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High throughput object-based image analysis of β-amyloid plaques in human and transgenic mouse brain

Harry D. Samaroo[a], Alan C. Opsahl[b], Jan Schreiber[c], Sharon M. O'Neill[a], Michael Marconi[a], Jessie Qian[b], Santos Carvajal-Gonzalez[a], Barbara Tate[a], Anthony J. Milici[a], Kelly R. Bales[a,*], Diane T. Stephenson[a]

[a] Neuroscience Biology, Pfizer Global Research & Development, Eastern Point Road, Groton, CT 06340, USA
[b] Investigative Pathology, Pfizer Global Research & Development, USA
[c] Definiens AG, Trappentreustrasse 1, 80339 München, Germany

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ABSTRACT

Advances in imaging technology have enabled automated approaches for quantitative image analysis. In this study, a high content object based image analysis method was developed for quantification of β-amyloid (Aβ) plaques in postmortem brains of Alzheimer's disease (AD) subjects and in transgenic mice over overexpressing Aβ. Digital images acquired from immunohistochemically stained sections of the superior frontal gyrus were analyzed for Aβ plaque burden using a Definiens object-based segmentation approach. Blinded evaluation of Aβ stained sections from AD and aged matched human subjects accurately identified AD cases with one exception. Brains from transgenic mice overexpressing Aβ (PS1APP mice) were also evaluated by our Definiens object based image analysis approach. We observed an age-dependent increase in the amount of Aβ plaque load that we quantified in both the hippocampus and cortex. From the contralateral hemisphere, we measured the amount of Aβ in brain homogenates biochemically and observed a significant correlation between our biochemical measurements and those that we measured by our object based Definiens system in the hippocampus. Assessment of Aβ plaque load in PS1APP mice using a manual segmentation technique (Image-Pro Plus) confirmed the results of our object-based image analysis approach. Image acquisition and analysis of 32 stained human slides and 100 mouse slides were executed in 8 h and 22 h, respectively supporting the relatively high throughput features of the Definiens platform. The data show that digital imaging combined with object based image analysis is a reliable and efficient approach to quantifying Aβ plaques in human and mouse brain.

1. Introduction

Technological advances in the field of image analysis allow for objective and efficient quantitative assessment of disease pathology, thus enabling precise studies of basic pathophysiology and disease progression. High content analytical methods in the nervous system are enhancing our ability to analyze complex data such as gene expression, cellular models and invertebrate model systems (Dragunow, 2008). The study of Alzheimer's disease (AD) is one area in which significant breakthroughs in imaging are advancing our understanding of disease pathogenesis (Weiner et al., 2010). At present, many novel therapies aimed at addressing the amyloid hypothesis are being tested in the clinic (Creed and Milgram, 2010). Given that noninvasive approaches for measuring amyloid plaques in the living human brain are becoming more widespread (Noble and Scarmeas, 2009), in vivo and postmortem evaluation of the effects of drug candidates on amyloid content in animal models represents a translational strategy to enhance confidence in the use of imaging biomarkers for advancing novel amyloid targeted drug candidates in patients. Pathologic assessment of disease relevant structures in AD is complemented and expanded by recent noninvasive imaging advances such as structural and functional neuroimaging. Conventional methods of image analysis in pathology are limited due to factors such as low throughput image acquisition, limited tissue sampling, and manual image analysis operation. In the nervous system in particular, there are additional sampling challenges inherent to the heterogeneity and complexity of the brain.

Virtual microscopy and automated image analysis (Rojo et al., 2009; Ying and Monticello, 2006) are recent technologies paving the way for a new era of high impact in the field of anatomic pathology. Virtual microscopy enables histopathological assessment of a specimen using a digital whole-slide image. The resulting virtual images allow for the capture of an area of interest at one magnification and its analysis in greater detail at a higher

* Corresponding author. Postal address: Pfizer Inc., Eastern Point Rd, MS 8220-3417, Groton, CT 06340, USA. Tel.: +1 860 715 6237.
E-mail address: Kelly.Bales@Pfizer.com (K.R. Bales).

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magnification. Importantly, whole slide scanning eliminates the need for sampling fields within a given specimen which allows for simplification of study designs, reduction of bias and increasing reproducibility. To date, several different types of scanning technologies exist for acquiring virtual images including progressive scanning (Nikon Coolscope, Zeiss MiraxScan), line scanning (Aperio ScanScope CS, XT, Hamamatsu NanoZoomer), and optical matrix (DMetrix DX-40) (Mulrane et al., 2008). Virtual microscopy is becoming widespread in teaching institutions (Kumar et al., 2004) and is being applied with increasing frequency to study the nervous system in normal and disease conditions. Examples of the impact of virtual microscopy in neuroscience are exemplified by the presence of large online image databases such as http://brainmaps.org/, the Allen Brain Atlas (http://www.brain-map.org/) and MicroBrightField's http://neuroinformatica.com/ with as much as 60 terabytes of storage (Mikula et al., 2007).

Automated image analysis is the application of complex computer algorithms to both process and interpret large batches of images in an objective and reproducible manner. Automated image analysis has advanced particularly in the area of cell based imaging applications (Evans et al., 2008; Narayan and Dragunow, 2010). Quantitative image analysis of virtual images can be applied at high magnification to entire tissue sections stained for a particular cellular or molecular marker in a relatively high throughput manner. Application of the algorithms to stitched and tiled images avoids the potential challenges or bias of random sampling. Furthermore, object-based image analysis enables definition of stained structures based on morphologic and relational features as opposed to pixels alone.

Definiens object-based image analysis software, originally designed as a tool for geospatial intelligence and remote sensing, is increasingly being applied to the biomedical sciences (Apfeldorfer et al., 2008; Baatz et al., 2009; Mech et al., 2011). The Definiens software uses algorithms (or rule sets) specific to each bioapplication to segment and classify objects based on color, shape, texture and details of their surroundings. Hence, it models the processes that underlie the way the human brain recognizes and interprets relational features of objects within an image. The interactive mode of Definiens allows for script development with a steep learning and progress curve. The execution environment uses a workspace concept in which the user may process many images offline and, if needed, in parallel on a computer cluster. Significant resources are required to develop a reliable rule set for the specified application. Once validated, a single rule set can be applied to literally hundreds of sections by automated computerized approaches requiring little to no supervision.

In this study, a high throughput object-based image analysis methodology using the Definiens software was established and validated to quantify immunohistochemically stained Aβ plaques in human and transgenic mouse postmortem brain specimens.

2. Materials and methods

2.1. Human brain tissues

Human post-mortem brain from pathologically confirmed cases of AD ($n = 7$) and neurologically normal controls ($n = 7$) was obtained from the Sun Health Research Institute (Sun City, AZ) (see Table 1). Tissue procurement and acquisition complied with informed consent and local IRB guidelines. All cases were selected so that there were no differences between the groups in terms of age, gender and postmortem delay interval (PMDI). The AD and non-AD cohorts had average ages of (80 ± 4 yrs) and (80 ± 2 yrs) at death. Average PMDI were (2.0 ± 0.2 h) and (2.7 ± 0.5 h) for AD and non-AD cases, respectively. Both males and females were included for both groups (Table 1). All brain tissue slabs were fixed in paraformaldehyde for 24–48 h and stored in glycol–formalin solution at −20 °C. Tissue was sampled from the superior frontal gyrus (cortex) and basal ganglia, regions differentially affected by AD neuropathology. Blocks of cortex and basal ganglia (approximately 4 cm^2) were embedded in paraffin and processed according to standard methods.

2.2. Generation of PS1APP transgenic mice

All animals used in this study were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. All procedures related to animal care and treatment were conducted according to the guidelines of the Institutional Animal Care and Use Committee at Pfizer, the National Research Council Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals, and The United States Department of Agriculture Animal Welfare Act and Animal Welfare Regulations. A transgenic mouse line overexpressing the human presenilin protein was created (Supplementary Fig. 1). A cDNA containing a mutation (G384A) in the human presenilin gene that is associated with familial AD was cloned into a vector containing the Thy1.1 promoter and insulators from the chicken β-globulin locus (Chung et al., 1997). This cDNA was then microinjected into nuclei of fertilized C57BL/6J (Jackson Labs) mouse embryos, as described (Hogan et al., 1986). Numerous founder lines were created from the initial microinjections. Quantitative PCR was used to confirm expression of the transgene in PolyA RNA isolated from brain tissue. A Taqman reaction was carried out using the following primers:

Human primer/probe set:

- forward primer: GGACAACCACCTGAGCAATACTG
- reverse primer: GGCTCCGTCTGTCGTTGTG
- probe: 6FAM-ACGTAGCCAGAATGACAATAGAGAACGGCAG-

Relative Expression Values were calculated by delta Ct method using GAPDH as the control. Based on PCR results indicating high levels of transgene expression one line (PS1 G384A) was selected and bred to homozygosity. Homozygous PS1 G384A transgenic mice were then crossed to APPswe mice (Hsiao et al., 1996), and offspring we backcrossed to PS1 G384A mice. The mice used for this study (PS1APP) were hemizygous for APPsw and homozygous for PS1 G384A.

2.3. Mouse brain tissues

Female PS1APP mice were evaluated at 4, 6, 8, and 12 months of age ($n = 6$ per group). Twelve month old wild type C57BL/6J ($n = 3$) and PS1 only animals ($n = 3$) were also evaluated in parallel. All animals were bred, group-housed, aged and genotyped at Charles River Laboratories (Wilmington, MA). Mice were euthanized with a lethal dose cocktail of ketamine (130 mg/kg), acepromazine (1.3 mg/kg), and xylazine (6.5 mg/kg). Brains were excised and bisected at the midline. One hemibrain was frozen on dry ice for Aβ ELISA measurements and the other was fixed for histology.

2.4. Aβ ELISA measurement

For biochemical evaluation of brain Aβ, hemibrains from PS1APP mice at age 4 ($n = 6$), 6 ($n = 4$) and 8 ($n = 4$) months were micro-dissected to yield cortex and hippocampus samples. The hippocampus was excised in its entirety first, and from the remaining tissue, a consistently sized portion of the frontal cortex was dissected. Brain samples were homogenized in a 1:10 dilution of 5 M Guanidine HCl (Sigma, St. Louis, MO) and incubated for 3 h at