

## Gadolinium-staining reveals amyloid plaques in the brain of Alzheimer's transgenic mice

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### Abstract

Detection of amyloid plaques in the brain by *in vivo* neuroimaging is a very promising biomarker approach for early diagnosis of Alzheimer's disease (AD) and evaluation of therapeutic efficacy. Here we describe a new method to detect amyloid plaques by *in vivo* magnetic resonance imaging (MRI) based on the intracerebroventricular injection of a nontargeted gadolinium (Gd)-based contrast agent, which rapidly diffuses throughout the brain and increases the signal and contrast of magnetic resonance (MR) images by shortening the T1 relaxation time. This gain in image sensitivity after *in vitro* and *in vivo* Gd staining significantly improves the detection and resolution of individual amyloid plaques in the cortex and hippocampus of AD transgenic mice. The improved image resolution is sensitive enough to demonstrate an age-dependent increase of amyloid plaque load and a good correlation between the amyloid load measured by  $\mu$ MRI and histology. These results provide the first demonstration that nontargeted Gd staining can enhance the detection of amyloid plaques to follow the progression of AD and to evaluate the activity of amyloid-lowering therapeutic strategies in longitudinal studies.

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### 1. Introduction

The formation of senile plaques composed of aggregated extracellular deposits of  $\beta$ -amyloid (A $\beta$ ) is 1 of the major neuropathological hallmarks of Alzheimer's disease (AD). The development of *in vivo* neuroimaging techniques to noninvasively detect amyloid plaques in the brain is a very promising approach for an earlier diagnosis of AD and can be used to evaluate the efficacy of anti-amyloid therapies in clinical trials. The ability to image amyloid load could also

provide a translational biomarker for longitudinal pharmacological studies in animal models and clinical trials.

Histological stains have been developed to detect amyloid plaques on brain sections. Besides this conventional postmortem technique, most neuroimaging studies of amyloid load in humans have been performed with positron emission tomography (PET) and several ligands have been developed that can detect amyloid in human brains (Klunk et al., 2004; Nordberg, 2007). However, the low spatial resolution of PET does not allow the visualization of individual plaques. Studies in animals have provided controversial results with some data suggesting that PET ligands such as the Pittsburgh compound B (PiB) can detect amyloid plaques (Maeda et al., 2007), while some other reports suggested that plaques are not detected by these ligands

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(Klunk et al., 2005). Various other imaging modalities have been developed to detect amyloid plaques (see Delatour et al., 2010 and Dhenain, 2008 for reviews). For example, optical imaging dyes such as AOI987, an oxazine dye, have been demonstrated to readily penetrate the intact blood-brain barrier (BBB) and to bind to amyloid plaques (Hintenstein et al., 2005). Using near-infrared optical imaging, a specific interaction of AOI987 with amyloid plaques was shown in mice *in vivo*, and confirmed by postmortem analysis of brain slices.

Only neuroimaging studies based on magnetic resonance imaging (MRI) have detected individual amyloid plaques in human brain samples (Meadowcroft et al., 2009) and in mouse models (Chamberlain et al., 2009; Dhenain et al., 2009; Jack et al., 2004). Several approaches have been evaluated based on the natural contrast of the plaques or with dedicated targeted contrast agents. The plaques appear as dark spots in T2, T2\*-weighted (T2\*w) or susceptibility-weighted images because of the presence of iron in the core of these lesions. However, in humans, the possibility to detect iron within plaques has been controversial (Dhenain et al., 2002; Meadowcroft et al., 2009). Also, iron accumulation in mice mainly occurs in old animals, which makes amyloid plaque detection very challenging in young animals. Using magnetic resonance (MR) contrast agents can be another option to detect amyloid plaques with MRI. One study using a fluorine-based MR contrast agent has been reported to detect amyloid plaques following intravenous injection of the agent in mice (Higuchi et al., 2005). Some other studies have been successful for early stage detection of amyloid plaques thanks to amyloid peptides tagged with MR contrast agents (gadolinium chelates or monocrySTALLINE iron oxide nanoparticles) in combination with compounds like mannitol that allow the contrast agent to cross the BBB (Poduslo et al., 2002; Sigurdsson et al., 2008; Wadghiri et al., 2003). However, these methods are technically difficult and still require extensive validation.

Staining tissue samples with gadolinium (Gd) chelates has previously been used to increase the signal- and contrast-to-noise ratios in MR images of whole mice (Johnson et al., 2002) or of brains (Benveniste et al., 2000; Dhenain et al., 2006; Kappeler et al., 2007). These protocols were based on the administration of a Gd chelate and formalin by intracardiac perfusion (active staining; Johnson et al., 2002) or by immersion of tissues in the Gd solution (passive staining; Dhenain et al., 2006). In the present study, we describe a new Gd staining method based on the injection of a nontargeted Gd agent into the cerebral ventricles that significantly improves the *in vivo* detection of amyloid plaques by  $\mu$ MRI in the brain of an APP/PS1 transgenic mouse model of amyloidosis (Blanchard et al., 2003). This method may also be applicable to detect amyloid plaques and evaluate the activity of anti-amyloid agents in longitudinal studies in the same animals.

## 2. Methods

### 2.1. Animals

Experiments were conducted on female APP/PS1 transgenic (Tg) mice overexpressing amyloid precursor protein (APP) and presenilin 1 (PS1) mutations associated with familial AD (double Thy1 APP751 SL Swedish (KM670/671NL) and London (V717I) mutations introduced in the human APP751 sequence  $\times$  HMG PS1 M146L transgenic mouse line) (Blanchard et al., 2003; Delatour et al., 2006). In these animals, amyloid deposition starts at the age of 2.5 months (Blanchard et al., 2003). Amyloid-free PS1 females were used as controls. *In vivo* images were recorded for 4 groups of animals at ages ( $\pm$  1 week) of 6, 9, 14, and 20 months. Six animals were followed longitudinally and were injected at 2 time points: 4 animals were first injected at 3.5 months and then at 8 months; 2 animals were first injected at 14.5 months and then at 17.5 months. Fourteen additional animals were used to evaluate neuroinflammation and animal weight up to 42 days following intracerebroventricular injection (ICV) of gadolinium. A total of 36 animals were used for this study (19 APP/PS1 and 17 PS1 controls).

### 2.2. Surgical procedure

The animals were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine, 10 mg kg<sup>-1</sup> (Imalgène® 500, Merial, Lyon, France) and medetomidine 0.68 mg/kg (Domitor®, Pfizer Santé Animale, Paris, France). After their heads were shaved, the mice were placed on a stereotaxic frame using ear bars and a tooth bar to hold them still. A heating pad was used to keep them at physiological temperature throughout the procedure. After a midline incision into the skin, the coordinates of the bregma were recorded for anterior-posterior (A/P) and lateral (L) references. The skull was bilaterally perforated with a Dremel at coordinates A/P -0.2 mm and L  $\pm$  1 mm, according to a stereotaxic atlas (Paxinos and Franklin, 2001). Blunt Hamilton syringes were used to inject gadoterate meglumine (Gd-DOTA, DOTAREM®, Guerbet, Aulnay-sous-Bois, France) into the lateral ventricles at coordinate -1.8 mm relative to the surface of the dura mater. A total volume of 1  $\mu$ L (0.5  $\mu$ mol) was injected in each side at a rate of 0.2  $\mu$ L/minute. Upon completion of the injections, the needles were very slowly withdrawn to minimize any outflow from pressure release and the skin was then sutured back. The anesthesia was reversed using atipamezole hydrochloride (Antisedan®, Pfizer) at a dose of 10 mg/kg to allow recovery before  $\mu$ MR imaging.

The effect of injection on animal's physiology was further evaluated by monitoring the weight of 17-month-old PS1 mice over 42 days after ICV injection of gadolinium ( $n = 6$ ) or saline ( $n = 6$ ).

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