signal, suggesting that cytotoxic edema is associated with myelin degradation and loss of white matter integrity [69]. It has been shown that low ADC signals seen in acute metabolic decompensation may resolve with appropriate treatment [70], although signals consistent with a vasogenic edematous pattern do not appear to be responsive to treatment [69]. The underlying pathophysiologic mechanisms responsible for these radiologic phenotypes in MSUD are incompletely understood, despite extensive characterization of associated signal abnormalities.

MRS has also been used to characterize neurochemical phenotypes in MSUD. Elevations in leucine, isoleucine and valine and their 2-oxo acids can be detected on MRS in a peak at 0.9 ppm. Heindel and colleagues [74] reported a nine-year-old patient with acute metabolic decompensation, in which elevated BCAA concentrations in brain were evident on MRS. MRS findings have also been reported for four MSUD patients during acute decompensation events [69]. In these patients, acute decompensation correlated with structural abnormalities on DWI and pronounced elevation of MSUD-related metabolites. These structural and chemical abnormalities normalized after recovery from decompensation.

To date, there have been no fMRI data reported on MSUD.

5.4. Methylmalonic acidemia (MMA)

Methylmalonic acidemia (MMA) is an autosomal recessive disorder of amino acid metabolism caused by deficient conversion of methylmalonic acid to succinic acid. This deficiency arises either from disruptions to the methylmalonyl-CoA mutase apoenzyme, or its coenzyme adenosyl cobalamine, with mutase deficiencies predicting more aggressive course and shorter survival periods than cobalamine deficiencies [75]. Increased methylmalonic acid levels are believed to inhibit succinate dehydrogenase, thereby disrupting the tricyclic acid cycle and the oxidative metabolism of glucose. MMA typically presents in early infancy, and is associated with vomiting, lethargy dehydration and

metabolic acidosis. Additionally, an array of neurologic symptoms is seen, including seizures, hypotonia, mental retardation, movement abnormalities, developmental delay, dyspraxia and paroxysmal deterioration secondary to infection. Radiologic abnormalities typically seen on CT and conventional MRI include marked signal abnormalities in the globus pallidus of the basal ganglia, which is presumed to reflect increased metabolic activity in this region in early development [76]. One case report has also provided evidence of reduced NAA and increased lactate signals on MRS which normalized with effective clinical management [77].

We are aware of five case studies in the literature that have used diffusion-based MRI to investigate the neurophysiologic bases of MMA. Studies using DWI to investigate ADC in MMA converge unanimously on a radiologic picture of restricted water diffusion in the globus pallidus [75–79]. Additionally, it has been shown that decreased ADC signals resolve with clinical interventions such as carnitine treatment [77] and reversal of acidosis with sodium bicarbonate [76]. It is believed that bioenergetic failure disrupts ATPase ion transport and homeostasis in affected neurons, causing cytotoxic edema, which restricts water diffusion and alters the ADC signal. Recently, DTI findings from 12 MMA patients demonstrated significant FA reductions in frontal, temporal and occipital white matter not seen on conventional MR images [79]. These findings suggest that, in addition to restricted water diffusion in globus pallidus neurons, MMA is associated with disturbances in white matter integrity throughout the brain, and that DTI is superior to other imaging modalities in identifying these diffuse lesions.

Neurocognitive lesions in MMA have yet to be investigated with fMRI.

5.5. Fabry disease (FD)

Fabry disease (FD) is an X-linked lysosomal storage disorder, characterized by decreased or absent activity of the lysosomal enzyme alpha galactosidase A due to mutation of the alpha galactosidase A gene at Xq22.1. This abnormality results in failure of conversion of globotriaosylceramide (Gb3) to lactosylceramide, with subsequent intracellular accumulation of glycosphingolipids, particularly Gb3 [80]. Glycosphingolipid deposits occur in the organs with a predilection for vascular endothelial and smooth muscle cells, myocardium, renal epithelium, cornea, and central nervous system. Clinical symptoms as a result of this accumulation include renal and cardiac failure, painful acroparaesthesias, angiokeratomas, hypohydrosis, corneal dystrophy (verticillata), and stroke [80]. Strokes occur in up to 25% of males and 21% of manifesting female carriers. It is believed to be due to impairment of endothelial function, altered cerebral blood flow and up regulation of prothrombotic factors. Chronic brain microbleeds are readily detected by Echo gradient (GRE)-weighted images, and their presence is associated with risk of brain hemorrhage [81]. The incidence of microbleeds is not certain. FLAIR and T2-weighted images reveal white matter disease and deep gray matter involvement. The findings in the right clinical setting are distinct enough that they should allow diagnosis. CT scan will demonstrate increased attenuation in the pulvinar region which corresponds to sites of hyperintensity on T1-weighted MRI (known as “the pulvinar sign”) [82].

Reisen et al. [82] performed Brain MRI prospectively in 51 consecutive patients from 7 different families with FD, with no prior history of cerebrovascular disease and referred to a single center for neurological evaluation between 2006 and 2008. In this cohort, 44.4% had subclinical evidence of small vessel disease on brain MRI. This finding was more frequent in older patients and in those with more cardiovascular risk factors. Females were affected as often as males. In addition to microbleeds, brain MRI revealed widespread evidence of small vessel disease. These findings have been seen in young children with FD as well [83]. Several studies have indicated that diffusion tensor imaging (DTI) is more accurate and

more sensitive in quantifying structural brain alterations in patients with Fabry disease and other disorders. Albrecht et al., [84,85] studied 25 clinically affected FD patients (10 men, 15 women, mean age 36.7) and 20 age matched healthy controls. There were significant difference in white matter integrity and coherence with mean diffusivity (MD) values significantly increased, mainly in regions of the frontal, temporal, central and parietal white matter. In gray matter, significant MD increases were detected only in the posterior thalamus bilaterally. Increases in diffusivity were pronounced in the periventricular white matter. These areas are supplied by long perforating arteries of small caliber. This pattern of diffusivity alterations can be explained by microangiopathy that is detected with more sensitivity on DTI than visible WMLs in FLAIR and T2 weighted MRI images. Additionally, MD elevations were detected in the posterior region of the thalamus which may suggest additional vulnerability of the posterior cerebral artery. There have been a small number of studies using ^1H MRS in Fabry disease. In the first one to be published, Tedeschi et al. [86] used multislice ^1H -MRSI to measure metabolite signal intensities of NAA, Cho, Cre, and Lac in the brain of Fabry disease patients to determine whether multislice ^1H -MRSI could detect cortical and subcortical neuronal involvement beyond the areas detected by routine T1/T2 MRI. The study involved nine male patients, aged 20 to 50 years and 20 age- and sex-matched healthy controls. The study demonstrated a diffuse pattern of neuronal involvement with significant reductions (as compared with controls) of NAA/Cre or NAA/Cho, or both, in the temporal, frontal, parietal and occipital cortex as well as in the thalamus and centrum semiovale white matter. Due to the absence of significant differences in the Cho/Cre ratio between patients and controls the authors concluded that the reduced NAA/Cre and NAA/Cho ratios were due to reduced NAA, either as a result of direct metabolic dysfunction of neurons secondary to $[\alpha]$ -galactosidase A deficiency and CTH accumulation or to subclinical ischemia. Because neuronal involvement extended beyond the areas of MRI-visible abnormalities, they suggested ^1H MRS could be useful for monitoring disease severity and response to future therapies.

6. Conclusion

Protection of the CNS from the toxic effects of metabolic dysfunction in urea cycle disorders (UCD) is a central concern for the clinical management of inborn errors of metabolism. Neuroimaging methodologies (using MRI scanning) allow physicians and researchers to characterize the pathophysiology of neurologic injury in IEMs, to track chronic and acute brain changes, and to evaluate the efficacy of therapeutic intervention. Combining imaging platforms in multimodal assessment batteries gives investigators a complex and varied perspective into the structural, functional and biochemical parameters of the central nervous system in IEMs. Functional MRI presents an especially promising and underutilized means of characterizing neurocognitive dysfunction in UCD. As neuroimaging methods become increasingly sophisticated, they are certain to play a critical role in the study and treatment of metabolic disease.

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Fig. 1.
FLAIR imaging (1B) shows area of white matter damage more clearly than on routine T2 weighted images (1A).

Fig. 2. Diffusion tensor imaging showing corticospinal tract fibers in a patient with arginase deficiency. The thickness of the bundle scales to the number of fibers, which in this case, is reduced from normal.

Fig. 3. Comparison of normal (left) and patient with OTCD is clearly demonstrated with decrease complexity of white matter fiber tracts in the corpus callosum.

Fig. 4. 28-year-old with PKU. Clinical T2 weighted images in coronal, axial and sagittal planes are on the left. Fluid attenuated (FLAIR) images on the right show areas of white matter injury more clearly.

Table 1

Comparison of imaging modalities and role in IEMs.

Modality	Principle	Application
Single photon emission computed tomography (SPECT)	Peripheral injections of gamma ray-emitting radiotracers, such as HMPAO, are administered. Radioactive decay produces gamma rays, which are captured by rotating gamma camera to produce a 3D image.	Radiotracers bound to ligands are used to determine chemical binding properties in living tissue. For example, SPECT is used to measure dopamine D2 receptor density in the brain.
Positron emission tomography (PET)	PET tracers are also administered peripherally, and emit positrons, which collide with electrons to emit two photons in opposite directions, detected by rotating cameras. PET signals are produced when photons separated by 180° are detected simultaneously.	As in SPECT, radiotracers bound to ligands are used to investigate chemical binding properties in living tissue. PET tracers may also be bound to glucose, as in fludeoxyglucose (FDG), to investigate <i>in vivo</i> metabolic activity.
Structural magnetic resonance imaging (MRI)	Differences in proton spins between tissue types allow this non-invasive modality to capture 3D images of living tissue. Protons aligned to high magnetic field are repeatedly excited by radiofrequency pulses, causing them to release electromagnetic energy as they “relax” back to alignment with field. This energy is captured by a “head-coil” receiver, and is computationally reconstructed into a 3D structural image of living tissue.	In the clinic, MRI is used to detect microstructural abnormalities in the human brain, such as brain tumors, strokes, traumatic injury, dysmyelination, and hydrocephalus. In the lab, structural MRI can be used to investigate volumetric differences throughout the brain between experimental and control populations. Imaging parameters can be adjusted to collect different image contrast qualities, such as T1, T2 and FLAIR.
Functional magnetic resonance imaging (fMRI)	Magnetic differences between oxygenated and deoxygenated hemoglobin allow active regions of the brain to image non-invasively in an MRI scanner. Firing neurons extract oxygen and glucose from cerebral vasculature. Compensatory blood flow increases to compensate for local oxygen debt, reaching peak flow 4–6 s after neuron firing. The peak of this hemodynamic response is detectable on MRI using a T2* pulse sequence.	fMRI has revolutionized the study of brain function, allowing researchers to administer cognitive tasks concurrently with fMRI scanning in order to map cognitive functions to individual structures and networks of structures within the brain. fMRI is highly valuable for clinical research in to neurologic and psychiatric illness, helping to identify neurocognitive and neurophysiological differences between clinical and healthy populations.
Diffusion-weighted and diffusion-tensor imaging (DWI and DTI)	These MR-based methods are used to image the randomness of water diffusion and the degree of ordered anisotropy that defines water movement along natural hydrophobic barriers within the brain, such as myelinated axons. DWI and DTI provide an index of brain structure.	Diffusion-based imaging techniques are highly sensitive to microstructural abnormalities invisible on traditional MRI, particularly within the brain’s white matter. Clinically, DWI and DTI have become a valuable tool for investigating both acute and chronic pathologic processes affecting white matter structural integrity. They are also used in clinical research studies to identify differences in white matter integrity between clinical and healthy populations.
Magnetic resonance spectroscopy (MRS)	The characteristic location and height of peaks along the MR spectrum are used to measure the concentration of chemicals and metabolites within living tissue. The 3D structure and chemical composition of organic molecules determine their spectroscopic resonance frequency and the extent of their “downfield” chemical shift on a mass spectrum.	Using a 3D voxel, researchers and clinicians can use MRS to determine the chemical profile within a given region of the brain. This can be used as clinical tool, as in inborn errors of metabolism that alter the concentration of metabolites within the brain, or by researchers seeking to identify neurochemical abnormalities in disease populations.