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Brain imaging in drug R&D

ALEX P. ZIJDENBOS[1,2], JASON P. LERCH[2], BARRY J. BEDELL[2], & ALAN C. EVANS[1,2]

[1]*Neuralyse Inc., LaSalle, Canada, and* [2]*McConnell Brain Imaging Centre, Montreal Neurological Institute, McGill University, Montreal, Canada*

Abstract
Magnetic resonance imaging (MRI), used as a clinical diagnostic tool since the early 1980s, is rapidly gaining traction as an integral part of the drug development process. Brain imaging research spans a wide area, covering both structure and function, and ranging from the physics and physiology associated with novel acquisition techniques, to the development of sophisticated image processing algorithms. This paper briefly describes two methods on either end of this spectrum: the "pipeline" framework for the fully automated morphometric analysis of brain imaging data, and molecular MRI, which holds promise for the non-invasive detection of molecular targets of new pharmacological compounds. The potential use of these technologies is illustrated by examples of their applications in multiple sclerosis, Alzheimer's disease, and oncology.

Keywords: *Magnetic resonance imaging, MRI, quantification, clinical trial, image analysis, morphometry*

Introduction

The use of magnetic resonance imaging (MRI) continues to expand. Initially (starting in the 1940s) used for spectroscopy, as an analytical method in the study of the composition of chemical compounds, it rapidly gained popularity as a radiological diagnostic tool throughout and following the 1980s. More recently, its use as a tool for the quantitative assessment of structure (anatomical MRI, aMRI) and function (functional MRI, fMRI) has rapidly been gaining momentum, especially above the neck – i.e. for imaging of the human brain. In this respect, the landmark interferon-beta-1b clinical trial (Paty et al. 1993), showing a clear correlation between MRI-measured brain lesion load and clinical findings in multiple sclerosis, placed neuroimaging firmly on the map of the drug development process.

This paper considers neuroimaging research within two broad categories: that focused on the physics and physiology of signal formation and that focused on signal analysis methodology. Two distinct areas will be highlighted: the characterization of neuroanatomy and neuropathology using novel data analysis techniques relying on "standard" aMRI data acquisition sequences; and an illustration of mMRI (molecular MRI), a novel data acquisition technique which allows for the *in vivo* characterization

Correspondence: Alex P. Zijdenbos, Ph.D., McConnell Bran Imaging Centre, Montreal Neurological Institute, 3801 University St., WB-208, Montreal, Quebec H3A 2B4, Canada. Tel: [+1] 514-398-5220. Fax: [+1] 514-398-8952. Email: alex@bic.mni.mcgill.ca

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of biological processes at the cellular and molecular level. On opposite ends of the imaging research spectrum, these methodologies are promising new tools for the drug development process.

Quantification of brain morphology

The quantitative analysis of "standard" magnetic resonance imaging (MRI) data has become increasingly important in both research and clinical studies aiming at human brain development, function, and pathology. Traditionally, these measurements are performed by human operators suffering from high intra- and interrater variabilities which may obscure a small treatment effect. The development of sophisticated image analysis techniques allows the extraction of a wide variety of "structural biomarkers" in an objective, reproducible and automated fashion. This includes the identification and quantification of various pathologies and lesions, different tissue types, specific brain structures, and cortical thickness measurements. We have developed a fully automatic "pipeline" image analysis framework which is able to not only produce a wide range of quantitative outputs on an individual's MRI brain scan, but also process large numbers of such data sets in an efficient and consistent fashion. This system has been applied to a large number of applications, as early as in 1997 when it was used for the fully automated quantification of multiple sclerosis lesion load for 2000 MRI brain scans, in the context of a phase III clinical trial (Evans et al. 1997, Weiner 1997, Zijdenbos et al. 2002).

Figure 1 shows a sample analysis pipeline, illustrating a number of processing steps present in most processing pipelines designed for structural, quantitative image analysis. The first stages of such pipelines are concerned with pre-processing and artefact reduction; MRI data typically suffers from a number of artefacts that affect the accuracy and reliability of automated image analysis, including different types of spatial variations of the MR signal and imaging noise. Many of these can be corrected or at least reduced using a variety of image processing algorithms (Zijdenbos et al. 2002).

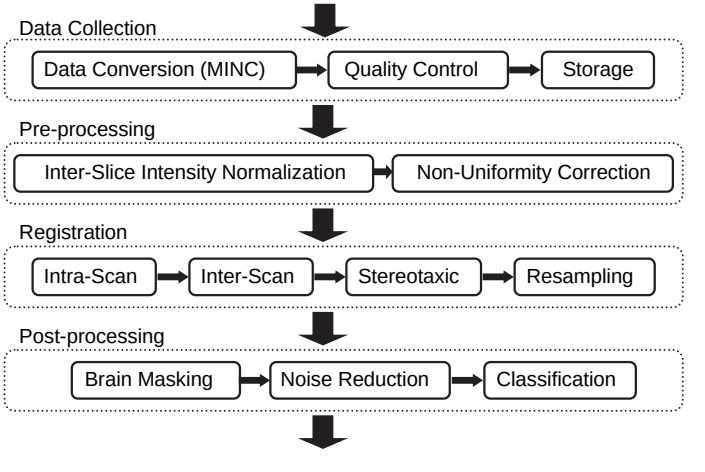

Figure 1. An example analysis pipeline for the quantitative analysis of structural MRI data.

Another central component of most automated analyses is spatial normalization (registration and resampling). The notion of a stereotaxic, brain-based coordinate system is critical for the analysis of large neuroimaging databases. For this purpose we rely on a coordinate space based on the atlas created by Talairach and Tournoux (Talairach et al. 1988) for neurosurgical applications. Variations on this concept are widely accepted in the brain mapping community to study functional activation (Evans et al. 1992, Fox et al. 1985, Frackowiak 1997) and anatomical variability (Ashburner et al. 1998, Mazziotta et al. 1995, Toga et al. 1996). This type of spatial normalization provides multiple advantages. First, it removes global differences (brain size) between subjects (Collins et al. 1994). Second, it provides a conceptual framework for the completely automated, 3D analysis across subjects. Third, it allows longitudinal and cross-site, cross-study, and cross-population analysis. Fourth, the Talairach space enables the use of spatial priors for classification and allows the use of spatial masks for postprocessing and anatomically driven hypothesis testing (Kamber et al. 1995, Riahi et al. 1998). Finally, it provides a framework for statistical analysis of the results based on established random field models (Worsley et al. 1992, 1996).

Tissue classification

The labelling of individual pixels or voxels with a particular tissue class is typically referred to as (voxel) classification. Most commonly in brain imaging work, voxels are labelled as belonging to one of the predominant tissue classes in the brain: white matter, grey matter, or cerebrospinal fluid (CSF). We have developed INSECT (Intensity Normalized Stereotaxic Environment for Classification of Tissues) which generates tissue class maps using an artificial neural network classifier (Zijdenbos et al. 2002), see Figure 2. For applications in neuropathology, INSECT can also be used to label voxels as belonging to areas affected by the pathology, e.g., white matter lesions in multiple sclerosis, or tumour areas in oncology. More recently, we are relying on accurate partial volume estimation in the classification process, which enhances the accuracy of volume estimation and aids other computational techniques such as the cortical thickness estimation method described below (Tohka et al. 2004).

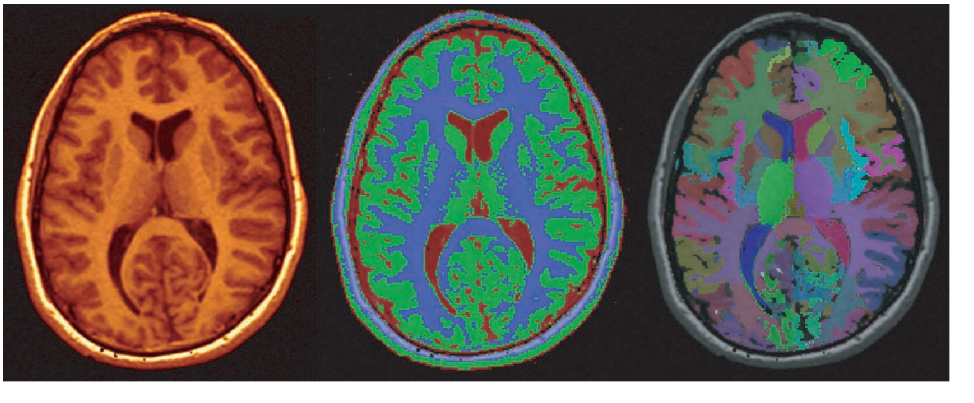

Figure 2. T1-weighted MRI scan (left), the corresponding INSECT-based tissue classification (middle) and the ANIMAL+INSECT brain structure segmentation result (right).

Anatomical segmentation

Anatomical segmentation – sometimes referred to as regional parcellation – is the assignment of neuroanatomical structural membership labels to voxels. Segmentation is a top-down process which frequently relies on higher-level knowledge of anatomy (e.g., atlases). This is both conceptually and methodologically distinct from tissue classification which is a data-driven, bottom-up process. ANIMAL (Automated Non-linear Image Matching and Anatomical Labelling) is an automated algorithm to perform this labelling in 3D (Collins et al. 1995). ANIMAL deforms one MRI volume to match another, previously labelled, MRI volume. It builds up a 3D non-linear deformation field in an iterative, multi-scale fashion. Anatomical labels are defined in the new volume by mapping the original labels through the derived 3D deformation field. It is possible to combine the top-down ANIMAL approach with the bottom-up INSECT classification to obtain improved segmentation results (Collins et al. 1999), see Figure 2.

Cortical thickness measurement

Rather than labelling individual voxels, it is possible to extract an explicit model of the cortex of the brain. In order to accomplish this we have developed ASP, an iterative method which yields 2D, tessellated mathematical models of the inner and outer cortical surfaces (MacDonald et al. 2000). ASP generates simple (non-self-intersecting) surfaces with spherical topologies using deformable models. Starting from the tissue classification, the process first deforms an initial spherical model towards the white/grey matter interface, using a combination of model- and image-based constraints. The white matter surface is then taken as a starting point for the expansion towards the pial surface. These two surfaces allow for the measurement of cortical thickness in 3D throughout the brain (Lerch et al. 2005). As a final step before statistical analysis the cortical thickness data are blurred using a surface based diffusion smoothing kernel (Chung et al. 2002), see Figure 3.

Molecular MRI

Although quantitative aMRI is capable of providing a wealth of information regarding normal and pathological processes, the structural changes seen on aMRI usually manifest late in the disease process, often after irreversible damage has occurred. Furthermore, aMRI is not capable of providing information regarding processes occurring at the cellular or molecular level. This information is generally provided from microscopic examination or molecular analysis of tissue. Although considered gold-standard diagnostic techniques, the reliance on tissue has several serious limitations, including its invasive nature and the inability to perform longitudinal studies on a single subject or animal. As such, recent years have witnessed the emergence of *in vivo* molecular imaging (Blankenberg 2003, Blasberg 2002, Cherry 2004, Heckl et al. 2004, Weissleder & Mahmood 2001). Although *in vivo* molecular imaging has traditionally been the domain of positron emission tomography (PET) and optical imaging, these techniques are limited by several factors including radiation dose concerns, poor spatial resolution, high cost, and limited availability. Recent attention, therefore, has turned to the use of MRI, which does not suffer such limitations, as a molecular imaging modality. It is certainly beyond the scope of this

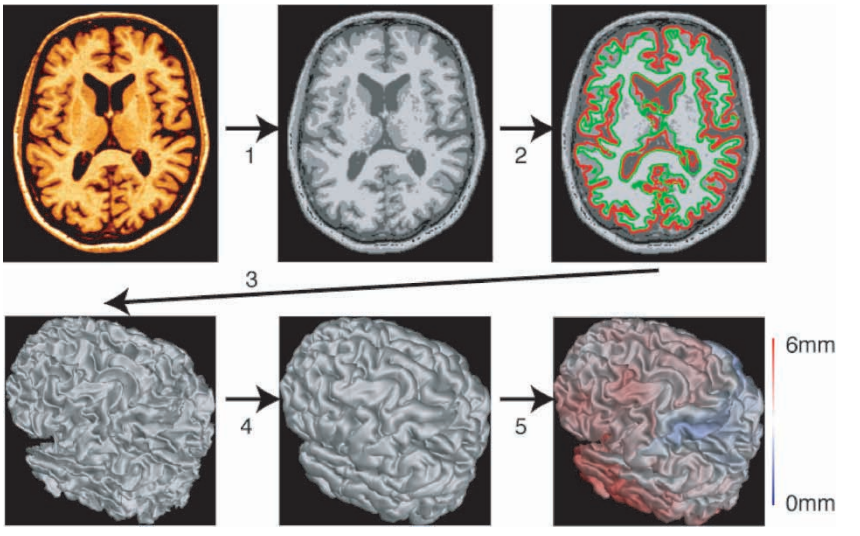

Figure 3. Native MRIs are registered to stereotaxic space and corrected for non-uniformity artefacts. They are then classified into white matter, grey matter, and CSF (1). Deformable models are fit to the white matter surface (3) and pial surface (4); the accuracy of the fit can be seen in (2), where the red line represents the pial surface, the green line the white matter surface. Once the two surfaces are extracted cortical thickness can be extracted and smoothed along the surface (5).

manuscript to review the burgeoning field of molecular MRI (mMRI), and we will focus, rather, on our recent work in this exciting field.

The emergence of mMRI has been lagging primarily due to the low detectability of conventional MRI contrast agents, which is generally several orders of magnitude below the *in vivo* concentrations of molecular targets (Nunn et al. 1997). We have overcome this obstacle by developing novel probes which do not rely on a single paramagnetic molecule binding to the molecular target (Bedell et al. 2004). Rather, our probes modulate microvascular dynamics, namely permeability and blood flow, which provides a much higher sensitivity than conventional probes. Quantitative analyses of changes in local microvascular permeability and/or blood flow following administration of a targeted probe provide an estimation of the relative tissue content of the molecular entity of interest.

Sample application areas

The MR imaging methodologies described here have a wide range of applications in the drug development process. Three illustrative examples are briefly described here.

Multiple sclerosis

Since the landmark Interferon-beta-1b trial in the early 1990s, a number of MRI-based surrogate endpoints are typically used in clinical trials in MS: (i) count and volume of MS plaques as visible in T2-weighted or FLAIR acquisitions; (ii) count of lesions showing signal enhancement following administration of gadolinium-DTPA; (iii) count of so-called "black holes" (Truyen et al. 1996) as visible on T1-weighted scans. Traditionally, these surrogate endpoints are quantified using assessment by a

human operator, either by or under supervision of a radiologist. Using modern image processing technology as described earlier, it is possible to completely automate the quantifications of these endpoints. This removes all operator-induced measurement variability and allows for more efficient analyses. In addition, results can be analysed using statistical techniques now ubiquitous in the brain mapping community. As an example, Figure 4 shows a 3-D probabilistic distribution of MS lesions, derived from a population of 462 MS patients. In essence, the question of whether a pharmaceutical compound has an effect on the burden of disease can be answered by a statistical comparison of two such lesion distributions, one derived from the placebo group, one from the active group. In addition, the application of standard voxel-wise statistical analysis techniques (Ashburner et al. 2000) allows the correlation of clinical outcomes against local brain morphology (Charil et al. 2003).

Alzheimer's disease

In Alzheimer's Disease (AD) the definitive pathological markers are amyloid plaques and neurofibrillary tangles. These are not visible in standard morphometric MRI, so the surrogate marker used is macroscopic atrophy, which co-locates with the microscopic hallmarks of the disease (Braak et al. 1991, Chetelat & Baron 2003, Fox et al. 2000, Gomez-Isla et al. 1997). In a recent study (Lerch et al. 2004), 19 AD patients and 17 controls were investigated using structural MRI and cortical thickness measurements. The results from this study clearly show statistically significant differences in cortical thickness between the two groups. Furthermore, the resulting maps of atrophy (Figure 5) clearly show region specificity of thickness decline in AD.

In conclusion, measures of cortical thickness from MRI provide a detailed view of AD, showing clear and believable differences between patients and controls. Moreover,

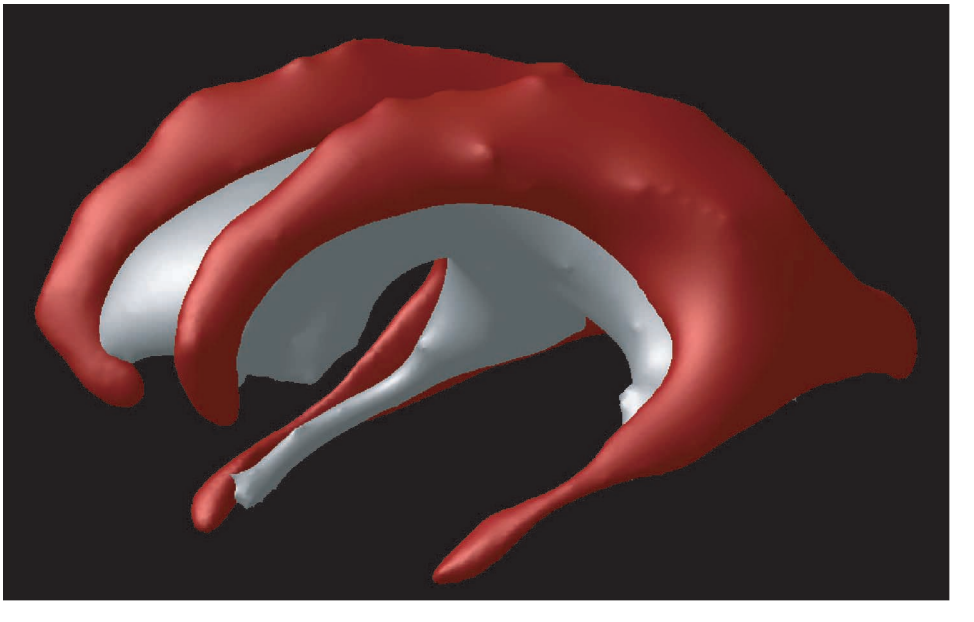

Figure 4. In red: 3D surface rendering of the MS lesion probability distribution derived from 462 MS patients. For reference, the ventricular system is rendered in white.

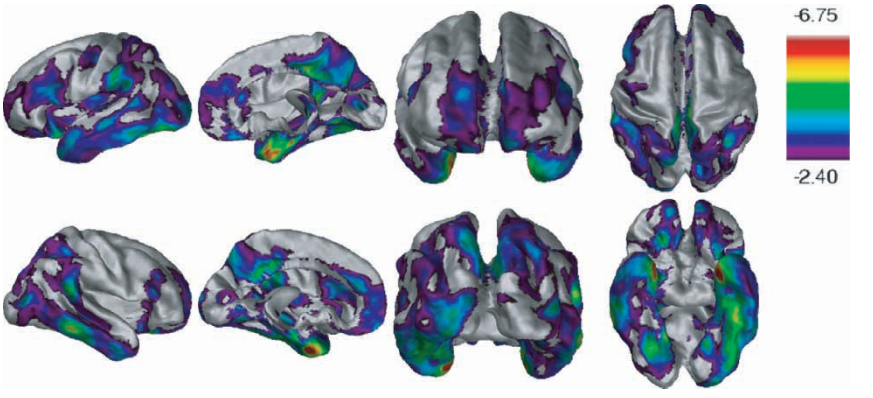

Figure 5. T-statistics of the difference between AD patients and normal elderly controls. The results show widespread atrophy with a specific focus on the temporal lobes and the limbic cortex.

since the technique is *in vivo*, progression can be monitored and correlations performed against cognitive scores, further elucidating the disease process.

Oncology

Oncology is an area where MRI has been utilized since its first use as a diagnostic imaging tool. As in MS, measurements of tumour size have been used as surrogate endpoints in oncology clinical trials, although the details of the quantification metrics used are still controversial (Mazumdar et al. 2004, Park et al. 2003). Nevertheless, the type of quantitative MRI analysis techniques described here, are able to not only identify tumours volumetrically, but also subtype the different components of the pathology (Figure 6). From this tissue characterization all currently debated tumour response criteria can be easily calculated.

Although the structural assessment of tumour response is commonplace in oncology clinical trials, molecular MRI is a potentially more rewarding technique for use in drug development. The drug development industry is currently moving away from conventional cytotoxic therapies and towards the generation of rationale targeted therapies, including growth-factor receptor inhibitors, anti-angiogenesis drugs, enzyme-activated prodrugs, and gene therapies. The ability to non-invasively detect the molecular targets of these new drugs and monitor their response to therapeutic intervention would greatly facilitate their development, shortening the time from the bench to clinical use.

We have specifically designed probes which allow for detection of (1) mediators of angiogenesis, (2) matrix metalloproteinases, and (3) reporter genes. We are currently evaluating the ability of these probes to image these targets *in vivo* in a rat C6 glioma model (Bedell et al. 2004). The C6 glioma is a model of the human brain tumour, glioblastoma multiforme, which is an aggressive tumour, generally resistant to currently available therapies, and carries a dismal prognosis. Both academic and research labs put intense effort into the development of drugs which specifically inhibit the actions of molecules such as vascular endothelial growth factor (VEGF), which promotes angiogenesis, and matrix metalloproteinases, which are involved in tumour invasion, metastasis, and angiogenesis (Kodera et al. 2000). The ability to non-invasively evaluate the efficacy of these new agents *in vivo* should bring these

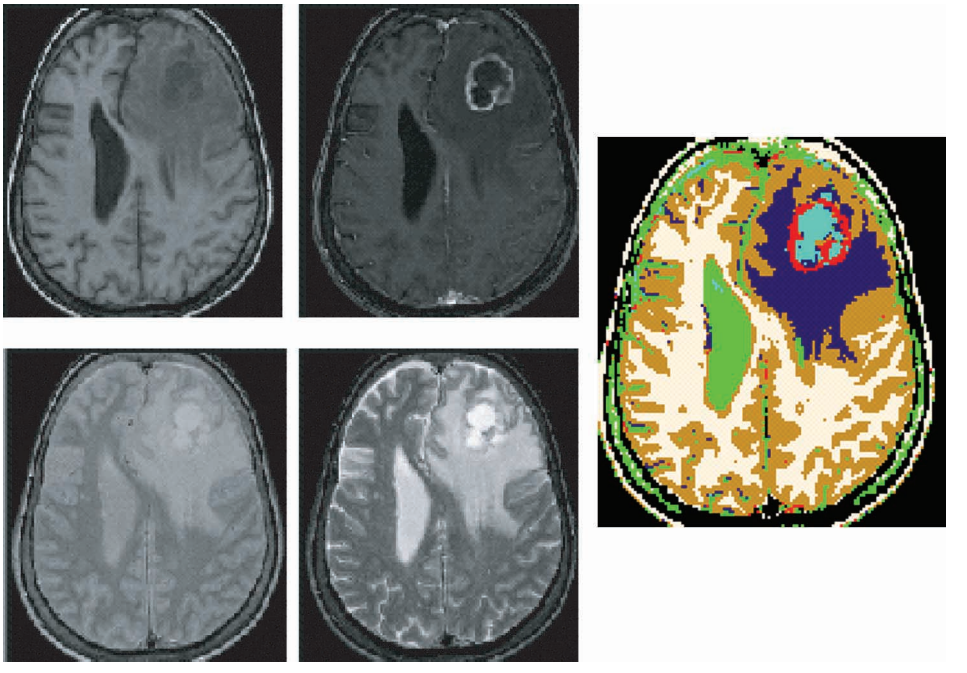

Figure 6. Classification and sub-typing of tumour components based on four MRI modalities. The four MRI images are, clockwise from top left: T1-weighted, T1-weighted following administration of gadolinium-DTPA, T2-weighted and proton-density-weighted. The colour image shows a 6-tissue-class classification based on these four input images, with white = white matter; brown = grey matter; green = cerebrospinal fluid; purple = oedema; red = enhancing ring; turquoise = necrotic centre.

treatments to patients more rapidly and at a lower cost to overburdened health-care systems.

Discussion

From a wide range of neuroimaging based technologies, we have addressed two distinct areas of research with strong potential in the drug development arena: sophisticated, quantitative analysis of imaging data acquired with traditional acquisition technology, and molecular MRI (mMRI), and exciting new image acquisition methodology. The potential uses, of which there many, of these techniques were illustrated for three different application areas: automatic lesion quantification in multiple sclerosis, cortical thickness measurements in AD, and early reports of mMRI applications in oncology. These applications provide a glimpse of the tremendous potential that imaging biomarkers have in drug research and discovery.

Given the logistical and algorithmic challenges posed by the automatic analysis of thousands of MRI data sets, we consider the "pipeline" framework, in combination with the concept of stereotaxic space, essential to the data processing of large image databases. The application of this framework to a phase III clinical trial in MS shows that it is possible to build and execute a fully automatic image processing pipeline which delivers the efficacy parameters dictated by such a study. Moreover, the ability to rapidly re-configure the processing pipeline and re-analyse the data in response to

changing requirements of the study is an important advantage over manual or semi-automatic approaches. Such a fully automated approach allows for the addition of new image processing techniques as they are developed to continually improve the potential research targets in drug R&D. To cite one example of the benefits provided by automated MRI analysis, in validation studies of the MS study described here (Zijdenbos et al. 2002), the average coefficient of variation (CV) of total lesion load assessments went from 44% for independent manual validation, to 27% for on site manual validation, to 0% for the automated image analysis pipeline. Moreover, the automated analysis of MRI provides an objective, reproducible *in vivo* metric of disease progression and remission, often in contrast to the neurological examination. To illustrate this distinction, consider that a small MS lesion in a critical brain region can result in a very poor clinical assessment score (EDSS) whereas a large lesion in a relatively silent area may only minimally affect the EDSS score. In these cases, the MRI burden of disease more properly reflects disease progression (Evans et al. 1997).

Similarly, the ability to quantify and localize atrophy using automated cortical thickness measurements is well illustrated here by application to Alzheimer's Disease (Lerch et al. 2004). The patterns of thinning co-locate well with the putative presence of microscopic pathological features (plaques and tangles), increasing confidence in these results. Lastly, the development of mMRI probes can, in the future, extend analysis to move beyond macroscopic anatomical changes to the study of particular chemical targets.

The unparalleled ability of MRI studies to follow patients longitudinally combined with automated analysis capabilities permits a direct view on disease progression and the potential influence of pharmacological interventions on that process.

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