REVIEW

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Anatomical, functional and molecular biomarker applications of magnetic resonance neuroimaging

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ABSTRACT MRI and magnetic resonance spectroscopy (MRS) along with computed tomography and PET are the most common imaging modalities used in the clinics to detect structural abnormalities and pathological conditions in the brain. MRI generates superb image resolution/contrast without radiation exposure that is associated with computed tomography and PET; MRS and spectroscopic imaging technologies allow us to measure changes in brain biochemistry. Increasingly, neurobiologists and MRI scientists are collaborating to solve neuroscience problems across sub-cellular through anatomical levels. To achieve successful cross-disciplinary collaborations, neurobiologists must have sufficient knowledge of magnetic resonance principles and applications in order to effectively communicate with their MRI colleagues. This review provides an overview of magnetic resonance techniques and how they can be used to gain insight into the active brain at the anatomical, functional and molecular levels with the goal of encouraging neurobiologists to include MRI/MRS as a research tool in their endeavors.

The use of nuclear magnetic resonance and MRI in biomedical research began nearly four decades ago. Since then, MRI/magnetic resonance spectroscopy (MRS) hardware, image acquisition techniques and reconstruction methods have evolved rapidly. These advances have helped neuroscientists gain insight into normal and diseases processes in living brains with unprecedented image resolution and image contrast. As such, the ability to evaluate magnetic resonance (MR) biomarkers of the brain can provide crucial opportunities for early detection of brain disease and disorder, facilitate early therapeutic intervention and aid drug development efforts. However, information obtained from MRI/MRS is based on image contrast and/or statistical correlations; hence, current MRI/MRS techniques may not provide adequate interpretations of true biological/pathological processes. There is a pressing need to correlate these noninvasive observables with the underlying molecular mechanisms of brain disease and disorders, knowledge that can only be obtained through neurobiological investigations.

Multidisciplinary collaborations between neurobiologists and MRI scientists are the key to transforming basic research into translational neuroscience. The goal of this review is to provide neurobiologists with the essential knowledge needed to engage in multidisciplinary research efforts involving MRI/MRS techniques. It will first provide a general background of MR, followed by descriptions of state-of-the-art MR methodologies and finally a broad overview of MR methods used to detect anatomic, functional and molecular biomarkers in brain disease and disorders. This report is not intended to provide an exhaustive list of MRI methods or MR biomarkers of the brain. Readers are encouraged to consult books and references for an in-depth discussion of specific topics of their interests.

KEYWORDS

- anatomical MRI • brain biomarkers • functional MRI • molecular MRI • MRI • MRS • neuroimaging

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MR image acquisition, reconstruction & analysis

An MR scanner is essentially a large magnet that generates a static magnetic field strength (B_0) when it is ramped up and at field. During an MRI scan, a subject is placed in the bore of the scanner, and the hydrogen protons (H^1) within the subject are exposed to the static magnetic field, causing their spins to precess around the B_0 direction. Radiofrequency (RF) excitation pulses are applied to the head or a section of the body under study through a transmission coil or an array of transmission coils, forcing the proton spins within this section to align with the direction of the RF pulses. After the RF pulses duration, these spins relax to their original states, depending on their environment and the MR relaxation properties of the various tissue types. Energy released due to relaxation of the spins is collected by a receive coil (or coils) into a raw data matrix called the k-space. The raw data are then 'reconstructed' to create MR images after Fourier transformation [1]. **Figure 1A & B** show an example of a k-space and its corresponding reconstructed image. The arrow lines on the k-space images describe the frequency and phase-encoding direction as the data are traditionally acquired (i.e., under Cartesian sampling) as discrete points. MR images are presented as collections of digital image voxels (3D boxes as opposed to pixels, which are 2D boxes), and the signal within each voxel represents all the MR visible protons within the voxel. MR scanners with higher MR field-strengths can generate better image quality and signal separation. An important confound associated with higher field-strength is greater image artifacts, for example, artifacts within the brain due to the air tissue interfaces at the ear canals. In addition, certain acute disease models that involve surgical manipulation within the imaging field of view can result in artifacts within the brain, for example, blood and fluid pooling in and around the surgical site and suture. Further, metallic objects (such as surgical clips/clamps or ear clips used for animal identifications) within the imaging field can cause severe image artifacts. Currently, the highest field strength of clinical MRI systems is 3 T. Field strengths of preclinical scanners (for human and animal research) can be higher, from 4 to 16.4 T [2].

• Spin-echo, gradient-echo MRI

Spin echo imaging (SE) is widely used in clinical MRI of brain, spine and neck. Gradient

echo imaging (GRE) is sensitive to micro-vessel bleeding and elevated iron content in the brain associated with stroke and neurodegenerative diseases. In addition, GRE imaging is often used in the molecular MRI arena to detect iron-tagged probes targeting low-concentration biological molecules. Detailed descriptions of SE and GRE imaging protocols and underlying physics can be found in Chapters 4 and 8 of an online textbook by Joseph P Hornak [3,4].

• T1-weighted, T2-weighted, diffusion-weighted MRI

While MR acquisition protocols involve many adjustable parameters, the two basic parameters in MR imaging protocol are the repetition time (the time between two consecutive excitation pulses, TR) and the echo time (the time between the excitation pulse and the echo, TE). Simplified pulse sequences for SE and GRE with the locations of TR and TE are presented in **Figures 1C & D**, respectively. By varying the lengths of TR and TE, one can acquire images with different image contrasts to emphasize specific brain structures of interest. **Figure 2B** shows the different features of T1-weighted (T1W) and T2-weighted (T2W) images of human brains, compared with images of the mouse brains with less features (**Figure 2A**). Conventional T1W images are acquired with both short TR and TE (TR <750 ms and TE <40 ms) [3] and provide anatomical images with good contrast between the gray and white matters. On the other hand, conventional T2W images require long TR and TE (TR >1500 ms and TE >75 ms) [5], and provide clear delineation of the cerebrospinal fluid (CSF; as bright areas) and good contrast for detecting white matter changes and brain lesions exhibiting abnormal fluid collection (i.e., edema). T2W GRE imaging is prone to magnetic field inhomogeneity, resulting in image artifacts particularly near the air–tissue interface which are exaggerated at high field (see arrows in **Figure 2C** with images acquired from a 9.4 T animal scanner).

Diffusion weighted imaging (DWI) is a contrast-enhanced MR technique that is sensitive to the diffusion of water molecules within tissue. The degree of diffusion weighting is controlled by the b values (in s/mm^2) with a maximum b value of around 1000 in clinical scanners [7]. Diffusion contrast enhances as the b values increase from zero to 3000 s/mm^2 (**Figure 3A**) and regions of cerebral ischemia

are revealed qualitatively as focal hyperintense areas (**Figure 3B**; acquired with $b = 1256 \text{ s/mm}^2$). Diffusion weighted images have been incorporated into general clinical imaging in the diagnosis of various types of cerebral ischemia and oncologic imaging.

• Echo planar imaging

In comparison to routine SE scans in which a line of k-space is filled sequentially and from left to right during each TR, single-shot echo planar imaging (EPI) scans fill k-space quickly, by rapidly switching the frequency encoding or readout gradients after a single RF excitation. Multi-shot EPI (phase encoding divided equally into several RF excitations) are also used to reduce the specific absorption rate and to minimize heating on tissue albeit at the cost of longer imaging times. Because of its extraordinary high temporal resolution, EPI is widely used in applications which require either fast image acquisition or signal averaging to improve signal-to-noise such as functional and diffusion MRI. However, EPI cannot achieve as high resolution as anatomical imaging within the same acquisition time. For example, EPI used for functional MRI studies at 3 T and below are often collected using voxel volumes of about 2–3 mm isotropic voxels, at less than a second per scan [8,9], while anatomical MRI can achieve 1–1.2 mm isotropic voxel within 5 min [10]. In addition, EPI is susceptible to B_0 field inhomogeneity and prone to geometric image distortion. Advanced mathematical algorithms have been developed to correct EPI geometric image distortion [11], alleviate or minimize motion artifacts and enhance temporal resolution. Fast EPI methods include partial Fourier, partial imaging and sparse sampling techniques, simultaneous multi-slice (SMS), multiband and simultaneous image refocused (SIR) EPI [12]. Recent development of EPI acquisition, reconstruction and correction methods have made EPI more appealing to neurobiologists and further development is needed before it can be applied to investigate *in vivo* human brain at the micron scale.

• Magnetic resonance spectroscopy

MRS is used as an adjunct to MRI in clinical settings to spatially map tissue metabolites in order to characterize neurological, psychiatric and metabolic diseases [13]. This technique measures concentrations of various metabolites within the tissue and provides biochemical

Figure 1. An example of raw data acquired from an MRI scanner, its corresponding magnetic resonance image and diagrams of standard magnetic resonance image sequences. (A) A raw data matrix obtained from magnetic resonance scanner vs **(B)** magnetic resonance image **(C)** spin echo and **(D)** gradient echo imaging pulse sequences.

(A & B) Courtesy of Dr Philip Zhe Sun at Massachusetts General Hospital (MA, USA).

characteristics of select brain regions. A ^1H -MRS spectrum consists of an array of peaks corresponding to a number of biochemical molecules that are important to brain metabolism (**Figure 4**); each metabolite is detected as one or more peaks with various chemical shifts (in ppm, x-axis). Several peaks of interest related to brain metabolism are NAA (N-acetylaspartate at 2.02 ppm) for neuronal metabolites, Cho (choline at 3.22 ppm) – containing compounds such as glycerophosphocholine and phosphocholine (as neurotransmitters), Cr (creatine at 3.02 ppm) for cellular energetics, Glx (glutamate and glutamine between 2 and 2.5 ppm) for neurotoxicity, Lac (Lactate at 1.33 ppm, not shown) for cellular respiration, Myo (myo-inositol at 3.56 ppm) as glial metabolite, Ala (alanine at 1.48 ppm, not shown) as an index for citric cycle metabolism, and lipids (Lip at 1.3 and 0.9 ppm) as an index of brain damage. Although Glx is a combined signal of glutamine and glutamate and is difficult to separate with clinical human MRI

Figure 2. MR image contrast can be manipulated by varying the acquisition parameters such as the time between two consecutive excitation pulses and the time between the excitation pulse and the echo and/or acquisition schemes such as spin echo or gradient echo imaging. Comparison between T1- and T2-weighted SE images of (A) mouse brain at 9.4 T, acquisition TR and TE are displayed in corresponding images of (B) human brain at 1.5 T. Panel (C) shows artifacts that are further exaggerated in GRE images at 9.4 T (with flip angle of 30°, top panel), most noticeably in the air–tissue interface, compared with the SE images acquired using the same TE and image resolution.

GRE: Gradient echo imaging; SE: Spin echo; T1W: T1-weighted; T2W: T2-weighted; TE: Time between the excitation pulse and the echo; TR: Time between two consecutive excitation pulses.

(B) Adapted with permission from [6].

scanners, separation of these two metabolites has become possible at higher field strengths such as 7 T [14]. Although somewhat quantitative, levels of metabolites detected using MRS are usually reported in relationship to a reference metabolites which can vary particularly in diseased brain [15]. Detailed discussion on the significant impact of ^1H -MRS on patient care and the needs in critical consideration of common data acquisition and processing procedures can be found in [16]. Lastly, MRS of other nuclei has also been explored, for example, ^{13}C -MRS experiments are used to follow the metabolic fate of ^{13}C -labeled substrates in the brain [17].

• MR contrast agents

MR contrast agents (CA) are chemical compounds whose presence can alter blood and tissue proton relaxation time and therefore enhance image contrast between different tissue types.

All exogenous MR CA cannot cross an intact blood–brain barrier (BBB); hypertonic mannitol injection, mechanical means such as intracerebroventricular injection [19] and focused ultrasound beams are methods used to facilitate the delivery of the MR CA into the brain parenchyma. Recent design of targeted polymeric nanoparticles for drug delivery across the BBB represents a potential way to deliver MR CA into the brain and allow MR visualization of certain cell types or disease markers [20].

Positive CAs

The presence of positive CA results in hyperintensity in T1W MRI. Gadolinium (Gd) enhanced MRI is routinely conducted in clinics to detect abnormal vascular structures and to locate areas of BBB leakage. Figure 5 shows T1W images acquired before (Figure 5A) and after Gd-DTPA injection revealing regions of

hyperintensity, indicative of BBB leakage (arrow in **Figure 5B**). Several Gd-based CA, under the trade names Ominiscan, Multihance, Magnevist and Prohance, are US FDA approved and used in clinical applications. However, Gd-based CA can be harmful to patients who have compromised renal function. Recent efforts to develop nonmetallic compounds that are bioorganic and biodegradable compounds, such as ^{19}F in the form of perfluorocarbon droplets [21] or organic radical contrast agent [22], may have potential clinical applications.

Negative CAs

The presence of negative CA results in hypointensity in T2W or T2*W MRI. The most widely used negative CA is superparamagnetic iron oxide nanoparticles (SPIOs or USPIOs) which provide the most signal relaxation per particle compared with Gd [19]. While SPIOs have relatively short blood half-life and are susceptible to tumor-associated macrophage extravasation, the surface of these particles can be chemically modified to include targeting moieties against biological targets for *in vivo* tracking of stem cell inoculation and propagation, inflammation progression, protein expression and gene expression in the brain.

Current MR methods to assess biomarkers in brain

This section describes MR methods that are used to evaluate biomarkers of the brain, how these methods are used and potential complications/confounds that should be taken into account in interpreting results derived from these methods.

• Anatomical MRI

Techniques and methods for anatomical MRI mentioned have been widely accepted and implemented in clinical scanners. Because the quality of MR images depends on scanners and varies among scans (even from the same scanner), parametric MR images allow quantitative measurements and longitudinal or some cross-platform comparisons.

T1 and T2 maps

T1 and T2 depict proton relaxation times of the tissue under study. **Figure 6A** shows a series of MR images acquired with a constant TR and TE but incremental inversion times showing drop in signal near the T1 value (at the 'null' point), permitting calculation of a parametric T1 map (**Figure 6B**). **Figure 6C** shows a series of SE images of two brain image slides that were acquired with a constant TR but incremental

Figure 3. Diffusion-weighted images of human brain and a mouse brain. (A) A series of diffusion weighted images of a human brain with increasing b values. **(B)** Hyperintense signals in DWI of a mouse brain reveal areas of metabolic disturbance in one side of the brain immediately after cerebral ischemia (arrows).

DWI: Diffusion-weighted imaging.

(A) Courtesy of Dr Kamil Ugurbil at University of Minnesota (MN, USA).

Figure 4. Typical ^1H -magnetic resonance spectroscopy of a normal brain reveals several prominent brain metabolites located at different chemical shift locations. Levels of any or multiple of these metabolites deviated from the normal range are indicative of disease progression.

Cr: Creatine; Myo: Myo-inositol; Cho: Choline; Glx: Glutamine and glutamate; MRS: Magnetic resonance spectroscopy; NAA: N-acetylaspartate.

Adapted with permission from [18].

TEs (20, 40, 60, 80 ms) for parametric T2 maps (Figure 6D). Note in Figure 6C, signal intensities of the images drop as TE increases. Similarly, T2* maps can be computed from a series of GRE images with decreasing TEs.

Maps of apparent diffusion coefficient, fractional anisotropy & tractography

Current applications of diffusion MRI are DWI, diffusion tensor imaging (DTI) and tractography. DTI technique detects the microscopic translational motion of water molecules in tissue, and is sensitive to various cellular changes and tissue abnormalities and permits the quantitative evaluation of gray and white matter pathology. The two most commonly used indices of water diffusion in tissue are the magnitude, expressed as the apparent diffusion coefficient (ADC), and the degree of anisotropy, often expressed as the fractional anisotropy (FA). ADC is calculated from a series of DWIs that are acquired with variable b values and is affected by the microstructure of the tissue and the diffusivity of

the water molecules. A set of DWI acquired at different gradient directions (Figure 7A for six directions) are used to compute FA and obtain tractography (tract) information (Figure 7B). FA has values between zero and one and is associated with the degree of functional anisotropy of the tissue and can be an indicator of tissue integrity. For example, normal white matter tracts have restricted diffusion path and their FA values are closer to one. Tractography maps allow visualization and delineating of the major white matter tracts within the brain, such as the corpus callosum (see arrow in Figure 7B, CC); also, fiber tract direction can be visualized based on the color notations (red for x-direction, green for y-direction and blue for z-direction or color combinations). Overall, FA provides an indirect maker of the microstructural properties of the white matter. ADC, combined with other DTI parameters like FA, has been shown to stage and predict ischemic brain injury over its temporal evolution [24]. A more detailed description of the principles and relevant clinical applications of diffusion MRI of the brain can be found in [25].

• Functional MRI

Functional MRI (fMRI) was first introduced in 1992 based on the concept of neurovascular coupling, that is, activation of a neuronal cell population induces an increase in blood volume, and consequently, blood oxygenation levels. It is important to note that fMRI studies on subjects with brain injuries or under the influence of stimulant may not reflect the underlying brain activities [26]. Furthermore, fMRI studies have been prone to biases due to small sample size, variable experimental paradigms and lack of standards in analytical methods for data interpretation, and further development is necessary to improve multicenter reproducibility [27]. Regardless, fMRI has contributed greatly in our better understanding of brain functions in health and disease.

Blood oxygenation level-dependent MRI

As oxygen is consumed as a result of elevated brain activity, the level of deoxygenated hemoglobin in the blood surrounding the active brain regions increases and results in a reduced signal in T2*W MR images. Blood oxygenation level-dependent (BOLD) MRI acquires repetitive GRE images of the whole brain as subjects perform certain tasks following a period of baseline acquisition (no task). Voxel-wide statistical

analyses are performed on the series of T2*W images acquired over time to identify brain regions that have significant signal changes from baseline [28]. Based on resting state MRI (rs-MRI) which does not involve tasks or cues, functional connectivity (fc-MRI) has emerged as a powerful tool in the clinical research arena to interrogate the intrinsic property of mental states [29,30]. By associating similar rs-MRI patterns among different brain regions, fc-MRI is used to evaluate brain conditions [31] and has deciphered detailed maps of complex neural systems, even across less well understood territories of the human brain [32,33]. However, fc-MRI is sensitive to head motion and the mental states of the participants which can confound data analyses and interpretations [33]. With a large collection of rs-MRI data, it is possible to pursue discovery science (as opposed to hypothesis-driven science) of brain function. For this reason, it was agreed by the fMRI community that sharing of large-scale data sets for data mining is needed. Without consensus on experimental paradigms and interpretation methods, it is almost impossible to aggregate fMRI or rs-MRI data sets from multiple data collection sites. Despite these challenges, the collection and sharing of large-scale rs-MRI data sets from multiple imaging sites was shown to be feasible by the 1000 Functional Connectomes Projects (FCP) and International Neuroimaging Data Initiative (INDI) [32].

Arterial spin-labeling MRI

Arterial spin-labeling (ASL) perfusion MRI takes advantages of protons in the arterial blood as a freely diffusible tracer and allows noninvasive quantification of cerebral blood flow. Two scans are involved in ASL technique. the first scan is acquired with the arterial blood flowing through the carotid artery 'spin-tagged' at the neck region before entering the head and a 'control scan' (without spin-tagging) is performed after. Brain perfusion maps are computed as the subtraction of these two scans which is then converted to ml/100 g/min [34]. Although ASL technique and maps are used in many preclinical investigations, their general acceptance by the clinical community is still lacking. This is attributed to the overabundance choices in labeling schemes, readout option and models to quantify cerebral blood flow. Therefore, a consensus approach is important to gain acceptance of ASL approach to the clinical community [35].

Figure 5. Gadolinium injection during MRI scans can reveal areas of compromised blood–brain barrier after a stroke. While ordinary SE image appears (A) normal (pre-Gd), Gd-enhanced MRI reveals areas of blood–brain barrier leakage due to (B) the retention of Gd-DTPA (post-Gd) after intravenous injection of Magnevist.

Adapted with permission from [23].

BBB: Blood–brain barrier; Gd: Gadolinium;

SE: Spin echo.

Dynamic contrast-enhanced MRI

Based on the temporal profile of signal changes within tissue following the injection of a CA, dynamic contrast-enhanced (DCE) MRI can be used to derive multiple and quantifiable parameters related to tissue perfusion, vessel permeability and fluid volume fractions [36]. These parameters can be used to evaluate the severity of stroke and the enhanced permeability and retention (EPR) effect in tumors.

IRON MRI

Iron oxide nanoparticles (e.g., MION, SPIO, SPION, USPIO, etc.) are injected intravenously as a bolus to animals prior to fMRI study to measure blood volume changes associated with brain activation. IRON MRI utilizing relatively high iron doses to shorten acquisition TE can achieve better time resolution and provide a roughly twofold greater sensitivity than BOLD MRI [37]. Pharmacological stimuli that give rise to complex temporal responses of cerebral blood volume can be captured using IRON MRI. Upon completion of IRON MRI, a General Linear Model (GLM) – a standard technique for fMRI data analysis [38] – is used to analyze the data and to assess the dynamic response of and interactions among neural receptors to drugs, a workflow dubbed phMRI. Unlike fMRI, phMRI faces challenges associated with drug-induced perturbations to hemodynamics and respiration which affect normal neurovascular coupling. Therefore, phMRI studies are performed in animals under control ventilation and close monitoring of blood O₂/

Figure 6. Series of images used to compute T1 or T2 maps. (A & B) T1 and T2 maps are computed from a series of spin-echo images with incremental inversion time for T1 map (inversion times ranging from 500 to 2500 ms) and (C & D) with incremental echo time for T2 map (TE of 20, 40, 60 and 80 ms).

(A & B) Courtesy of Dr Phillip Zhe Sun at Massachusetts General Hospital (MA, USA).

CO₂ level. To date, IRON MRI has been used for rs-MRI and fMRI in human using ferumoxytol (USPIO) [39,40] but phMRI studies have not been applied to human [26].

Manganese-enhanced MRI

Manganese-enhanced MRI (MEMRI) directly measures neuronal activation and has only been applied in animal studies. This technique requires injection of a manganese solution into the CSF; manganese has been shown to be acutely and fatally neurotoxic. Manganese entry into the cells is based on the phenomenon that calcium ions (Ca²⁺) enter neurons through voltage-gated calcium channels as neurons are activated. MEMRI uses Mn²⁺ ions, which are MR visible and enter neuron like Ca²⁺ upon neuronal activation. Accumulation of Mn²⁺ inside neurons shortens the T1 of neurons and results in hyperintensities in T1W images [41] (Figure 8A) which reveals neuronal formation in the hippocampus (Figure 8B).

• Molecular MRI

With the significant advancements in MR hardware/software development, high-throughput screens of nanoparticle libraries and novel methods for targeted MR CAs, MRI has emerged as a

powerful tool for imaging molecules and molecular events.

Protein targeting or gene transcript (mRNA) targeting MRI

Modern laboratory techniques and drug development are increasingly focusing on the molecular bases of disease, targeting earlier physiologically relevant events or indicators rather than treating the symptoms. In order to monitor these early indicators in living subjects, there have been tremendous efforts in the design of MR contrast probes that can image activities of biological molecules such as DNA, RNA, mRNA, microRNA, proteins and metabolites. MR CAs such as SPIOs are used to label targeting moieties for the biological molecules to gauge their ubiquitous presence or deviation from normal levels in active biological systems.

Chemical exchange saturation transfer

Chemical exchange saturation transfer (CEST) method is a contrast enhancement technique that allows the indirect detection of molecules with exchangeable protons [42]. Endogenous metabolites with exchangeable protons that can be used for *in vivo* MR investigation are called diamagnetic CEST agents. Exchangeable

protons in these endogenous CEST agents include amide protons, amine groups on small metabolites such as glutamate (Glu) and creatine (Cr), hydroxyl groups on glycosaminoglycan (GAG), glycogen, Myo-inositol (Myo), and glucose [43]. Another class of CEST agents is called paramagnetic CEST (PARACEST) agents, which are exogenous molecules with exchangeable protons combined with a paramagnetic metal ion of the lanthanide family [42].

Nuclear hyperpolarization

Although MRS lack sensitivity to detect low abundant ^{13}C in nature, dynamic nuclear polarization techniques (DNP) can enhance ^{13}C signal by 10,000-fold or greater (termed hyperpolarization) [44], allowing the use of ^{13}C MR spectroscopic imaging for *in vivo* studies of metabolites such as pyruvate, bicarbonate, fumarate and urea with much-enhanced resolution. However, synthesis of hyperpolarized (HP) probes involves specialized instruments and precludes general clinical applications of such technology at this time; a review in the applications of ^{13}C and HP- ^{13}C spectroscopic imaging of brain metabolism can be found in [17].

MR biomarkers of brain

In this section, four most prevalent brain diseases/disorders – neurodegenerative diseases, addiction and mental illnesses, stroke, brain tumor – and related anatomical, functional and molecular MR biomarkers are presented. Although some of these biomarkers are sufficiently robust and are used in clinical decision making, most of these biomarkers are trends that were based on correlations and/or association analyses of group studies. MR biomarkers based on group analyses/data correlations should only be used as indications of certain disease condition for individual patient. The power of MR biomarkers is the ability to track changes in physiological indices (such as anatomy, functions and molecular signatures) of individual subject as the disease progresses or after therapeutic intervention. **Table 1** summarizes the MR biomarkers which are described below.

• Neurodegenerative diseases

Anatomical biomarkers

T1W images for the determination of brain cortical thickness is currently accepted as an Alzheimer's disease (AD) diagnostic criteria

Figure 7. Magnetic resonance images used to compute the diffusion tensor parametric maps.

(A) A series of diffusion-weighted images are acquired at different gradient directions (no diffusion weighting, positive x, negative x, positive y, negative y, positive z and negative z, referring to the coordinate to the right). (B) Maps of apparent diffusion coefficients, fractional anisotropy and tractography (tract) are generated.

ADC: Apparent diffusion coefficient; FA: Fractional anisotropy.

Figure 8. Schematic image of manganese-enhanced MRI. (A) Manganese ions act as calcium ion analog and enter neurons through calcium channel upon neuronal activation. **(B)** Retention of manganese ions in neurons clearly delineate neuronal formation in the hippocampus.

Adapted with permission from [41].

MEMRI: Manganese-enhanced MRI.

for patient recruitment. Specifically, analyses of human brain MRI collected under the Alzheimer's Disease Neuroimaging Initiative have shown that imaging biomarkers of AD signatures such as regional cortical thinning predict AD dementia in healthy elderly and individuals with mild cognitive impairment [45], corroborate with regions of hypometabolism in individuals with obvious amyloid- β plaques detected using fluorodeoxyglucose-PET (FDG-PET) [46] and are associated with lower

A β and higher tau concentrations in the CSF [47]. In addition, morphological and volumetric analyses of T1W images were sufficiently robust in differentiating AD from frontotemporal lobar degeneration patients [48], correlating the extent of brain atrophy with the degree of motor deficit in chronic stroke patients [49] and establishing the association between network-dependent white matter hyperintensities and impaired executive functioning in mild cognitive impairment patients [50]. Analyses based on combined T1W, T2W and/or T2*W MRI data from multiple clinical studies suggested that the influence of abnormal white matter changes and microbleeds are strongly associated with the clinical course of AD [51]. High-resolution DTI and corresponding tractography of the medial temporal lobe have been used to identify additional anatomical abnormalities in AD patients [52]. Combined DTI and tractography data also reveal general atrophy in the substantia nigra pars compacta (SNc) [53] and reduced connectivity between the SN and several parts of the brain in Parkinson's disease patients compared with normal subjects [54]. By cross-examining the anatomical image data and gene profiling from blood collected from 740 subjects, genome-wide association studies (GWAS) show significant association between the image-derived measure (temporal lobe volume, highlighted areas in the brain image, **Figure 9**) and a specific single nucleotide polymorphism (SNP) of a glutamate receptor variant (*GRIN2b*) relevant to neurodegeneration in AD [55].

Functional biomarkers

ASL was shown to have sufficient sensitivity and specificity to correlate regions of reduced cerebral blood flow with patterns of altered brain glucose metabolism (determined by FDG-PET) in AD patients [55,56] at rest. Human group studies of fc-MRI suggested that resting tremor in Parkinson's disease patients may result from a pathological interaction between the basal ganglia and the cerebellothalamic circuit [57].

Molecular biomarkers

1H -MRS based molecular biomarkers associated with neurodegeneration include decreased Glu [58,59] and elevated total Cho (tCho) levels [60]. Elevation in the Myo may be associated with glial or microglial activation as the result of regional neuronal damage [15].

• Addiction & mental illness

Anatomical biomarkers

Brain volumetric analyses based on structural MRI have been used as an image biomarker of schizophrenia (SZ). A systematic meta-analysis of region-of-interest brain volumetric MRI acquired from multiple studies indicates medium-sized reduction of insula volume in patients with SZ compared with control subjects [61]. Although the research literature provides ample evidence that SZ has neuroanatomical correlates that can be seen at group levels, structural MRI cannot be used to identify SZ at the individual level [62,63]. DTI has been used to test the hypothesis that mental disorders such as attention-deficit and hyperactivity disorder (ADHD) and SZ are the result of disruptions of certain connections of brain fibers at group levels. In one study, DTI brain scans of adults with ADHD revealed a divergence of the microstructure of the corpus callosum (CC) from the control group, which may point to an abnormal lateralization in adults with ADHD [64]. Based on meta-analyses of DTI data, white matter alterations including fiber integrity and fiber track connectivity are apparent from the preclinical to the chronic stages of SZ with the affected brain regions within corticospinal track, interhemispheric connections, long association white matter tracts, cerebello–thalamo–cortical circuits and limbic system [65].

Functional biomarkers

Pharmacological MRI in rodents showed that psychostimulants such as cocaine and

amphetamine produce responses in the dopaminergic pathway (also known as the pleasure pathway) which include the striatum, nucleus accumbens and pre-frontal cortex. In addition, phMRI reveals the dynamics regulation of the neurotransmitter systems associated with mu-opioid receptor agonists in rats [66] and their interaction with different receptors in the brain [26]. With regard to mental illness, ASL in human group studies identified elevated blood perfusion in the cortex (**Figure 10**) as one of the MRI biomarkers for the diagnosis of post-traumatic stress disorders in veterans of wars [67]. By performing independent component analysis to cross-examine empirically chosen single-nucleotide polymorphisms (SNPs) and fc-MRI, researchers were able to performed GWAS to identify a functional brain network in the thalamus, anterior and posterior cingulate gyri which was significantly associated with SNPs in chromosome 7q21 and 5q35 in SZ patients [68].

Molecular biomarkers

The most relevant neurochemicals associated with addiction are the neurotransmitters GABA and glutamate, which were found to be lower in adolescents who were chronic marijuana users, based on ¹H-MRS [69]. Meta-data analyses also revealed that substantial NAA reductions in the frontal lobe, medial temporal lobe gray and white matter, cerebellum, and parietal cortex are strongly associated with SZ [70]. Using SPIO-labeled DNA-based molecular probes targeting specific mRNA sequences, molecular MRI

Table 1. Summary of MR biomarkers mentioned in this review.

Disease categories	Anatomical MRI	Functional MRI	Molecular MR	
			MRS	MRI
Neurodegenerative	H: T1W (cortical thinning) H: T2W (bright), T2*W(dark) H: FA ↓, tract ×	H: ASL ↓ H: fc-fMRI ×	H: Glu ↓ H: tCho, Myo ↑	
Addiction/mental	H: T1W (insula volume ↓) H: FA ↓, tract ×	H: ASL(cortex↑)	H: GABA, Glu, NAA↓	A: Fos mRNA ↑ A: GFAP mRNA ↓
Stroke	H: Gd-T1W (bright) H: T2W (bright) H: Acute ADC ↓ H: FA ↓	H: fc-fMRI ×	H: Lac↑/ tNAA↓	A: APT, pH↓ A: P-, E-selectin ↑ A: VCAM-1 ↑ A: MPO ↑ A: GFAP mRNA ↑
Brain tumor	H: Gd-T1W (bright) H: Perfusion ↑ H: ADC ↑	H: fc-fMRI (image guided surgery)	H: Cho, Lac, Lip ↑ H: NAA, Cr ↓ H: tCho, tNAA ↑ H: Myo↑ H: 2HG↑	

A: Pre-clinical applications only; H: Human applications; × implies disruptions.

Figure 9. Genome-wide association study identified a common glutamate receptor variant (*GRIN2b*) with suggestive evidence of association with temporal lobe volume and increased risk for Alzheimer's disease in 740 brain MRI scans.

Adapted with permission from [55].

was able to detect changes in mRNA levels in brain regions associated with the dopaminergic pathway following acute or chronic exposure to amphetamine in live rodents. Specifically, it was observed in living mice that c-Fos and FosB mRNA and activator protein 1 (AP-1, a transcription factor) were elevated after acute amphetamine exposure [19,71–72] but ΔFosB (a splice variant of FosB) mRNA were elevated only after chronic amphetamine exposure [72]. This technique also detects the downregulation of glial fibrillary acidic protein (GFAP) mRNA after acute amphetamine exposure and identifies regions that were most susceptible to neurotoxic damage after chronic exposure [73].

• Ischemic stroke

Anatomical biomarkers

Gd-enhanced T1W images can reveal areas of BBB leakage after stroke where Gd is trapped in brain tissue, causing an area of hyperintensity. T2W images (and T2 maps) can reveal regions of water accumulation and is an accepted technique for predicting ischemic lesions beyond the acute stage (between 10 and 48 h after stroke) and is also a robust marker of final infarction volume determined by histopathology [74]. Areas of reduced ADC during the acute stage are indicative of metabolic disturbance [23] but areas of reduced ADC but with normal T2 may have less severe injury [74]. Areas of normal-appearing tissue on DWI with significantly reduced perfusion are considered at high risk for infarct growth if the reperfusion is not established early; these areas are called the ischemic

penumbra [74]. Recent trials using standardized and quantitative MR techniques for brain diffusion and perfusion have demonstrated that the mismatch concept can aid in penumbral selection with great confidence [75].

Functional biomarkers

Truncated or abnormal functional connectivities observed using fc-MRI can provide insight into the pathophysiology associated with stroke-induced deficits and could help to optimize individual treatment regimens [76]. Combination of serial rs-MRI, DTI and behavioral testing in rats that underwent experimental unilateral stroke demonstrated that preserved structural integrity of the corticospinal tract of the stroke hemisphere appears to be a critical factor for good functional outcome and could be a primary target for therapeutic repair strategies [77].

Molecular biomarkers

¹H-MRS can be used to evaluate the severity of ischemia and neuronal dysfunction and damage after the hyperacute phase of stroke (>4.5 h) for prognostic purpose [16]. It was noted that presence of Lac, in addition to reductions in tNAA, has been observed in large infarcts associated with poor outcomes [78]. Visible Lac levels within the first month following a traumatic brain injury could predict outcomes for children with non-accidental trauma [79]. Amide proton transfer (APT) technique (a variant form of CEST imaging) can quantitatively measure amide protein and tissue pH that is associated with the heterogeneous pathologies in stroke lesions [80,81]. Iron

oxide nanoparticles such as SPIOs can be injected into the circulation to track monocyte infiltration in and around ischemic lesions; iron-oxide labeled probes targeting P- or E-selectin, or vascular cell adhesion molecule 1 (VCAM-1) can be used to detect inflamed, activated endothelium after stroke [82]. Injection of Gd-based enzyme-activatable agents which cause signal hyperintensity upon reaction with myeloperoxidase (MPO) can be used to detect activated neutrophil and macrophages after stroke [83]. SIPO-labeled molecular probes targeting mRNA of GFAP or mRNA of Nestin can be used to detect stroke-induced glial activation and progenitor cell migration associated with stroke recovery, respectively [84].

• Brain tumor

Anatomical biomarkers

Gd-enhanced T1W images are used in the clinics to delineate brain tumors. A randomized multicenter clinical trial of recurrent glioblastoma (GBM) patients treated with bevacizumab found that Gd-enhanced T1W subtraction maps improved visualization and aided prediction of patient survival in recurrent GBM [85]. DCE MRI using ferumoxytol (an iron-oxide-based MR CA) and perfusion MRI for tumor blood volume measurement (rCBV) can help clinicians in differentiating true tumor progression from pseudoprogression in GBM patients exhibiting apparent tumor progression after chemoradiotherapy, hence a potential prognostic biomarker [86]. Quantitative diffusion MRI based on ADC can be used to assess lesion heterogeneity and can serve as a robust biomarker for determining the stage and progression of brain tumors or the efficacy of therapy [87]. Early changes in mean ADC were highly correlated with subsequent tumor response to radiation therapy [88]. With the ability to visualize relevant white matter tracts, DTI allows neurosurgeons to guide their surgical procedures in the resection of both high- and low-grade gliomas for better patient neurological outcomes [89].

Functional biomarkers

fMRI is used in presurgical planning for tumor resections. Specifically, neurosurgeons can preoperatively map eloquent functional brain regions (i.e., motor, sensory, language and visual) to make informed decisions about surgical trajectories so that damage can be minimized during tumor resection [90].

Molecular biomarkers

^1H -MRS can reveal common metabolite changes in brain tumors, including elevations in Cho, Lac and Lip, as well as decreases in NAA and Cr. The prominent signal at 1.3 ppm from lipids present in cytoplasmic droplets associated with necrosis or hypoxia is generally associated with higher tumor grade and poor survival [91]. An increase in tCho/tNAA in the peritumoral regions reflects tumor invasiveness and can be used to differentiate high-grade gliomas from brain metastases [92]. Myo signals are used to differentiate low- and high-grade gliomas. Further, ^1H -MRS has been used to identify a glioma subtype with isocitrate dehydrogenase mutations utilizing an *in vivo* fingerprinting technique based on levels of 2-hydroxyglutamate [93]. APT MRI that was mentioned in the ischemic stroke section can be used to image amide protein to differentiate glioma and radiation necrosis after brain tumor therapy in rats [94]. The ratios of lactate-to-pyruvate after the infusion of HP ^{13}C -pyruvate and HP-MRS are being used clinically to monitor efficacy of Everolimus in treating orthotopic GBM tumors [95] and to evaluate the heterogeneous metabolic profile of pyruvate in GBM tumors [96]. Based on combined data from The Cancer Genome Atlas (TCGA) and imaging of the corresponding TCGA patients provided by The Cancer Imaging Archive, MR imaging phenotypes such as deep white matter tract involvement and ependymal enhancement are strongly linked to molecules

Figure 10. Arterial spin-labeling MRI of human brains. ASL images of patients diagnosed to have PTSD show increased perfusion in the cortex (B) compared with non-PTSD subjects in (A).

ASL: Arterial spin-labeling; PTSD: Post-traumatic stress disorders.

Adapted with permission from [67].

associated with mitochondrial dysfunction, *myc* oncogene activation and inhibition of NF- κ B inhibitor- α [97].

Conclusion & future perspective

MR biomarkers of the brain have been of great interest in recent years as they are the only means for providing direct or surrogate measurements of active brains besides biopsy. However, anatomical and functional MR images are based on image contrast and/or statistical analyses and may or may not provide adequate interpretations of biological/pathological processes. These noninvasive observables need to be better understood with the help of neurobiological investigations. Emerging molecular MRI techniques, although mostly preclinical, have shown promise in revealing molecular signature of intact brains. With increasingly sophisticated molecular MRI techniques, MRI of molecular events in the live human brain at the cellular level is within reach. In conclusion,

with quantitative interpretation methods and established relevance with underlying molecular events, MR biomarkers are poised to make significant contribution toward better understanding of live brain and improving clinical management of brain disease/disorder.

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EXECUTIVE SUMMARY

- MRI and magnetic resonance (MR) spectroscopy techniques are based on contrast and statistical analyses and may or may not provide adequate interpretation of biological/pathological processes.
- The power of MR biomarkers is the ability to track changes in anatomical, functional or molecular signature in individual subject during disease progression or therapeutic treatments.
- Combination of multiple MR biomarkers across anatomical, functional and molecular levels will provide more accurate information for clinical decision making.
- Molecular MR biomarkers will likely play an important role in aiding disease diagnosis and characterization at the gene and protein levels in the near future.

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