Kognitive und neurostrukturelle Korrelate von CADASIL als Modellerkrankung einer vaskulären Demenz

Dissertation
zum Erwerb des Doktorgrades der Humanbiologie
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
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aus
Engelskirchen
Jahr
2013
Mit Genehmigung der Medizinischen Fakultät
der Universität München

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Tag der mündlichen Prüfung: 20.11.2013
Danksagung

Ein ganz herzliches Dankeschön an meinen Doktorvater, Prof. Dr. Martin Dichgans, für die fachliche Unterstützung, Bereitstellung der Ressourcen und die Inspiration. Zutiefst zu Dank verpflichtet bin ich Dr. med. Marco Düring, ohne den diese Arbeit nicht möglich gewesen wäre. Danke für Diskussionen, Anregungen und sehr produktive gemeinsame Forschungsarbeiten.

Allen anderen Mitarbeitern des ISD danke ich für die gute Zusammenarbeit, angenehme kollegiale Atmosphäre und über die Fachbereiche hinausgehende freundschaftliche Verbindungen.

Last, but not least danke ich meiner Familie: den kleinen und großen Mitgliedern, den nahen und fernen Verwandten, für die moralische und seelische Unterstützung und für den nicht enden wollenden Glauben an mich.
Zusammenfassung


In der zweiten Publikation wurde ein Faktor untersucht, der diesen Zusammenhang zwischen Funktion und Struktur moderiert, die Kognitive Reserve. Ein häufig postuliertes Modell der kognitiven Reserve als aktiver Kompensationsmechanismus konnte in den CADASIL-Daten bestätigt werden. Patienten mit höherer Schulbildung (als Operationalisierung kognitiver Reserve) zeigten bei gleichem Ausmaß an Pathologie weniger Beeinträchtigung in Bearbeitungsgeschwindigkeit und Exekutivfunktionen als weniger Gebildete, jedoch nur bei geringem und mittlerem Ausmaß an Pathologie.
Abstract

The subject of this thesis is cognitive impairment following cerebrovascular disease. Due to the heterogenity of „vascular cognitive impairment“ (VCI) and the frequent comorbidity with Alzheimer's Dementia, a monogenous form of microangiopathy (cerebral autosomal dominant angiopathy with subcortical infarcts and leucoencephalopathy, CADASIL) is used as a model of pure VCI. The first publication used voxel-based lesion-symptom-mapping to investigate the relationship between lesions in frontal subcortical tracts and CADASIL-specific cognitive impairment. The strongest correlation was found for cognitive processing speed and lesions in the anterior thalamic radiation as well as the Forceps Minor. An additional regression analysis showed that instead of the total amount of brain damage, lesion load in previously identified locations is significant for specific functional impairment.

The second publication explored a factor moderating the relationship between structure and function, namely Cognitive Reserve. A frequently proposed model of cognitive reserve as an active compensation mechanism could be confirmed in the CADASIL population. Patients with higher formal education (as proxy for cognitive reserve) showed less impairment in processing speed and executive function at the same amount of pathology than less educated patients. This difference however was only present for low and medium levels of pathology.
1 Einleitung

1.1 Vaskuläre Demenz und vaskuläre kognitive Störungen

Einleitung.

Abb.1: Mechanismen der vaskulären kognitiven Störung.

Multiple Territorialinfarkte sowie ein einzelner strategischer Infarkt können kognitive Beeinträchtigung und in letzter Konsequenz eine Demenz zur Folge haben. Mikroangiopathien („small vessel disease“) führen zu lakunären Läsionen, großflächigen Läsionen der weißen Substanz („white matter hyperintensities“, WMH; siehe Kap. 1.2) sowie Atrophie und bedingen dadurch ebenfalls kognitive Beeinträchtigung und die subkortikale ischemische vaskuläre Demenz.

Einleitung.


1.2 CADASIL

Bei 30-40% der Patienten ist das erste klinische Symptom eine Migräne mit Aura, die früh beginnen kann (bereits mit Mitte 20), jedoch im späteren Verlauf wieder abnimmt. Im MRT-Bild lassen sich zum selben Zeitpunkt bereits WMH feststellen. Ischämische Schlaganfälle sind ab Anfang 30 zu beobachten. Es folgen kognitive Beeinträchtigungen, allen voran eine exekutive Dysfunktion, sowie affektive Störungen und im späteren Verlauf Apathie, motorische Beeinträchtigungen und Demenz.

Aufgrund des frühen Beginns der Erkrankung und der charakteristischen neuropsychologischen Defizite sowie der MRT-Charakteristika ist CADASIL geeignet als Modellerkrankung für eine reine subkortikale ischämische vaskuläre Demenz (SIVD) (Abb.3).

1.2.1 Kognitive Beeinträchtigung bei CADASIL

Kognitive Beeinträchtigung ist die zweithäufigste klinische Manifestation von CADASIL (Reyes, Viswanathan et al. 2009; Chabriat et al. 2009). In den meisten Fällen sind zunächst die Exekutivfunktionen betroffen (Dichgans 2009). Mit fortschreitender Erkrankung werden auch andere kognitive Funktionen eingeschränkt; insbesondere Aufmerksamkeit, visuell-konstruktive und visuell-räumliche Fähigkeiten und später auch verbale und visuelle Merkfähigkeit (Peters, Opherk et al. 2005). Der Vergleich einer Gruppe von 65 CADA-
Einleitung


1.2.2 MRT-Charakteristika von CADASIL

Einleitung

Abb. 4: CADASIL-typische Veränderungen im MRT.

1.2.3 Korrelation von kognitiven und Bildgebungsparametern

Das Vorhandensein sowie das Volumen von Lakunen korreliert mit kognitiver Beeinträchtigung bei CADASIL (Liem, van der Grond et al. 2007; Viswanathan, Gschwendtner et al. 2007). Dabei konnte gezeigt werden, dass Lakunen einen unabhängigen Einfluss haben, wenn andere Variablen wie Alter, WMH und Mikroblutungen auspartialisert werden. Das globale Hirnvolumen hat sich sowohl im Querschnitt (Jouvent, Viswanathan et al. 2007) als auch longitudinal (Peters, Holtmannspötter et al. 2006) als unabhängiger Prädiktor kognitiver Funktionen erwiesen. Der Zusammenhang zwischen WMH und kognitiver Beeinträchtigung bei CADASIL ist dabei weniger eindeutig. In univariaten Modellen korrelieren WMH zwar mit kognitiven Parametern (Holtmannspötter,

1.3 Strukturelle Bildgebung

1.3.1 Läsionsstudien


Ein weiteres statistisches Problem bei VLSM ist die Korrektur für multiples Testen. Da der gleiche Mittelwertsvergleich pro Voxel, d.h. weit über 1000mal, durchgeführt wird, entsteht eine Alpha-Fehler-Kumulation. Das übliche Korrekturverfahren, die Bonferroni-Korrektur, ist bei der Vielzahl der Tests zu konservativ, da bei diesem Verfahren das Alpha-Fehler-Niveau an die Anzahl der durchgeführten Tests angepasst wird.
Für $k$ Tests gilt also $i=1...k \quad \alpha(korr.)=\frac{\alpha}{k}$. Bei großem $k$ geht das Alpha-Niveau gegen 0 und es ist dementsprechend wahrscheinlich, ein Signal in den Daten zu übersehen. Eine Alternative bietet das False Discovery Rate (FDR) - Verfahren, bei dem das Verhältnis falsch Positiver zu korrekt erkannten Signal kontrolliert wird. Die FDR ist das erwartete Verhältnis Falsch-Positiver über alle signifikanten Hypothesen; d.h. es wird a priori eine Rate Falsch Positiver in Abhängigkeit vom Alpha-Fehler-Niveau festgelegt. Wenn beispielsweise die Nullhypothesen bei 1000 Tests experimentell zurückgewiesen wurden und das Fehlerniveau auf 0.10 festgelegt ist, würden 100 der Zurückweisungen als Falsch-Positive klassifiziert.

Um individuelle Gehirne statistisch miteinander zu vergleichen, werden diese üblicherweise in einen Standardraum transferiert, z.B. den auf 152 gesunden Gehirnen basierenden Standardraum, der vom Montreal Neurological Institute (MNI) entwickelt wurde. Die Transformation von krankhaft veränderten Gehirnen ist dabei im VLSM eine zusätzliche Herausforderung, da sowohl Läsionen als auch Atrophie eine Veränderung des Signals darstellen, für die Standardalgorithmen zur Transformation oft nicht geeignet sind.

1.4 Kognitive Reserve

1.4.1 Kognitive Leistung und Hirnschädigung


1.4.2 „Brain Reserve“ und „Kognitive Reserve“

Höhere Bildung wird nach dem aktuellen Forschungsstand als Indikator für bessere Kompensation von Hirnpathologie betrachtet. Stern (2009) unterscheidet dabei zwischen einem sogenannten „passiven“ und „aktiven“ Erklärungsmodell. Das passive Modell oder die „Brain Reserve“- Hypothese postulierte, dass es Eigenschaften des Gehirns gibt, die manchen Individuen erlaubt...

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1.4.3 Kognitive Reserve als universelles Konzept

Die Beobachtungen von Stern basieren hauptsächlich auf Studien zur AD und gesunden älteren Menschen. Es gibt jedoch Hinweise, dass es sich um ein Konzept handelt, das in jeder Art von Hirnerkrankung eine moderierende Rolle spielt. So gibt es Untersuchungen zu kognitiver Reserve bei Patienten mit Humanem Immundefizienz-Virus (HIV) (Farinpour, Miller et al. 2003), Schizophrenie, bipolaren Störungen und Depression (Barnett, Salmond et al. 2006), Schädel-Hirn-Trauma (Kesler, Adams et al. 2003) sowie Multipler Sklerose (Sumowski, Wylie et al. 2010).


1.5 Fragestellung

1.6 Literaturverzeichnis Einleitung


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2 Strategic Role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL

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Abstract
Cerebral small vessel disease is the most common cause of vascular cognitive impairment. It typically manifests with lacunar infarcts and ischemic white matter lesions. However, little is known on how these lesions relate to the cognitive symptoms. Previous studies have found a poor correlation between the burden of ischemic lesions and cognitive symptoms thus leaving much of the variance in cognitive performance unexplained. The objective of the current study was to investigate the relationship between the location of subcortical ischemic lesions and cognitive symptoms in small vessel disease. We applied a voxel-based lesion-symptom mapping approach to data from 215 patients with CADASIL, a genetically defined small vessel disease with mutations in the NOTCH3 gene. All patients were examined by magnetic resonance imaging and comprehensive neuropsychological testing. Lacunar lesions and white matter lesions were segmented on 3DT1 and fluid attenuated inversion recovery sequences, respectively. One hundred and forty five subjects had a total of 854 lacunar lesions (range: 1 to 13 per individual). The normalized volume of white matter hyperintensities ranged from 0.0425 % to 21.5 % of the intracranial cavity. Significant clusters for cognitive performance were detected for both lacunar lesions and white matter hyperintensities. The most prominent results were obtained on a compound score for processing speed, the predominantly affected cognitive domain in this group of patients. Strategic locations included the anterior parts of the thalamus, the genu and anterior limb of the internal capsule, the anterior corona radiata and the genu of the corpus callosum. By combining the lesion-symptom mapping data with information from a probabilistic white matter atlas we found that the majority of the processing speed clusters projected on the anterior thalamic radiation and the forceps minor. In multivariate models that included demographic parameters, brain atrophy, and the volume of ischemic lesions, regional volumes of lacunar lesions and white matter hyperintensities in the anterior thalamic radiation predicted performance in processing speed tasks whereas there was no independent contribution of the global volume of ischemic lesions. These observations emphasize the importance of lesion
location for both lacunar and ischemic white matter lesions. Our findings further highlight the anterior thalamic radiation as a major anatomical structure impacting on processing speed. Together these findings provide strong support for a central role of frontal subcortical circuits in cerebral small vessel disease and vascular cognitive impairment.
2.1 Introduction

Vascular cognitive impairment (VCI) is the second most common cause of dementia following Alzheimer’s disease. VCI has been associated with various lesion patterns including multiple infarcts, single strategic infarcts and incomplete ischemic lesions mainly in the cerebral white matter (O’Brien et al., 2003; Román et al., 2002; Selnes and Vinters, 2006). However, the exact relationship between these lesions and cognitive impairment is still strongly debated.

MR imaging and autopsy studies have demonstrated an impact of the total burden of ischemic lesions on cognitive status. An association between lesion volumes and cognitive performance has been demonstrated for infarcts (Erkinjuntti et al., 1988, Pohjasvaara et al., 1998; Tatemichi et al., 1993), lacunar infarcts (Liem et al., 2007; Mungas et al., 2005) and white matter lesions (O’Brien et al., 2002; Prins et al., 2005). In general however, correlations between volumetric measures and cognitive performance have been modest leaving much of the clinical variance unexplained.

Another increasingly recognized factor is lesion location. Thus, for example, lacunar infarcts in the thalamus and basal ganglia were found to have a larger impact on cognition than infarcts in the deep white matter (Gold et al., 2005).

In the Rotterdam Scan Study, periventricular but not subcortical white matter lesions were associated with both cognitive function (de Groot et al., 2000) and cognitive decline during follow-up (De Groot et al., 2002). This finding, although still somewhat controversial (Debette et al., 2007; Delano-Wood et al., 2008, Smith et al., 2000), has been attributed to a disruption of functionally important neuronal tracts traversing through the periventricular and deep white matter.

Extending these observations recent studies have focused on major cognitive domains. In the Rotterdam Scan Study, thalamic infarcts were associated with a decline in memory performance, whereas non-thalamic infarcts were associated with a decline in psychomotor speed (Vermeer et al., 2003). These relationships were further influenced by the presence of additional infarcts adding to the notion that there is an interplay between the burden of ischemic
lesions and their spatial distribution in determining cognitive status (Saczynski et al., 2009).

Patients with VCI often show deficits of attention and executive function with slowing of information processing (Selnes and Vinters, 2006). This profile has been related to the frequent occurrence of vascular lesions in brain structures harboring frontal-subcortical circuits (Chui, 2007; Cummings, 1989) and is particularly prominent in patients with cerebral small vessel disease (SVD), the most common cause of VCI (Jokinen et al., 2009; O’Brien et al., 2003; Prins et al., 2005, Román et al., 2002).

SVD typically manifests with two types of lesions: cavitating lacunar lesions (LL) and incomplete ischemic lesions that are hyperintense on fluid-attenuated inversion recovery (FLAIR) and T2-weighted MRI. The latter are typically located within the white matter and are thus termed white matter hyperintensities (WMH) but may likewise involve the deep grey matter (Jacqmin et al., 2010; van Straaten et al., 2003). The same changes are also found in patients with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), a hereditary SVD caused by mutations in the \textit{NOTCH3} gene (Chabriat et al., 2009; Opherk, 2004). Because of an early onset, age-related pathologies, such as Alzheimer-type changes, are very uncommon in these patients. Thus, CADASIL has become a model for studying the mechanisms of SVD and VCI in particular (Chabriat et al., 2009; Charlton et al., 2006; Dichgans et al., 2008; Jouvent et al., 2008; Peters et al., 2005).

In this study we explored the role of strategic lesions for cognitive deficits in CADASIL. We hypothesized that strategic locations can be detected for both LL and WMH and that locations may vary between cognitive domains. We further speculated that strategic locations for LL and WMH affect similar brain regions or white matter tracts. Finally, we hypothesized that the total burden of lesions within strategic white matter tracts may be more predictive for cognitive performance than the global burden of LL and WMH in the brain. To address these questions in a systematic manner we used a hypothesis-free voxel-based lesion-symptom mapping approach.
2.2 Methods

2.2.1 Study cohort and neuropsychological testing

320 CADASIL patients from an ongoing prospective study (Klinikum Großhadern, University of Munich, Germany and Hopital Lariboisière, Paris, France) were evaluated for analysis. In all subjects the diagnosis had been confirmed either by skin biopsy or by genetic testing (Joutel et al., 1997; Peters et al., 2005). Neuropsychological testing was done using the following tests: trail making test parts A and B, block design, digit span, similarities, verbal fluency, free recall, and delayed free recall. Raw test scores were transformed into age- and education-corrected Z scores based on reference values obtained from healthy subjects (Tombaugh, 2004; Troyer, 2000; Van der Linden et al., 1993; Wechsler, 2006).

50 patients were excluded based on their MRI scans for the following reasons: insufficient image quality such as motion artifacts (N=15); territorial infarctions (N=3); difficulties in registering images to standard space (N=32; see below). Thus, images from 270 subjects were available in standard space. Of those, 55 patients had to be excluded because of failure to adequately perform or complete all neuropsychological tests. These patients did not differ significantly from the remaining subjects in terms of normalized WMH volume (Mann-Whitney-U-Test; p=0.085) but had slightly higher normalized LL volume (p<0.05). The final sample available for the voxel-based lesion-symptom mapping approach consisted of 215 subjects.

2.2.2 MR imaging

MRI scans were performed on 1.5 Tesla systems: Siemens Vision (Munich) and General Electric Medical Systems Signa (Paris and Munich). Sequence parameters for the 3DT1 and FLAIR protocols have previously been published (Jouvent et al., 2007) (see table 1).
Strategic Role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL.

Table 1. MRI sequence parameters

<table>
<thead>
<tr>
<th>scanner</th>
<th>TR [ms]</th>
<th>TE [ms]</th>
<th>TI [ms]</th>
<th>slice [mm]</th>
<th>voxel [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3DT1</td>
<td>Munich Vision</td>
<td>11.4</td>
<td>4.4</td>
<td>-</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Munich Signa</td>
<td>22</td>
<td>6</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Paris</td>
<td>8.6</td>
<td>1.9</td>
<td>-</td>
<td>0.8</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Munich Vision</td>
<td>4284</td>
<td>110</td>
<td>1428</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Munich Signa</td>
<td>8402</td>
<td>151</td>
<td>2002</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Paris</td>
<td>8402</td>
<td>161</td>
<td>2002</td>
<td>5.5</td>
</tr>
</tbody>
</table>

TR: Repetition time, TE: Echo time, TI: Inversion time
All sequences were done without interslice gap

2.2.3 Generation of lesion maps

Lesion maps were generated using custom 2D and 3D image editing tools provided by BioClinica SAS (Lyon, France). LL maps were created using a slightly modified version of our previously published protocol (Viswanathan et al., 2007): hypointense lesions on the T1-weighted images with a signal identical to cerebrospinal fluid, sharp delineation and a diameter >2 mm were segmented as LL. Virchow-Robin spaces were excluded by size <2 mm and their typical orientation along perforating vessels or perpendicular to the brain surface (Doubal et al., 2010). Particular attention was paid to brain regions typically containing Virchow-Robin spaces (Kwee and Kwee, 2007). In difficult cases decisions were reached by consensus between two or more experienced readers. Lesions hyperintense on FLAIR images were labeled WMH although some of these lesions also affected the subcortical grey matter. WMH maps were generated as previously described (Viswanathan et al., 2006).

The intracranial cavity (brain parenchyma plus cerebrospinal fluid space) was determined using an automated 3D image segmentation algorithm on the T2 sequence followed by manual correction. Lesion volumes were calculated from lesion maps and divided by intracranial cavity for normalization. The
intra- and inter-rater reliability for these procedures has been shown to be high (Viswanathan et al., 2010; Viswanathan et al., 2006). The overlap of lesion masks between raters as judged by the Dice coefficient was good for LL masks (0.88) and excellent for WMH (0.98).

Brain volume was estimated from native T1 images with SIENAX (Smith et al., 2001, Smith et al., 2002), part of FSL (Smith et al., 2004; Woolrich et al., 2009). SIENAX extracts brain and skull images from whole-head input data to calculate brain volume (Smith, 2002). Results were manually checked and parameters optimized if necessary. Brain parenchymal fraction (BPF) was calculated by dividing total brain volume by intracranial cavity.

Tools from FSL (most recent version, August 2008) were used for all subsequent processing steps. For registration to standard space a lesion masking approach was applied to enhance registration quality and to better preserve anatomical structures (Brett et al., 2001). In brief, cost-function masks were created by subtracting lesion maps from brain masks in T1 space. Linear (FSL flirt) and nonlinear (FSL fnirt) registration of T1 images were done with standard parameters to a 1 mm Montreal Neurological Institute 152 (MNI) template provided within FSL. Rigorous quality checks were done at all steps by superimposing results onto the target image. Where needed, parameters were adjusted to optimize the registration process. Thirty-two subjects were excluded because of pronounced image distortion mostly due to atrophy. After quality control of T1 images in MNI space, the warp fields were used to co-register corresponding lesions maps to a 2 mm MNI template. To correct for small registration errors, modest smoothing (mean filtering, Gaussian kernel 4 mm, threshold 0.5) was applied to LL maps.

### 2.2.4 Voxel-based lesion-symptom mapping

Analyses were done on compound scores for major cognitive domains. Compound scores were defined by principal component analysis (PCA, confirmatory analysis). PCA seeks for combinations of variables in order to extract the maximum variance from the dataset. These combinations are
represented by factors. A three-factor model was used to describe our data and varimax rotation was applied to maximize the factor load of the tests. Only neuropsychological tests clearly loading on one of the three factors were considered for the generation of compound scores. Non-parametric mapping (NPM, most recent version, April 2010) was used to relate lesion location to cognitive performance (Rorden et al., 2007) (settings: Brunner-Munzel test, 4000 permutations for univariate analysis). Voxels affected in less than 4 subjects were not considered for analysis. Correction for multiple testing was achieved by permutation generated familywise error thresholds ($p_{fwe} < 0.01$ for WMH) or false-detection-rate ($p_{fdr} < 0.05$ for LL). Voxels reaching statistical significance were projected on major white matter tracts as identified on a probabilistic white matter tract atlas (Hua et al., 2008) provided within FSL (JHU-ICBM-tracts, maximum probability map). Images are displayed according to neurological convention (right side displayed on the right).

2.2.5 Region-of-interest based multiple regression analysis

Next, we used information from the white matter parcellation atlas (maximum probability map, thresholded at a probability of 0.1) to create regions-of-interest (ROIs) for major white matter tracts in MNI space. These ROIs were then used to calculate regional volumes in standard space for LL and WMH within specific white matter tracts. Regional volumes were entered as independent variables in stepwise multiple linear regression model (SPSS, version 19).
2.3 Results

Demographic, clinical, and MR imaging characteristics of the study cohort are provided in table 2. The median normalized lesion volume (in percentage of intracranial cavity) was 0.00526 for LL (interquartile range 0.0219) and 5.54 for WMH (interquartile range 5.96).

<table>
<thead>
<tr>
<th>Table 2: Characteristics of the study cohort (n=215)</th>
</tr>
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<tbody>
<tr>
<td><strong>Demographic Characteristics</strong></td>
</tr>
<tr>
<td>Age [years], mean (SD)</td>
</tr>
<tr>
<td>Education [years], mean (SD)</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td><strong>Clinical Features</strong></td>
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<tr>
<td>Symptomatic</td>
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<tr>
<td>Prior clinically apparent stroke</td>
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<tr>
<td>Years since first stroke, median (IQR)</td>
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<tr>
<td>Migraine history</td>
</tr>
<tr>
<td>History for depression</td>
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<tr>
<td><strong>Vascular Risk Factors</strong></td>
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<tr>
<td>Current smoker</td>
</tr>
<tr>
<td>Smoking History</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Hypercholesterolemia</td>
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<tr>
<td>Diabetes</td>
</tr>
<tr>
<td><strong>Clinical Scores</strong></td>
</tr>
<tr>
<td>MDRS, median (IQR)</td>
</tr>
<tr>
<td>Modified Rankin scale, median (IQR)</td>
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<tr>
<td>Modified Rankin scale &gt; 2</td>
</tr>
<tr>
<td>NIHSS, median (IQR)</td>
</tr>
<tr>
<td>Barthel index, median (IQR)</td>
</tr>
<tr>
<td><strong>Imaging Characteristics, mean (SD)</strong></td>
</tr>
<tr>
<td>Normalized LLV [%]</td>
</tr>
<tr>
<td>Normalized WMHV [%]</td>
</tr>
<tr>
<td>BPF [%]</td>
</tr>
</tbody>
</table>

IQR: interquartile range
MDRS: Mattis dementia rating scale
NIHSS: NIH stroke scale
BPF: Brain Parenchymal Fraction
LLV: Lacunar lesion volume
WMHV: White matter hyperintensities volume
2.3.1 Lesion prevalence maps

Overall, there were 854 LL present in 145 subjects (mean number per patient in the entire cohort of 215 patients = 4, range 0 to 13). As illustrated by the lesion prevalence maps frequent locations included the thalamus, the anterior limb of the internal capsule, anterior parts of the striatum and globus pallidus, the centrum semiovale and the pons (figure 1a).

Figure 1: Lesion probability maps for lacunar lesions (LL) (A) and white matter hyperintensities (WMH) (B) in Montreal Neurological Institute 152 (MNI) standard space (N=215). The statistical maps are superimposed onto the MNI T1 template. R = right.

The anatomical distribution of LL was similar between subgroups stratified for the normalized LL volume (figure 2).
WMH were detected in all subjects. They were found in the majority of voxels representing white matter and several voxels representing central grey matter (figure 1b). WMH were most prevalent in the periventricular and deep white matter and were less prevalent in the internal capsule and central grey matter. The overall pattern was highly symmetrical between the left and right hemisphere with a spread towards subcortical white matter as the overall volume of lesions increased (figure 3).
2.3.2 Cognitive profile and major cognitive domains

Figure 4a shows the cognitive profile in the entire study sample. Principal component analysis (PCA) of individual test scores revealed 3 factors explaining 88% of raw score variance. Factor 1 (37% variance) was determined by timed tests requiring visual executive functions (trail making test parts A and B, block design). Factor 2 (33% variance) is a verbal memory factor, determined largely by immediate and delayed verbal recall, with minor contribution of verbal fluency and verbal similarities. Factor 3 (18% variance) was marked by digit span, again with minor contribution of verbal fluency and verbal similarities. All subsequent analyses were done on scores derived from...
the PCA: a compound score reflecting processing speed (PC1), a compound score for memory (PC2), and a third score represented by the digit span (PC3) (figure 4a).

Figure 4: A: Cognitive profile of the study cohort (N=215; TMT: trail making test). B: Profiles in subgroups stratified for the normalized LL volume (low = first and second quartile of patients with lesions; high = third and fourth quartile). C: Profiles in subgroups stratified for normalized WMH volume (in quartiles). Compound scores for psychomotor speed (PC1), memory (PC2) and a score represented by digit span (PC3) were derived from principal component analysis as described in the text.
Figures 4b and 4c show the cognitive profiles in subgroups stratified for the volume of LL and WMH, respectively. Between-group differences with regard to lesion volumes were found for processing speed (MANOVA; LL: $p < 0.001$, WMH: $p < 0.01$) and memory (LL: $p < 0.001$, WMH: $p < 0.05$) but not for the digit span.

2.3.3 Voxel-based lesion-symptom mapping

Processing speed (PC1): Nonparametric voxel-based lesion-symptom mapping for the processing speed score revealed significant clusters for both LL and WMH following correction for testing of multiple voxels (figure 5A and B, table 3).
Figure 5: Voxel-based lesion-symptom mapping results for processing speed (panels A and B) and memory (panel C) in MNI space. A: Significant clusters for processing speed after nonparametric mapping and correction for multiple testing. Orange (LL) and light green (WMH) indicate voxels remaining significant after adding normalized LL volume (for LL) and normalized WMH volume (for WMH) as a covariate. R = right. B: Significant clusters for LL (red) and WMH (green) projected on major white matter tracts (JHU-ICBM-DTI atlas). Depicted are the forceps minor, the anterior thalamic radiation, the forceps major and the corticospinal tract. C: Significant clusters (WMH) for memory. Voxels that remained significant after correction for global normalized WMH volume are depicted in light green. The statistical maps are superimposed onto the MNI T1 template. R = right. There were no significant clusters in slices outside the region shown in panels A to C.
For LL significant clusters were found bilaterally in the anterior part of the thalamus (extending to the capsular genu on the right) and the anterior limb of the internal capsule (figure 3a, upper panel; MNI z 0 to 15). Additional clusters were seen in the left genu of the corpus callosum (z 0 and 5) and the left anterior corona radiata (z 20). When adding normalized LL volume as a covariate only the thalamic clusters remained significant.

For WMH significant clusters were found bilaterally in the genu of the corpus callosum (z 0 to 15), the anterior limb of the internal capsule (z 0 to 15) and the anterior corona radiata (z 15) (figure 3a, lower panel). Significant clusters were further detected in the posterior limb of the left internal capsule extending to the centrum semiovale (z 10 to 25), and the splenium (z 15 to 25). Several of these clusters remained significant when adding normalized WMH volume as a covariate.

Memory (PC2): For the memory score significant clusters were found for WMH predominantly in the splenium of the corpus callosum (figure 5c, z 10-20). Several voxels remained significant when adding normalized WMH volume as a covariate.

Digit span (PC3): No significant voxels were identified for digit span.
Strategic Role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL.

Table 3: Significant clusters for processing speed in MNI space and lesion prevalence at these locations

A) Lacunar lesions

<table>
<thead>
<tr>
<th>No.</th>
<th>MNI coordinates</th>
<th>Contributing lacunes (n)</th>
<th>Anatomical structure</th>
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<tbody>
<tr>
<td>1</td>
<td>-8 -14 5</td>
<td>9</td>
<td>thalamus</td>
<td>left</td>
</tr>
<tr>
<td>2</td>
<td>11 -4 9</td>
<td>13</td>
<td>thalamus</td>
<td>right</td>
</tr>
<tr>
<td>3</td>
<td>-20 9 16</td>
<td>21</td>
<td>internal capsule, anterior limb</td>
<td>left</td>
</tr>
<tr>
<td>4</td>
<td>22 11 11</td>
<td>15</td>
<td>internal capsule, anterior limb</td>
<td>right</td>
</tr>
<tr>
<td>5</td>
<td>-12 32 2</td>
<td>6</td>
<td>corpus callosum, genu</td>
<td>left</td>
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</table>

B) White matter hyperintensities

<table>
<thead>
<tr>
<th>No.</th>
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<th>Mean WMH Prevalence (%)</th>
<th>Anatomical structure</th>
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<tr>
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<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
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<td>right</td>
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<tr>
<td>4</td>
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<td>19</td>
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<tr>
<td>5</td>
<td>11 36 4</td>
<td>24</td>
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<td>right</td>
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<td>-24 -18 16</td>
<td>43</td>
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</table>

2.3.4 Identification of strategically relevant white matter tracts

To examine the spatial relationship between strategic lesions and major white matter tracts we projected significant clusters from the lesion-symptom mapping on a JHU-ICBM white matter atlas registered to MNI space. As shown in figure 5b, the majority of processing speed clusters projected on the anterior thalamic radiation (ATR), the forceps minor (Fmin), the forceps major and the left corticospinal tract. The most prominently involved structures were the ATR (bilateral clusters for both LL and WMH) and the Fmin (bilateral clusters for LL, unilateral cluster for WMH). Clusters for the memory score mostly projected on the forceps major (figure 5c).
2.3.5 Region-of-interest based regression models for processing speed

In a last step, we assessed whether the cumulative burden of lesions within strategic white matter tracts is more predictive for cognitive performance than the global burden of LL and WMH in the brain. Given the prominent role of processing speed and of frontal white matter tracts (ATR and Fmin, figure 5b) we focused on these components. Regional volumes of LL and WMH projecting on the ATR and Fmin were measured in standard space (figure 6) and entered into stepwise multiple regression models with processing speed as dependent variable. In a model that included both regional and global lesion volumes, LL in the ATR explained most of the variance (adjusted R square = 0.15) followed by WMH in the ATR and LL in the Fmin (table 4).

Figure 6: Segmentation of lesions projecting on the ATR: left: FLAIR image of a patient in native space (resliced according to MNI template); middle: same image after co-registration to MNI standard space with the ATR superimposed in half transparency; right: MNI T1 template illustrating the segmentation of LL and WMH inside the ATR. The regional lesion volumes were used in multivariate analyses.

Global lesion volumes and WMH in the Fmin did not contribute to the model. After adding BPF, age, sex and years of formal education to the model BPF explained most of the variance, followed by the regional volumes of LL and WMH in the ATR. None of the other variables contributed to the model.
Strategic Role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL.

Table 4: Stepwise multiple linear regression analysis on processing speed

**Model A**
Independent variables: LLV (global), LLV (ATR), LLV (Fmin), WMHV (global), WMHV (ATR), WMHV (Fmin)

<table>
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<th>Variable</th>
<th>p</th>
<th>Standardized beta</th>
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<tr>
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<td>2</td>
<td>WMHV (ATR)</td>
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<td>3</td>
<td>LLV (Fmin)</td>
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**Model B**
Variables as in model A, with addition of BPF, age, sex and formal education (years)

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>p</th>
<th>Standardized beta</th>
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</thead>
<tbody>
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<td>BPF</td>
<td>&lt; 0.000</td>
<td>0.267</td>
</tr>
<tr>
<td>2</td>
<td>LLV (ATR)</td>
<td>&lt; 0.000</td>
<td>-0.265</td>
</tr>
<tr>
<td>3</td>
<td>WMHV (ATR)</td>
<td>&lt; 0.001</td>
<td>-0.213</td>
</tr>
</tbody>
</table>

ATR: Anterior thalamic radiation
Fmin: Forceps minor
2.4 Discussion

This study demonstrates a possible link between the distribution of subcortical ischemic lesions and the profile of cognitive symptoms in patients with CADASIL, a genetic cause of SVD and vascular cognitive impairment. Using a voxel-based approach we identified several locations for LL and WMH that were significantly associated with performance in distinct cognitive domains. The most prominent results were obtained for processing speed, the predominantly affected domain in our patients. The results on LL and WMH are complementary and identify the ATR and Fmin as functionally important anatomical structures. A strategic role of the ATR is further suggested by the multivariate models showing that the volume of LL and WMH projecting on this white matter tract independently predict cognitive deficits. Together, these observations highlight the role of frontal-subcortical circuits in SVD and VCI.

To our knowledge this is the first lesion-symptom mapping study on subcortical ischemic lesions and cognitive performance using voxel-based methods. Our findings on LL and processing speed add to earlier reports on patients who developed dementia in the context of small infarcts in the thalamus (Auchus et al., 2002; Kalashnikova et al., 1999; Szirmai et al., 2002), genu (Tatemichi et al., 1992) and anterior limb (Kalashnikova et al., 1999) of the internal capsule and anterior part of the corpus callosum (Auchus et al., 2002). In fact, the processing speed clusters for LL match remarkably well with locations previously reported to be associated with strategic infarct dementia (Tatemichi et al., 1995).

In the Rotterdam Scan Study, silent thalamic infarcts were associated with a decline in memory whereas non-thalamic infarcts were associated with a decline in psychomotor speed (Gold et al., 2005; Vermeer et al., 2007; Vermeer et al., 2003). We found no significant clusters for LL and memory, possibly because memory was relatively preserved in our cohort. However, the results on processing speed broadly agree with those from the Rotterdam Scan Study while providing a more detailed account of infarct locations associated with processing speed.
A major finding of this study was that WMH in distinct, partially overlapping brain regions also associate with processing speed. The identified clusters extend on earlier sporadic reports, which found processing speed to be associated with WMH in the caudate nucleus, internal capsule, and thalamus (Burton et al., 2003; O’Brien et al., 2002). However, these studies were small, hypothesis-driven, and did not control for the total burden of lesions. In the current study, many of the WMH clusters for processing speed remained significant when controlling for lesion volumes. We therefore hypothesize that these clusters represent true strategic locations. In support of this, there was considerable overlap between processing speed clusters for WMH and LL with regard to functional anatomical structures such as the ATR.

Some of the WMH clusters for processing speed projected on the left corticospinal tract. This finding likely reflects the effects of motor impairment on our processing speed tasks, all of which involved manual skills. In fact, we feel that the corticospinal tract clusters can be viewed as an internal validation of the voxel-based lesion-symptom mapping. However, this could not be formally assessed as we did not control for hand motor function. Also, we cannot exclude the possibility that motor impairments contributed to some of the other clusters.

We also found single clusters for WMH and memory in the splenium of the corpus callosum. There is some evidence for a role of the splenium in episodic memory (Voineskos et al., 2010) whereas there is little data on verbal memory the main aspect covered by our compound score. However, we feel the findings on memory must be interpreted cautiously since memory was relatively preserved in our cohort. This might also explain why we found no significant clusters for memory and LL.

Adding information from the JHU-ICBM white matter atlas we found that many of the processing speed clusters project on the ATR and Fmin. As a major white matter tract, the ATR carries reciprocal projections between the thalamus, prefrontal cortex, and striatum, which participate in prefrontal-subcortical circuits (Behrens et al., 2003). There is a broad literature suggesting an involvement of these circuits in processing speed and executive
functioning (Mega and Cummings, 1994; Tekin and Cummings, 2002). Our findings add to this concept while emphasizing a strategic role of subcortical ischemic lesions. A role of the ATR in processing speed is also suggested by a recent study on patients with first episode psychosis (Pérez-Iglesias et al., 2010). Likewise, our finding of significant clusters in the Fmin agree with data showing that microstructural integrity of the genu of the corpus callosum influences interhemispheric processing speed (Schulte et al., 2005).

In regression models accounting for global and regional measures of disease burden regional volumes of LL and WMH in the ATR both had an independent influence on processing speed. In contrast, there was no independent contribution of the global volume of LL and WMH when regional volumes were included in the model. This finding may help to resolve some of the controversy between studies that have emphasized the importance of lesion location versus lesion volumes (Gold, 2009). Our data suggest that both aspects are important but that the cumulative volume of lesions within specific functional networks may be clinically more relevant than the total volume of lesions within the brain. Still, the explained variance in our models is only modest, indicating a potential role of other factors. Such factors might include microscopic damage within brain tissue appearing normal on T2-weighted images (O’Sullivan et al., 2005) or individual compensatory mechanisms such as cognitive reserve, which has been shown to modify the relationship between pathology and cognitive performance (Brickman et al., 2009).

The lesion prevalence maps demonstrate clear predilection sites for LL and WMH with an early spread of lesions into anatomical structures found here to be strategically important. Frequent locations for LL included the striatum, the internal capsule, and the thalamus, whereas WMH tended to accrue in the periventricular white matter. This pattern matches with that reported for sporadic SVD (Enzinger et al., 2006). It has been suggested that the cognitive profile of VCI with frequent impairments of executive function and processing speed in part relates to the frequent spread of lesions into anatomical structures harboring frontal-subcortical circuits (Chui, 2007; Cummings, 1989).
Our study supports this concept while demonstrating a role for both LL and WMH.

There are limitations to our study that should be considered. First, we might have missed functionally relevant locations because there were too few lesions. This is most obvious for LL. Chances to detect significant associations were therefore higher in locations commonly affected in CADASIL, although the issue is likely to be less relevant for WMH, which were more widespread and exhibited higher lesion frequencies than LL (figure 1). A significant proportion of patients had to be excluded because of difficulties in registering images or failure to complete all the neuropsychological tests. This together with potential biases in patient recruitment might have lead to a selection of patients who were less impaired. Second, we cannot exclude errors associated with normalization and registration processes. However, we consider these errors to be small as all images were rigorously controlled by visual inspection. Finally, we did not include other imaging modalities such as DTI, which are more sensitive in capturing subtle structural abnormalities (O’Sullivan et al., 2004; Pérez-Iglesias et al., 2010; Turken et al., 2008). Thus, effects from more widespread microscopic lesions might have been missed.

The main strengths of this study are the homogeneity of the sample, a large sample size and the hypothesis-free approach using voxel-based methods. Studies in typically older patients with sporadic SVD are complicated by a high prevalence of co-morbid age-related pathologies (Schneider et al., 2007), which may interfere with studying the mechanisms of VCI. The average age in our patients was well below the age at which typical age-related causes of cognitive impairment commence. Thus, we are confident, that our findings truly relate to the underlying SVD.

In summary, we identified strategic locations for subcortical ischemic lesions impacting on processing speed as a major cognitive aspect of VCI. Significant clusters were found for both lacunar lesions and white matter hyperintensities. The results are complementary and identify the anterior thalamic radiation and the forceps minor as key anatomical structures. Taken together, our observations emphasize the role of frontal-subcortical circuits in subcortical
Strategic Role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL.

ischemic vascular disease and related cognitive impairment. Future studies in sporadic patients may show whether these findings are generalizable to other types of SVD.
2.5 References


Strategic Role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL.


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Kalashnikova LA, Gulevskaya TS, Kashina EM. Disorders of higher mental function due to single infarctions in the thalamus and in the area of the thalamofrontal tracts. Neurosci Behav Physiol. 1999 Jan 1;29(4):397-403.


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Strategic Role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL.


3 Education modifies the relation of vascular pathology to cognitive function: cognitive reserve in CADASIL

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\textsuperscript{b} Department of Neurology, Medical Centre, Ludwig-Maximilians-University, Marchioninistr. 15, 81377 Munich, Germany

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\textsuperscript{1} These authors contributed equally to this work
Abstract
A clinical impact of cognitive reserve (CR) has been demonstrated in Alzheimer's disease whereas its role in vascular cognitive impairment (VCI) is largely unknown. In this study we investigated the impact of CR in patients with CADASIL, a genetic variant of pure VCI. 247 NOTCH3 mutation carriers from a two-center study were investigated using detailed neuropsychological and neuroimaging protocols. CR was operationalized as years of formal education. Brain pathology was assessed by MRI using normalized brain volume and lacunar lesion volume as proxies. Multivariate analyses were done for each structural measure with scores of processing speed, executive function and memory as dependent variables. Additional linear regression models were conducted with interaction terms for education x brain volume and education x lacunar lesion volume. Education had an independent impact on cognitive performance in subjects with mild and moderate degrees of brain pathology whereas there was no significant influence of education on cognition in patients with severe MRI changes. This interaction was found for processing speed, the cognitive domain most impaired in our patients. Our findings demonstrate an interaction of education and brain pathology in regard to cognitive impairment: The effect of education seems most pronounced in early disease stages but may ultimately be overwhelmed by the pathological changes. The results extend the concept of CR to VCI.
3.1 Introduction

Cognitive response to brain damage in dementia can vary to a considerable degree (Stern, 2002). To account for this observation, the “Cognitive Reserve Hypothesis” proposes that there is an individual threshold for the amount of brain damage a person can sustain (Stern, 2009). Differences between individuals in this model are seen as a result of compensatory mechanisms, such as more flexible use of existing networks or recruitment of additional networks. Stern suggested that this cognitive reserve mediates the relationship of neuropathology and cognitive function with a distinct pattern as illustrated in figure 1.

![Figure 1: The cognitive reserve model (adapted from Stern, 2009)](image)

The illustration shows the proposed slope of cognitive function with increasing brain pathology, depending on the level of cognitive reserve. Patients with higher cognitive reserve show better cognitive performance than patients with less reserve. However, this effect vanishes at higher degrees of pathology.
Cognitive reserve (CR) has primarily been reported in patients with Alzheimer’s disease (AD), mild cognitive impairment (Stern et al., 1999) and healthy aging (Stern et al., 2005). Education has often been used as a proxy to examine CR (Ngandu et al., 2007). Several studies have shown that patients with higher education are less likely to present with cognitive deficits than subjects with lower education at the same level of pathology (Bennett et al., 2003; Ngandu et al., 2007; Roe et al., 2007). However, after diagnosis of dementia, higher educated individuals show more rapid disease progression and lower survival rates (Kemppainen et al., 2008).

Studies in healthy elderly (Fritsch et al., 2007) suggest that CR might be applicable more broadly. Few studies exist on other fields of brain damage, such as stroke (Elkins et al., 2006), traumatic brain injury (Kesler et al., 2003), and vascular cognitive impairment (VCI) (McGurn et al., 2008). VCI is considered the second most common cause of dementia following AD (Stevens et al., 2002) and is etiologically heterogeneous with various causes and mechanisms including multiple small or large infarcts, strategic infarcts, and incomplete ischemic lesions of the cerebral white matter (Jagust, 2001; O'Brien et al., 2003; Roman et al., 2002). The most common form of VCI is related to small vessel disease. Interpreting findings on VCI remains difficult because of frequent overlap between vascular and neurodegenerative pathology in the elderly (Heyman et al., 1998). Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a hereditary small vessel disease caused by mutations in the \( \text{NOTCH3} \) gene (Joutel et al., 1996), is regarded a model of “pure” VCI (Chabriat et al., 2009; Dichgans et al., 2008). Due to the early age of onset, concurrent neurodegenerative pathology is rare. Thus, CADASIL allows an unbiased approach to studying the effects of vascular pathology on cognitive performance. Like sporadic patients with age-related small vessel disease, \( \text{NOTCH3} \) mutation carriers exhibit a distinct cognitive profile with pronounced deficits in executive function and processing speed, whereas memory is relatively preserved (Dichgans, 2009; Peters et al., 2005).
Neuroimaging studies have demonstrated correlations between cognitive scores and imaging measures of VCI, in particular lacunar lesion volume and brain atrophy (Peters et al., 2006; Viswanathan et al., 2010). However, the strength of these correlations is moderate suggesting a modifying influence from other factors, such as CR (Duering et al., 2011). The aim of this study was to determine the role of CR in CADASIL as a model for pure VCI. We wanted to assess whether the relationship between brain pathology and cognitive deficits is modulated by educational level. We hypothesized that higher educated subjects would show better performance at the same level of pathology than less educated subjects, but that this effect would decrease or vanish with larger amounts of pathology. Thus, we set out to examine the interaction between education and pathology exceeding any a priori advantage higher educated patients might have.


3.2 Methods

3.2.1 Subjects

A total of 313 consecutive subjects were drawn from an ongoing prospective two-centre cohort study of patients with CADASIL. Subjects were evaluated at Klinikum der Universität München, Germany and Lariboisière Hospital, Paris, France between 10/2003 and 03/2010. In all cases diagnosis was confirmed by identification of a typical mutation in the NOTCH3 gene or by skin biopsy. All study participants underwent detailed clinical and neuropsychological assessment as well as cranial MRI. Neuropsychological testing and MRI acquisition as well as test scoring and image analyses were obtained blinded to clinical information. Educational attainment was assessed as formal years of education by questioning the participant or, in case of severe cognitive impairment, the primary caregiver.

23 patients (7.9%) were excluded because they had experienced a stroke within the preceding 3 months. There were no cases of alcohol dependence, drug abuse, or organic brain disease other than CADASIL. Images of 13 of the remaining 290 patients had to be excluded for quality reasons such as motion artifacts; 2 patients had experienced a territorial infarct and were excluded because of possibly confounding large vessel disease. 22 subjects were excluded due to confounded neuropsychological testing (aphasia, motor deficits affecting the dominant hand, severe dementia). Six patients suffered from diabetes; those were also excluded from further analysis since diabetes is associated with white matter disease (Hsu et al., 2012) and brain atrophy (van Elderen et al., 2010). Thus 247 patients (78.9%) (table 1) served as the final study sample.

The study was approved by the ethics committees of both institutions. Written and informed consent was obtained from all patients participating in the study.
Education modifies the relation of vascular pathology to cognitive function: cognitive reserve in CADASIL.

Table 1: Demographics and clinical characteristics of the study group (n=247)

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<thead>
<tr>
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<th>Education ≤ 10 years (n=117)</th>
<th>Education &gt; 10 years (n=130)</th>
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<tr>
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Clinical scores

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Vascular risk factors

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<td>Smoking*, n (%)</td>
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Imaging characteristics

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<td>BPF [%], median (IQR)</td>
<td>82.8 (6.79)</td>
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<td>nLLV [%], median (IQR)</td>
<td>0.065 (0.0023)</td>
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<td>nWMHV [%], median (IQR)</td>
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<td>6.57 (7.13)</td>
<td>5.47 (7.84)</td>
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MDRS, Mattis Dementia Rating Scale; mRS, modified Rankin Scale; MADRS, Montgomery Asberg Depression Rating Scale; BPF, brain parenchymal fraction; nLLV, normalized lacunar lesion volume; nWMHV, normalized white matter hyperintensities volume; IQR, interquartile range; SD, standard deviation; n.s., not significant. * current or past smoker.
2.2. Neuropsychological testing

We applied a battery of neuropsychological tests tapping processing speed, working memory, reasoning, visuospatial ability, executive function, and verbal memory (see table 2 for an overview of the applied tests). Neuropsychological testing was done on the previous or same day as the MRI examinations. Effects of education on cognitive tasks were analyzed using the raw tests scores as well as age- and education corrected norm values (Z scores) based on healthy comparisons from current literature (table 2).

Table 2: Neuropsychological tests

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<tr>
<td>Trail Making Test</td>
<td>Tombaugh, 2004</td>
<td>Matrix A time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Matrix B time</td>
</tr>
<tr>
<td>Wechsler Adult Intelligence Scale, Revised (WAIS-R)</td>
<td>Tewes, 2006</td>
<td>Similarities</td>
</tr>
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<td>Block Design</td>
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<td></td>
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<td>Digit Span</td>
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<tr>
<td>Verbal Fluency</td>
<td>Troyer, 2000</td>
<td>Semantic Fluency Task (Animal Naming)</td>
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<tr>
<td>Grober &amp; Buschke Free and Cued Selective Reminding Task</td>
<td>van der Linden M., 1993</td>
<td>Free Recall 1-3</td>
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<td></td>
<td></td>
<td>Delayed Free Recall</td>
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</table>

Z scores were created in order to cancel out the a priori advantage higher educated subjects might have in neuropsychological tests, thus making education a proxy for cognitive reserve. For tests relying on verbal input, norms from samples of the respective language were applied (French or German, respectively). To assign the applied tasks to neuropsychological domains we conducted a confirmatory factor analysis (principal components analysis [PCA]; three-factor solution; varimax rotation). PCA suggested a factor encompassing the timed measures of the Trail Making Test A (TMT-A) and B (TMT-B), and Block Design. This factor explained 32.81% of the overall variance in cognitive performance and was best described as “processing speed”. PCA further identified a factor of Verbal Fluency, Digit Span, and Similarities (30.53% of variance), which was deemed “executive function”, and a factor of Free Recall and Delayed Recall (16.90% of variance; total
explained variance = 80.24% after varimax rotation), most likely reflecting “verbal memory”. Subsequently, we used compound scores of neuropsychological domains, calculated as mean of the Z scores of those variables loading highest on the components identified by PCA (Duering et al., 2011).

2.3. Neuroimaging

MRI scans were performed on 1.5 Tesla systems (Siemens Vision [Munich, n=41] or General Electric Medical Systems Signa [Paris, n=146; Munich, n=60]). 3DT1 sequences, fluid-attenuated inversion recovery (FLAIR) and T2-weighted images were obtained. MRIs were processed and analyzed by Bio-Imaging Technologies SAS in Lyon, France.

Lacunar lesion volume was assessed using our previously published (Viswanathan et al., 2007) protocol using isotropic 1 mm resolution 3DT1 images: Hypointense lesions on the T1-weighted images with a signal identical to that of cerebrospinal fluid (CSF) and a diameter of more than 2mm (to exclude Virchow-Robin spaces) were defined as lacunar lesions. This method has proven to have a good intra- and interrater reliability (Viswanathan et al., 2006). Lacunar lesion volume was then normalized to the intracranial cavity (ICC), which includes brain parenchyma and CSF spaces. ICC was assessed using an automated 3D image segmentation algorithm on T2 weighted images in order to precisely determine the boundary between CSF and skull. ICC maps were manually corrected by trained neurologists.

Brain tissue volume was estimated from 3DT1 images with SIENAX, part of FSL (Smith et al., 2004; Smith et al., 2002). Results were checked visually by trained neurologists. Even after manual correction the brain extraction algorithm failed on some images, so brain volume could only be obtained for 231 (93.5%) of the patients. Finally, brain parenchymal fraction (BPF) was calculated by dividing total brain volume by ICC.
2.4. Statistical analysis

Statistical analysis was conducted with the SPSS software package, version 18.

For multivariate analysis, subjects were stratified into two educational groups, one with 10 years or less and one with more than 10 years of education. Normalized lacunar lesion volume (nLLV) and brain parenchymal fraction (BPF) were each divided into three groups based on the volume distribution. BPF was normally distributed, so 3 groups were formed using mean BPF ± 0.5 SD as cut-offs (high BPF: n=72; medium BPF: n=77; low BPF: n=82). nLLV was skewed to the left, reflecting that a large number of patients had no or few lacunar lesion volume. Therefore, patients were stratified into groups with no lacunar lesions (n=76), low lesion volume (n=114) and high lesion volume (n=57) using the mean lesion volume as cut-off. We chose mean lesion volume instead of performing a median split to ensure a sufficient difference in disease severity between the low and high load groups while maintaining a balance of group size.

Due to the difference in sample size for nLLV (n=247) and BPF (n=231), we conducted a separate 2 (educational level) x 3 (structural brain measure group) multivariate analysis of variance (MANOVA) for each structural measure (nLLV and BPF) using the compound scores for processing speed, executive function and verbal memory as dependent variables. Age, hypertension, hypercholesterolemia, modified Rankin scale (mRS) – a measure for motor disability – as well as study center were entered as covariables in the multivariate model (see group differences in results section). Although education groups also differed in global cognitive performance (Mattis Dementia Rating Scale, MDRS), it was not entered as a covariable since our aim was to treat cognitive parameters as dependent variables. A priori differences in cognitive performance due to the educational level were addressed by using education-corrected values. Post-hoc group comparisons were Bonferroni-corrected for multiple testing.
We further calculated regression models using continuous variables and interaction terms. First, to partial out the influence of the covariables used in the multivariate model, a linear regression analysis was performed for each cognitive score using age, hypertension, hypercholesterolemia, mRS and study center as predictors. In a second step, linear regression was performed on the standardized residual scores from the first analysis using nLLV, BPF, years of education as well as the interaction terms nLLV x education and BPF x education as predictor variables.
3.3 Results

3.3.1 Sample characteristics and cognitive domains

The demographic characteristics of the study sample are provided in table 1. Overall, patients were relatively mildly affected, most of them showing subclinical or mild cognitive impairment. The median modified Rankin Scale Score was 0 (Interquartile Range, IQR=1) and the median Mattis Dementia Rating Scale Score was 141 (IQR=8).

In total, 146 patients were assessed on the Paris site and 101 in Munich. Patients from the Paris center were more likely to suffer from arterial hypertension (chi square=8.73, p<0.01) and hypercholesterolemia (chi square=11.9, p<0.01). They tended to be less educated (two-sample t-test; t=-0.964, p<0.05), more cognitively impaired (as measured by MDRS; t=-2.19, p<0.05) and older (t=2.17, p<0.05) and had higher normalized white matter hyperintensities volume (nWMHV) (t=-2.01, p<0.05). To account for these center differences, center was added to the multivariate model as a covariate.

The neuropsychological profile of the 247 subjects is displayed in figure 2A. Figure 2B shows the compound scores for the three factors stratified by nLLV or BPF; processing speed showed the highest variation with progressing pathology.
Education modifies the relation of vascular pathology to cognitive function: cognitive reserve in CADASIL.

Figure 2: Cognitive profiles of the patient sample
(A) Profile of neuropsychological performance in the 247 patients expressed by Z scores with healthy controls as a reference (boxplots are shown without outliers). The three components derived by principal components analysis are depicted on top. The most prominent deficits are found in tests of processing speed. TMT, Trail Making Test.
(B) Compound scores of the three cognitive domains (mean score + standard error) stratified by the three levels of normalized lacunar lesion volume (nLLV; n=247) and brain parenchymal fraction (BPF; n=231). The highest variability in performance depending on lesion load can be seen for processing speed.
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3.3.2 A priori group differences

Educational groups were balanced with regard to gender, BPF, nWMHV, current smoking, smoking history, depression and alcohol consumption. Overall, less educated patients had higher nLLV (two-sample t-test; t=3.26, p<0.01), tended to be older (t=2.02, p<0.05) and were more likely to have hypertension (chi square=4.04, p<0.05) and hypercholesterolemia (chi square=12.7, p<0.01). Less educated patients also reported a higher level of mobility impairment (mRS, t=3.26, p <0.01) and performed worse in a global measure of cognitive ability (MDRS, t=-4.10, p<0.001). Overall, both groups were only mildly to moderately impaired. Since there was no difference in nLLV between educational groups within the no, low and high nLLV group we did not treat nLLV as a confounder in multivariate analysis.

Analysis on raw test scores revealed an overall effect of education on performance in all cognitive subtasks (two-sample t-test; p values ranging from 0.002 to 0.045), also reflected in the difference in global cognitive ability between the two education groups. There was a significant age effect on all tasks (bivariate Pearson correlations; all p values <= 0.001). Thus, all subsequent analyses were performed using the age- and education corrected Z scores to solely focus on the interaction of education, brain pathology and cognitive performance.

3.3.3 Effects of education and structural brain measures on cognitive function

Processing speed: Patients with lower BPF performed worse on processing speed tasks (main effect of BPF: F=6.1, df1=5, df2=225, p<0.01). There was a trend for patients with high nLLV to have reduced processing speed, however, this effect failed to reach significance (main effect of nLLV: F=2.629, df1=5, df2=241, p=0.075). When using age- and education corrected values there was no overall effect of education on processing speed, but there was a significant education x nLLV interaction (F=3.225, df1=5, df2=241, p<0.05) which showed the following pattern in post-hoc analysis: Higher educated
patients performed significantly better than less educated patients in the no (t=-4.032, p<0.001) and low nLLV group (t=-3.319, p<0.001), whereas there was no educational effect in the high nLLV group. Although the education x BPF interaction was not significant (F=0.531, df1=5, df2=225, p=0.589), a similar relationship was found for BPF in group comparisons: In the high BPF group, higher educated patients performed better than less educated patients (t=-3.327, p<0.01) (figure 3, all group comparisons were Bonferroni-corrected). In contrast, there was no significant effect of education on processing speed in the medium and low BPF groups.

Executive function: Using norm values corrected for age and education, there were no differences in performance on executive function tasks regarding nLLV and BPF (no significant main effects). There was also no overall effect of education on executive function tasks. The interactions of education and brain pathology were not significant in multivariate analysis.

Verbal memory: No significant main effects of nLLV, BPF or education could be found for the verbal memory score. Also, there were no significant interactions or group differences after correction for multiple testing.
Figure 3. Effects of the interaction between educational level and pathology on processing speed and executive function
Processing speed performance (mean score ± standard error) in the two educational groups at different levels of brain pathology. Patients with higher education perform better with no or a low amount of pathology, but performance does not differ between the two educational groups with higher amounts of pathology. Interaction plots are shown for normalized lacunar lesion volume (nLLV; n=247, left panel), and brain parenchymal fraction (BPF; n=231, right panel).

*p<0.01, **p<0.001, n.s., not significant.

3.3.4 Linear regression analyses
Processing speed: Controlling for age, arterial hypertension, hypercholesterolemia, motor impairment (mRS) and center effects by using the standardized residuals, linear regression analysis on the processing speed score yielded nLLV and the nLLV x education interaction as significant predictors (n= 231, total adjusted R²=0.104, see table 3).

Executive function: After controlling for covariables, all predictor variables failed to reach significance in the regression model for executive function.
Verbal Memory: After correction for covariables, only lacunar lesion volume (n=231, standardized beta=-0.576, p<0.05, adjusted $R^2=0.108$) was a significant predictor for verbal memory.
3.4 Discussion

This study on a well-defined cohort of patients with cerebral small vessel disease demonstrates a role of CR in VCI. The main finding can be summarized as follows: Using compound scores corrected for age and education, an additional effect of education was only found in patients with low amount of brain pathology whereas there was no such effect in patients with advanced brain damage. This modifying effect of education on the relationship between brain pathology and cognitive performance was demonstrated for two established markers of vascular brain damage (nLLV and BPF) and for an important cognitive domain (processing speed). The interaction between education and nLLV could be demonstrated in a group comparison between two educational groups as well as a linear regression analysis treating education as a continuous variable.

The observed pattern (figures 3) closely resembles the model of cognitive reserve (Stern, 2009) (figure 1): Patients with high reserve showed better cognitive performance at the same level of pathology, but only for low or moderate amounts of brain damage. No differences were found for higher amounts of pathology. This supports the concept of an active mechanism allowing higher educated patients to compensate for brain damage until an individual threshold is reached and the amount of brain damage overpowers cognitive reserve mechanisms.

The cognitive reserve effect was observed in processing speed tasks, the cognitive domain most prominently affected in our subjects. The most likely explanation for this observation is that patients were not as severely impaired in executive function and memory compared to processing speed and therefore did not vary sufficiently in performance (see figure 2B).

The effect observed in this study exceeds the common finding that higher educated subjects perform better in neuropsychological tasks (O'Connor et al., 1991; Sarnacki, 1979). Using age- and education corrected values, we demonstrate an additional advantage for higher educated patients but only
with low and medium amounts of pathology. The fact that higher educated patients tended to perform even worse than less educated patients at the highest load of lacunar lesions (figure 3) might reflect the more rapid cognitive decline for higher educated patients with advanced pathology as seen in other studies (Kemppainen et al., 2008; Stern, 2009).

So far, most studies on CR have focused on neurodegenerative disease (Stern et al., 1999). By design, confounding effects from unrecognized AD pathology were excluded in our study. Our findings on lacunar lesions (LL) extend the concept of CR to subcortical ischemic lesions. A study on participants from the Cardiovascular Health Study (CHS) found an effect of education on the Modified Mini-Mental State Examination Scores in subjects with incident infarcts (Elkins et al., 2006). However, infarct mechanisms were not examined in detail, and because of the relatively high age of this cohort a confounding effect from neurodegenerative disease cannot be excluded. A second study from Scotland found that lower premorbid cognitive ability is a risk factor for VCI. However, this study provided no data on brain structure or cognitive testing in adults (McGurn et al., 2008) and is thus difficult to compare. A recent study on neurologically healthy older adults demonstrated an effect of CR on the clinical expression of white matter hyperintensities (WMH) (Brickman et al., 2009). In the current study interaction effects were strongest for lacunar lesions (LL). This may in part relate to a greater impact of LL on cognitive performance compared with WMH (Viswanathan et al., 2010).

The underlying neurophysiology of CR in VCI remains to be elucidated. Up to date, not much is known about the exact mechanisms that lead from ischemic tissue damage to cognitive impairment. Recently, again using CADASIL as model for pure VCI, we were able to show that an important mechanism for the impairment of processing speed might be the disruption of frontal subcortical neuronal circuits through ischemic lesions (Duering et al., 2011). Inter-individual differences in the ability to compensate for damages within these neuronal networks (Buckner, 2004) might form the basis for CR in VCI.
Limitations of this study include the cross-sectional design and the use of a single proxy for CR. The inclusion of other proxies for CR, which was not possible due to the retrospective nature of this study, might have resulted in a more comprehensive understanding of CR in VCI. Overall, participants from this study were relatively mildly affected thus precluding a thorough analysis of cognitive aspects other than processing speed, which is already impaired in early disease stages. The fact that the multivariate analysis with processing speed and BPF did not reach significance is another limitation. Since post-hoc results pointed in the same direction as results with nLLV, we felt that the trend seen with BPF is worth reporting.

The concept of CR has originally been proposed to account for the obvious disjunction between levels of brain damage and clinical outcomes (Stern, 2009). This disjunction between MRI proxies of ischemic brain damage and clinical outcomes is broadly recognized and has major practical implications such as challenges in developing surrogate markers for clinical trials and the well-known difficulties in establishing valid diagnostic criteria for VCI. The current study suggests that CR should be considered when studying VCI.
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