LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Aus dem Institut für Schlaganfall- und Demenzforschung
Direktor: Prof.Dr. Martin Dichgans

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
Dipl.-Psych. Nikola Zieren
aus
Engelskirchen

Jahr
2013

Mit Genehmigung der Medizinischen Fakultät
der Universität München

Berichterstatter:	Prof. Dr. Martin Dichgans
Mitberichterstatter:	Priv.Doz.Dr. Jennifer Linn Prof.Dr. Gerhard F. Hamann
Mitbetreuung durch den promovierten Mitarbeiter:	Dr. med. Marco Düring
Dekan:	Prof. Dr. med. Dr. h.c. M. Reiser, FACR, FRCR
Tag der mündlichen Prüfung:	20.11.2013

Inhalt

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

Das Thema dieser Arbeit ist kognitive Beeinträchtigung nach zerebrovaskulärer Schädigung. Aufgrund der Heterogenität des Krankheitsbildes der „vaskulären kognitiven Beeinträchtigung“ (Vascular Cognitive Impairment, VCI) und der häufigen Komorbidität mit Alzheimerdemenz wird eine monogen vererbte Mikroangiopathie (zerebrale autosomal dominante Angiopathie mit subkortikalen Infarkten und Leukenzephalopathie, CADASIL) als Modellerkrankung einer reinen VCI herangezogen. In der ersten der beiden Publikationen wurde mittels voxelbasiertem Läsions-Symptom-Mapping der Zusammenhang zwischen Läsionen in frontalen subkortikalen Arealen und CADASIL-typischen kognitiven Einschränkungen untersucht. Der stärkste Zusammenhang bestand zwischen kognitiver Bearbeitungsgeschwindigkeit und Läsionen in der anterioren Thalamusstrahlung sowie der Forceps Minor. Eine zusätzliche Regressionsanalyse konnte zeigen, dass nicht das Gesamtausmaß der Schädigung entscheidend ist für spezifische Funktionsbeeinträchtigung, sondern die Läsionslast in den zuvor identifzierten Lokalisationen.

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

Demenz und erworbene kognitive Störungen haben häufig mehrere zugrundeliegende Ursachen. Zwar ist die häufigste bekannte Ursache neurodegenerative Pathologie wie bei der Alzheimerdemenz (AD), oft jedoch spielt auch zerebrovaskuläre Schädigung eine zusätzliche Rolle und beeinflusst den Verlauf der Erkrankung. Ebenfalls treten bei reiner zerebrovaskulärer Pathologie kognitive Beeinträchtigungen auf, die bis zur Demenz führen können. Unter dem Begriff der „Vaskulären Demenz“ (VaD) werden verschiedene klinisch identifizierbare Störungen zusammengefasst (O'Brien, Erkinjuntti et al. 2003): Multi-Infarkt-Demenz, Demenz nach einem singulären Schlaganfall, sowie die meist mikroangiopathiebedingte, subkortikale ischemische vaskuläre Demenz (siehe Abb.1).

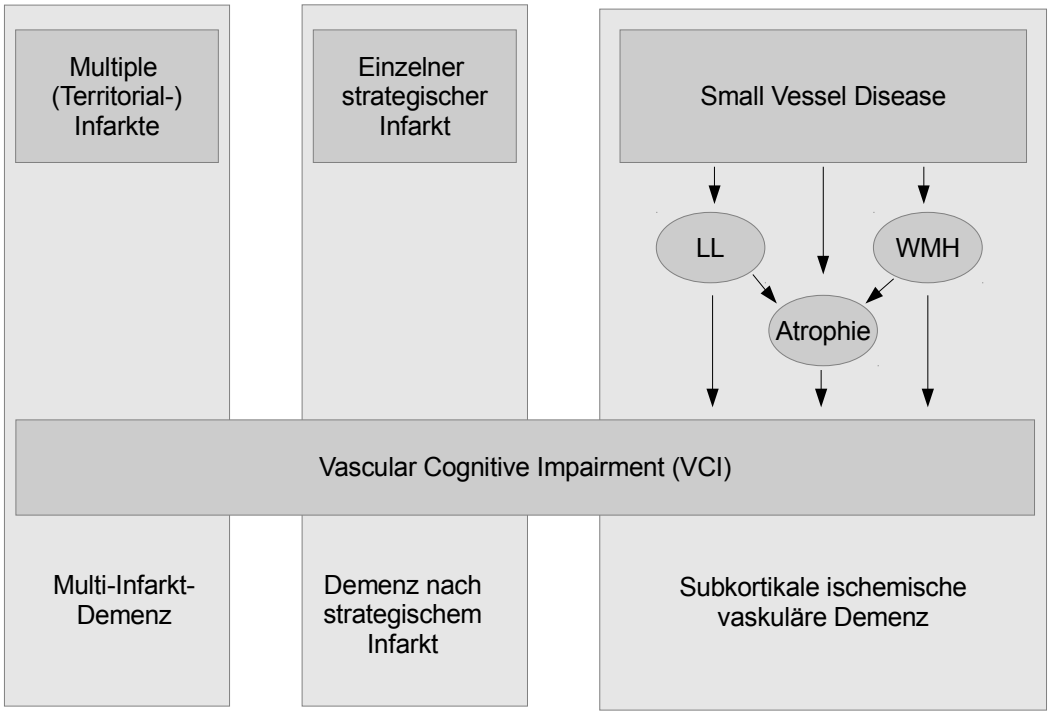

Abb.1: Mechanismen der vaskulären kognitiven Störung.

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 kogntivie Beeinträchtigung und die subkortikale ischemische vaskuläre Demenz.

So heterogen wie die zugrundeliegende Hirnschädigung ist auch das klinische Erscheinungsbild sowie das Ausmaß und die Art der kognitiven Störung. So zeichnet sich die VaD oftmals durch relativ gut erhaltene Gedächtnisfunktionen aus bei gleichzeitigem Vorliegen einer Störung der Exekutivfunktionen (O'Brien, Erkinjuntti et al. 2003). Immer noch ist in gängigen Diagnosesystemen wie DSM-IV oder ICD-10 das Vorliegen einer Gedächtnisstörung Bedingung für die Diagnose einer Demenz. Zusätzlich fordern die Diagnosesysteme zur Diagnose einer Demenz das Vorliegen einer Alltagsbeeinträchtigung. Die Erhebung einer durch kognitive Dysfunktion hervogerufenen Alltagsbeeinträchtigung ist bei VaD zusätzlich erschwert, da durch die häufigen fokalneurologischen Defizite (Ataxie, Hemiparese)

ebenfalls eine Einschränkung der Alltagsfähigkeiten vorliegen kann (O'Brien, Erkinjuntti et al. 2003; Barnett, Salmond et al. 2006; Pinkston, Alekseeva et al. 2009). Daher wird im Falle einer VaD auch oft von „Vascular Cognitive Impairment“ (VCI) gesprochen. In dieser Arbeit wird der Begriff VCI verwendet, da die Diagnose bzw. die Entwicklung einer Demenz als Folge vaskulärer Ereignisse nur als letzte Konsequenz kognitiver Beeinträchtigungen betrachtet wird.

Die beschriebene Heterogenität der VaD sowie überlappende Pathologien (Risikofaktoren bei DAT, Atrophie und alzheimertypische Pathologie bei VaD) macht eine eindeutige Abgrenzung im klinischen Setting oft schwierig (Leys, Henon et al. 2005). Zur Untersuchung des spezifischen Einflusses vaskulärer Erkrankungen auf Struktur und Funktion wird daher in dieser Arbeit eine Stichprobe von Patienten mit genetisch bedingter Mikroangiopathie als Modellerkrankung für eine reine VCI herangezogen.

1.2 CADASIL

CADASIL steht für „*C*erebral *A*utosomal *D*ominant *A*rteriopathy with *S*ubcortical *I*nfarcts and *L*eucoencephalopathy“. Es handelt sich hierbei um eine monogen vererbte Mikroangiopathie durch Mutation im *NOTCH3*-Gen. Das erste Symptom ist häufig eine Migräneerkrankung mit oder ohne Aura, die im Durchschnitt im Alter von 30 Jahren beginnt (Chabriat, Vahedi et al. 1995). Im durchschnittlichen Alter von 49 Jahren beginnen subkortikale ischämische Schlaganfälle, oft in Abwesenheit klassischer Risikofaktoren wie Rauchen, Übergewicht, Bluthochdruck (Chabriat, Joutel et al. 2009). Diese subkortikalen ischämischen Ereignisse führen zu einem charakteristischen auffälligen MRT-Bild (siehe 1.2.2). Die Folgen sind neben sensomotorischen Beeinträchtigungen häufig affektive Störungen wie Depression, bipolare Störungen (Dichgans, Mayer et al. 1998) und Apathie (Reyes, Viswanathan et al. 2009) sowie eine kognitive Beeinträchtigung (Abb.2).

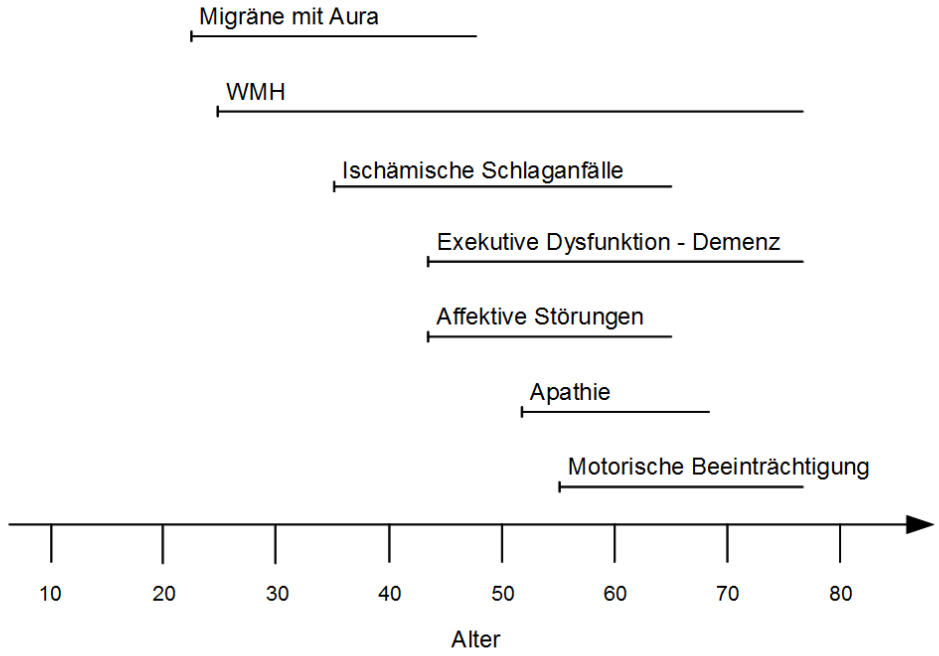

Abb.2: Krankheitsverlauf von CADASIL.

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).

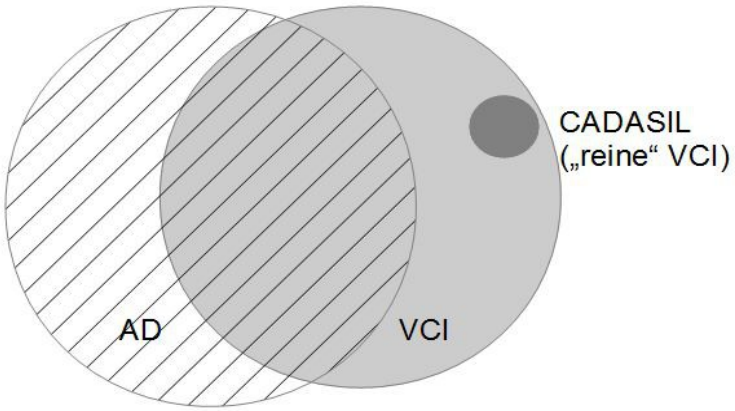

Abb.3: CADASIL als „reine“ VCI.

Alzheimer- und vaskuläre Pathologie kommen häufig gemeinsam vor, insbesondere mit fortschreitendem Alter. CADASIL als monogen bedingte Mikroangiopathie mit frühem Beginn lässt sich als „reine“ Form vaskulärer kognitiver Beeinträchtigung (Vascular Cognitive Impairment, VCI) verstehen und zeigt keine Überlappung mit AD.

Durch das junge Alter der Patienten bei klinischer Manifestation ist es unwahrscheinlich, bei ihnen Alzheimerpathologie vorzufinden. Auch histologisch gibt es bei diesen Patienten keine Hinweise auf Alzheimerpathologie (Formichi, Parnetti et al. 2010; Paquet, Jouvent et al. 2010). CADASIL kann zudem eindeutig über die Molekulargenetik diagnostiziert werden. Die kognitiven und klinischen Charakteristika von CADASIL und sporadischer SIVD gleichen sich weitgehend (Charlton, Morris et al. 2006).

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-

SIL-Patienten mit einem gesunden Kontrollkollektiv zeigte die deutlichsten Beeinträchtigungen in der Bearbeitungszeit des Trail Making Test A und B sowie in den Fehlerraten des Stroop-Tests. Insbesondere bei schwerer betroffenen Patienten waren zusätzlich die semantische Wortflüssigkeit sowie die Fehlerrate in einem Zahlen-Durchstreich-Test beeinträchtigt. Beeinträchtigungen der verbalen Merkfähigkeit zeigen sich vor allem im freien Abruf, nicht jedoch in gestützter Reproduktions- oder Wiedererkennensleistung (Buffon, Porcher et al. 2006).

1.2.2 MRT-Charakteristika von CADASIL

Typische Veränderungen des Gehirns werden bei CADASIL häufig schon 10-15 Jahre vor einer klinischen Manifestation sichtbar (Tournier-Lasserve, Joutel et al. 1993). Im MRT sind die frühesten und häufigsten Veränderung als hyperintenses Signal in T2-gewichteten Bildern sichtbar. Es handelt sich dabei um großflächige Läsionen, vornehmlich in der weißen Substanz, daher werden sie in der Literatur auch als „White Matter Hyperintensities“ (WMH) bezeichnet (Abbildung 4A). Sie treten zunächst periventrikulär, später auch diffuser und großflächiger in der Capsula interna, der Corona radiata, dem Centrum semiovale und dem anterioren Temporallappen auf (O'Sullivan, Jarosz et al. 2001; Markus, Martin et al. 2002). Jedoch sind auch Teile der subkortikalen grauen Substanz betroffen, hier v. a. die Basalganglien und der Thalamus (Markus, Martin et al. 2002; Chabriat, Joutel et al. 2009). Im Verlauf der Erkrankung werden auch Lakunen sichtbar, die gewöhnlich in den selben Arealen wie die WMH erscheinen (Chabriat, Levy et al. 1998) (Abbildung 4B). Hierbei handelt es sich um kleine, vom umliegenden Gewebe gut abgrenzbare flüssigkeitsgefüllte Gewebsdefekte mit einem Durchmesser > 2mm. Bei etwa einem Drittel der Patienten sind zerebrale Mikroblutungen nachweisbar (Viswanathan, Guichard et al. 2006) (Abb. 4C). In der Diffusionstensorbildgebung werden schon in frühen Erkrankungsstadien mikrostrukturelle Veränderungen sichtbar (verminderte FA, erhöhte MD) (Chabriat, Pappata et al. 1999; Holtmannspötter, Peters et al. 2005) (Abb. 4D). Im späteren Verlauf zeigt das MRT eine Atrophie des Gesamthirns (Peters, Holtmannspötter et al. 2006).

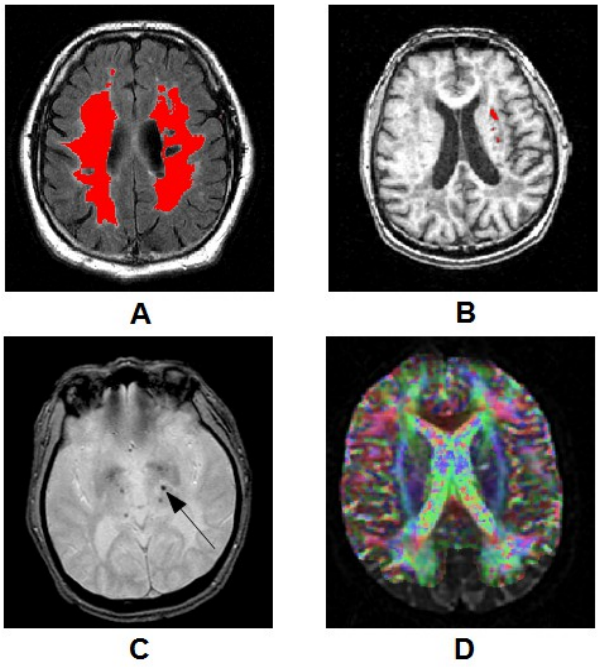

Abb. 4: CADASIL-typische Veränderungen im MRT.

***A:** „White Matter Hyperintensities (WMH), großflächige Läsionen, vorwiegend in der weißen Substanz (rot markiert). **B:** Lakunäre Läsionen (LL), kleine Gewebsdefekte (rot markiert). **C:** Zerebrale Mikroblutungen (Pfeil). **D:** Mikrostrukturelle Veränderungen in der Diffusionstensorbildgebung.*

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 auspartialisiert 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,

Peters et al. 2005), in multivariaten Modellen erklären sie jedoch meist keinen separaten Varianzanteil (Viswanathan, Godin et al. 2008).
Korrelative Ansätze können nur bedingt über funktionelle Zusammenhänge aufklären. Wie die bisherigen Studien zeigen, gibt es Varianzanteile, die durch die reine Korrelation nicht erklärt werden können. Immer häufiger werden daher strukturelle Bildgebungsdaten mit Hilfe fortgeschrittener statistischer Verfahren ausgewertet, um eine bessere Aussage über den Zusammenhang zwischen Läsion und Funktion treffen zu können.

1.3 Strukturelle Bildgebung

1.3.1 Läsionsstudien

Mit Hilfe der strukturellen Bildgebung wird Größe und Lokalisation von Läsionen mit neuropsychologischen Parametern korreliert, um eine Aussage zu treffen, welche Funktionen in dem betroffenen Gewebe lokalisiert sind. Ein klassischer Ansatz hierfür besteht darin, das Volumen der Läsion pro Patient zu berechnen und dadurch das Ausmaß der Läsionslast mit dem Ausmaß funktionellen Ausfalls in Zusammenhang zu bringen (Prins, Fujima et al. 2005; Liem, van der Grond et al. 2007). Die Korrelationen zwischen Läsionsvolumen und kognitiver Beeinträchtigung sind meist jedoch nur moderat, was auf einen hohen Anteil ungeklärter Varianz durch dieses Verfahren hindeutet. Eine weitere Möglichkeit sind Läsionsstudien, in denen die Läsionen einzelner Patienten und ihre Funktionsausfälle verglichen werden und anhand von doppelter Dissoziation die Areale identifiziert werden, die mit der speziellen Funktion in Verbindung stehen (Dronkers 1996). Dabei ist es entweder möglich, Patienten nach Läsion zu gruppieren (Vergleich einer Gruppe von Patienten mit Läsion in einer bestimmten Region mit einer Gruppe, die dort keine Läsion haben) oder nach Verhalten (Funktionsbeeinträchtigung vs. keine Beeinträchtigung) (Bates, Wilson et al. 2003).

1.3.2 Voxelbasiertes Läsions-Symptom-Mapping (VLSM)

Ein Nachteil läsionsbasierter Ansätze besteht in der Limitierung der Bereiche im Gehirn, in denen nach Zusammenhängen gesucht wird, auf sogenannte „Regions of Interest“ (ROI) und dadurch eventuell der Verlust von Information über das übrige Gehirn. Des weiteren sind durch den Verlauf der Blutgefäße bestimmte Bereiche des Gehirns häufiger von Schädigung betroffen als andere, so dass auch hierdurch die vorurteilsfreie Untersuchung des Zusammenhangs zwischen Schädigung und Beeinträchtigung erschwert wird. Voxelbasiertes Läsions-Symptom-Mapping (VLSM) verwendet robuste statistische Methoden, um kontinuierliche Merkmale zwischen Probanden auf Voxelbasis zu vergleichen. Dabei werden für jedes Voxel diejenigen Patienten, die eine Schädigung in diesem Voxel (binär) haben, hinsichtlich einer (kontiunierlichen) Funktionseinschränkung mit den Patienten verglichen, die dort keine Schädigung haben. Rorden et al. (Rorden, Karnath et al. 2007) schlagen dabei zum Gruppenvergleich einen nichtparametrischen Test vor, da der üblicherweise verwendete t-Test voraussetzt, dass das Merkmal, das verglichen wird, normalverteilt ist und die zwei Gruppen, die verglichen werden, Varianzhomogenität aufweisen. Neuropsychologische Tests sind häufig nicht normalverteilt. Des weiteren ist oft der Mittelwert, der im t-Test zwischen den Gruppen verglichen wird, ein unsensitives Maß z.B. bei schief verteilten Daten. Eine Anwendung des t-Tests bei nicht gegebenen Verteilungsvoraussetzungen kann zu falsch-negativen wie auch falsch-positiven Ergebnissen führen. Ein Test, der sich auch für schief verteilte Stichproben auf Rangskalenniveau als robust erwiesen hat, ist der Test von Brunner und Munzel (Brunner 2000). Die Adaption für kleine Anzahl Probanden pro Gruppe, wie im Läsions-Symptom-Mapping erforderlich, erfolgte von Rorden et al. (2007).

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 $i = 1...\mathrm{k} \quad alpha(korr.) = \frac{alpha}{k}$. 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 erkanntem 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.

Auch die Verfahren des VLSM stoßen hinsichtlich der Varianzaufklärung an eine Grenze, wenn es um den Zusammenhang zwischen Läsion und Funktion geht, insbesondere bei neuropsychologischen Defiziten. Neuropsychologische Funktionsausfälle können multifaktoriell bedingt sein und neuropsychologische Tests messen nicht immer eine unmittelbar zuordenbare Funktion. Ein Test zur Bearbeitungsgeschwindigkeit z.B. umfasst kognitive Flexibilität, motorische Fähigkeiten, visuell-räumliches Vorstellungsvermögen und selektive Aufmerksamkeit. Selbst bei gezielter Betrachtung einzelner Funktionen bleiben individuelle Unterschiede in der Bearbeitung der Aufgabe. Die Schlussfolgerung liegt nahe, dass es moderierende Faktoren gibt, die die individuelle Beeinträchtigung beeinflussen. Eine davon ist die Kognitive Reserve.

1.4 Kognitive Reserve

1.4.1 Kognitive Leistung und Hirnschädigung

Zwischen dem Ausmaß der Hirnschädigung und den neuropsychologischen Defiziten einer Person besteht oft kein direkter Zusammenhang (Stern 2002). So fanden z.B. prospektive Studien, dass bei bis zu 25% älterer Menschen, bei denen post mortem die pathologischen Kriterien für Alzheimerkrankheit erfüllt waren, bis zum Tode unauffällige neuropsychologische Testergebnisse vorlagen (Ince 2001). Ein Faktor, der das Verhältnis von Schädigung und Funktionsstörung beeinflusst, scheint die formale Schulbildung zu sein. Dabei liegt die Schlussfolgerung nahe, dass höher Gebildete in kognitiven Tests besser abschneiden als weniger Gebildete, da sie geübter in Leistungsaufgaben sind (O'Connor, Pollitt et al. 1991). Die Beziehung zwischen Bildung und Hirnpathologie geht aber über diesen „testmanship bias“ hinaus. So fanden z.B. Bennett und Kollegen (Bennett, Wilson et al. 2003) in einer prospektiven Studie mit älteren Geistlichen („Religious Orders Study“), dass höhere Schulbildung nicht nur die kognitive Leistung vorhersagt, sondern dass eine Interaktion zwischen Bildung und Alzheimerpathologie besteht. Dabei hatten höher Gebildete einen weniger starken Zusammenhang zwischen Alzheimerpathologie und kognitiven Funktionen als weniger Gebildete. Dieser Zusammenhang war unterschiedlich stark je nach Maß für die Pathologie sowie die kognitive Funktion. Einen besonders ausgeprägten Effekt fanden die Autoren für Bearbeitungsgeschwindigkeit, semantisches Gedächtnis und Arbeitsgedächtnis. Weniger deutlich war der Zusammenhang bei episodischem Gedächtnis und räumlich-visuellen Fähigkeiten.

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 postuliert, dass es Eigenschaften des Gehirns gibt, die manchen Individuen erlau-

ben, besser mit vorhandener Schädigung umzugehen als anderen. Als potenzielle Eigenschaften werden dabei die Größe des Gehirns und die Anzahl der Neuronen oder Synapsen diskutiert, aber ebenso durch Lebenserfahrung variierbare Komponenten wie neuronale Plastizität und Resistenz gegen Zelltod (Katzman 1993; Satz, Morgenstern et al. 1993). Mit dieser Modellvorstellung geht die Annahme einher, dass die gleiche Schädigung auf jedes Gehirn den gleichen Einfluss hat und es einen festen „Schwellenwert" gibt, ab dem das Individuum als Folge der Schädigung kognitiv beeinträchtigt ist. Aktive Kompensationsmechanismen finden in diesem Modell keine Berücksichtigung. Stern (Stern, Alexander et al. 1992) postuliert daher das Modell der „Kognitiven Reserve". Hier wird davon ausgegangen, dass bei gleichem Ausmaß der Schädigung unterschiedliche Strategien der Aufgabenbearbeitung und Kompensation dafür verantwortlich sind, dass Individuen unterschiedlich gute kognitive Leistung zeigen und damit ein individueller Schwellenwert existiert, ab dem ein Individuum kognitive Beeinträchtigung erlebt. Dabei werden als mögliche Kompensationsmechanismen die bessere Ausnutzung vorhandener neuraler Netzwerke oder das Hinzuziehen anderer Netzwerke, die bei Gesunden für die Aufgabe normalerweise nicht verwendet werden, diskutiert.

Unter der Annahme, dass pathologische Veränderungen des Gehirns schon existieren, bevor kognitive Beeinträchtigung evident wird, postuliert Stern (2009) folgenden modifizierenden Einfluss der kognitiven Reserve: Patienten mit hoher kognitiver Reserve zeigen grundsätzlich bessere Leistung in kognitiven Aufgaben. Daher muss ein höheres Ausmaß an Pathologie vorhanden sein, bis kognitive Beeinträchtigung bei diesen Patienten evident wird bzw. bis die Diagnose einer Demenz erfolgt. Dies trägt auch der scheinbar paradoxen Beobachtung Rechnung, dass nach der Diagnose einer Demenz Patienten mit höherer Bildung einen schnelleren Krankheitsverlauf und weniger gute Überlebensraten zeigen (Kemppainen, Aalto et al. 2008). Ab einem bestimmten Ausmaß an Hirnschädigung kann diese nicht mehr kompensiert werden und Reservefaktoren spielen keine Rolle mehr.

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).

Auch im Bereich vaskulärer Hirnschädigung wird kognitive Reserve diskutiert. Insbesondere beim Einfluss von WMH auf kognitive Beeinträchtigung wurden Reservefaktoren als Moderator in den vergangenen Jahren immer wieder beobachtet (Galluzzi, Lanni et al. 2008). Dabei fand sich einen Zusammenhang zwischen höherer Reserve (gemessen an formeller Bildung und der Leistung in einem veränderungsinvarianten Intelligenztest) und höherem Ausmaß an WMH bei gleichem Niveau kognitiver Leistung (Brickman, Siedlecki et al. 2011). Dieser Zusammenhang war besonders deutlich für Aufmerksamkeit (Bearbeitungsgeschwindigkeit) und Exekutivfunktionen.

Abschließend muss betont werden, dass, obwohl die meisten Studien formale Schulbildung als Operationalisierung für kognitive Reserve verwenden, andere Faktoren ebenfalls Reserve abbilden können. So fanden eine Reihe von Autoren (Evans, Beckett et al. 1993; Stern, Gurland et al. 1994; Stern, Alexander et al. 1995), dass formale Schulbildung, Ausbildungsniveau bzw. berufliche Laufbahn, Freizeitaktivitäten und prämorbider IQ jeweils unabhängig zur Aufklärung der Varianz kognitiver Reserve beitragen.

1.5 Fragestellung

Die vorliegenden Arbeiten beschäftigen sich mit dem Zusammenhang zwischen Hirnschädigung und kognitiver Beeinträchtigung bei CADASIL. Die erste Arbeit stellt mit Hilfe voxelbasierter Läsions-Symptom-Kartierung den Zusammenhang zwischen der Schädigung bestimmter Hirnareale und typischen Defiziten bei vaskulärer kognitiver Beeinträchtigung (VCI) dar. CADASIL dient in der Unteruchung dabei als Modellerkrankung der reinen VCI. In der zweiten Arbeit wird das Konzept der kognitiven Reserve in einer Stichprobe von CADASIL-Patienten untersucht. Der moderierende Einfluss formaler Schulbildung auf den Zusammenhang zwischen kognitiven Defiziten und CADASIL-typischer Hirnpathologie, Lakunenvolumen und globales Hirnvolumen, wird dargestellt.

1.6 Literaturverzeichnis Einleitung

Barnett, J. H., C. H. Salmond, et al. (2006). Cognitive reserve in neuropsychiatry. Psychol Med 36(8): 1053-1064.

Bates, E., S. M. Wilson, et al. (2003). Voxel-based lesion-symptom mapping. Nat Neurosci 6(5): 448-450.

Bennett, D. A., R. S. Wilson, et al. (2003). Education modifies the relation of AD pathology to level of cognitive function in older persons. Neurology 60(12): 1909-1915.

Brickman, A. M., K. L. Siedlecki, et al. (2011). White matter hyperintensities and cognition: testing the reserve hypothesis. Neurobiol Aging 32(9): 1588-1598.

Brunner, E. M., U (2000). The nonparametric Behrens-Fisher problem. Asymptotic theory and a small sample approximation. Biometrical Journal 42(1): 17-25.

Buffon, F., R. Porcher, et al. (2006). Cognitive profile in CADASIL. J Neurol Neurosurg Psychiatry 77(2): 175-180.

Chabriat, H., A. Joutel, et al. (2009). Cadasil. Lancet Neurol 8(7): 643-653.

Chabriat, H., C. Levy, et al. (1998). Patterns of MRI lesions in CADASIL. Neurology 51(2): 452-457.

Chabriat, H., S. Pappata, et al. (1999). Clinical severity in CADASIL related to ultrastructural damage in white matter: in vivo study with diffusion tensor MRI. Stroke 30(12): 2637-2643.

Chabriat, H., K. Vahedi, et al. (1995). Clinical spectrum of CADASIL: a study of 7 families. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Lancet 346(8980): 934-939.

Dichgans, M. (2009). Cognition in CADASIL. Stroke 40(3 Suppl): S45-47.

Dichgans, M., M. Mayer, et al. (1998). The phenotypic spectrum of CADASIL: clinical findings in 102 cases. Ann Neurol 44(5): 731-739.

Dronkers, N. F. (1996). A new brain region for coordinating speech articulation. Nature 384(6605): 159-161.

Evans, D. A., L. A. Beckett, et al. (1993). Level of education and change in cognitive function in a community population of older persons. Ann Epidemiol 3(1): 71-77.

Farinpour, R., E. N. Miller, et al. (2003). Psychosocial risk factors of HIV morbidity and mortality: findings from the Multicenter AIDS Cohort Study (MACS). J Clin Exp Neuropsychol 25(5): 654-670.

Formichi, P., L. Parnetti, et al. (2010). CSF Biomarkers Profile in CADASIL-A Model of Pure Vascular Dementia: Usefulness in Differential Diagnosis in the Dementia Disorder. Int J Alzheimers Dis 2010.

Galluzzi, S., C. Lanni, et al. (2008). White matter lesions in the elderly: pathophysiological hypothesis on the effect on brain plasticity and reserve. J Neurol Sci 273(1-2): 3-9.

Holtmannspötter, M., N. Peters, et al. (2005). Diffusion magnetic resonance histograms as a surrogate marker and predictor of disease progression in CADASIL: a two-year follow-up study. Stroke 36(12): 2559-2565.

Ince, P. (2001). Dementia with Lewy bodies. Adv Exp Med Biol 487: 135-145.

Jouvent, E., A. Viswanathan, et al. (2007). Brain atrophy is related to lacunar lesions and tissue microstructural changes in CADASIL. Stroke 38(6): 1786-1790.

Katzman, R. (1993). Education and the prevalence of dementia and Alzheimer's disease. Neurology 43(1): 13-20.

Kemppainen, N. M., S. Aalto, et al. (2008). Cognitive reserve hypothesis: Pittsburgh Compound B and fluorodeoxyglucose positron emission tomography in relation to education in mild Alzheimer's disease. Ann Neurol 63(1): 112-118.

Kesler, S. R., H. F. Adams, et al. (2003). Premorbid intellectual functioning, education, and brain size in traumatic brain injury: an investigation of the cognitive reserve hypothesis. Appl Neuropsychol 10(3): 153-162.

Leys, D., H. Henon, et al. (2005). Poststroke dementia. Lancet Neurol 4(11): 752-759.

Liem, M. K., J. van der Grond, et al. (2007). Lacunar infarcts are the main correlate with cognitive dysfunction in CADASIL. Stroke 38(3): 923-928.

Markus, H. S., R. J. Martin, et al. (2002). Diagnostic strategies in CADASIL. Neurology 59(8): 1134-1138.

O'Brien, J. T., T. Erkinjuntti, et al. (2003). Vascular cognitive impairment. Lancet Neurol 2(2): 89-98.

O'Connor, D. W., P. A. Pollitt, et al. (1991). The influence of education and social class on the diagnosis of dementia in a community population. Psychol Med 21(1): 219-224.

O'Sullivan, M., J. M. Jarosz, et al. (2001). MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. Neurology 56(5): 628-634.

Paquet, C., E. Jouvent, et al. (2010). A cortical form of CADASIL with cerebral Abeta amyloidosis. Acta Neuropathol 120(6): 813-820.

Peters, N., M. Holtmannspötter, et al. (2006). Brain volume changes in CADASIL: a serial MRI study in pure subcortical ischemic vascular disease. Neurology 66(10): 1517-1522.

Peters, N., C. Opherk, et al. (2005). The pattern of cognitive performance in CADASIL: a monogenic condition leading to subcortical ischemic vascular dementia. Am J Psychiatry 162(11): 2078-2085.

Pinkston, J. B., N. Alekseeva, et al. (2009). Stroke and dementia. Neurol Res 31(8): 824-831.

Prins, M. L., L. S. Fujima, et al. (2005). Age-dependent reduction of cortical contusion volume by ketones after traumatic brain injury. J Neurosci Res 82(3): 413-420.

Reyes, S., A. Viswanathan, et al. (2009). Apathy: a major symptom in CADASIL. Neurology 72(10): 905-910.

Rorden, C., H. O. Karnath, et al. (2007). Improving lesion-symptom mapping. J Cogn Neurosci 19(7): 1081-1088.

Satz, P., H. Morgenstern, et al. (1993). Low education as a possible risk factor for cognitive abnormalities in HIV-1: findings from the multicenter AIDS Cohort Study (MACS). J Acquir Immune Defic Syndr 6(5): 503-511.

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc 8(3): 448-460.

Stern, Y. (2009). Cognitive reserve. Neuropsychologia 47(10): 2015-2028.

Stern, Y., G. E. Alexander, et al. (1992). Inverse relationship between education and parietotemporal perfusion deficit in Alzheimer's disease. Ann Neurol 32(3): 371-375.

Stern, Y., G. E. Alexander, et al. (1995). Relationship between lifetime occupation and parietal flow: implications for a reserve against Alzheimer's disease pathology. Neurology 45(1): 55-60.

Stern, Y., B. Gurland, et al. (1994). Influence of education and occupation on the incidence of Alzheimer's disease. JAMA 271(13): 1004-1010.

Sumowski, J. F., G. R. Wylie, et al. (2010). Premorbid cognitive leisure independently contributes to cognitive reserve in multiple sclerosis. Neurology 75(16): 1428-1431.

Tournier-Lasserve, E., A. Joutel, et al. (1993). Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. Nat Genet 3(3): 256-259.

Viswanathan, A., O. Godin, et al. (2008). Impact of MRI markers in subcortical vascular dementia: A multi-modal analysis in CADASIL. Neurobiol Aging.

Viswanathan, A., A. Gschwendtner, et al. (2007). Lacunar lesions are independently associated with disability and cognitive impairment in CADASIL. Neurology 69(2): 172-179.

Viswanathan, A., J. P. Guichard, et al. (2006). Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. Brain 129(Pt 9): 2375-2383.

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

Marco Duering[1,*], Nikola Zieren[1,*], Dominique Hervé[2], Eric Jouvent[2], Nils Peters[3], Chahin Pachai[4], Christian Opherk[1], Hugues Chabriat[2], Martin Dichgans[1]

1 Institute for Stroke and Dementia Research, Ludwig-Maximilians-University, Munich, Germany
2 Department of Neurology, CHU Lariboisière, Assistance Publique des Hôpitaux de Paris, France
3 Department of Neurology, Ludwig-Maximilians-University, Munich, Germany
4 Bioclinica SAS, Lyon, France
* These authors contributed equally to this work

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 *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).

Table 1. MRI sequence parameters

	scanner	TR [ms]	TE [ms]	TI [ms]	slice [mm]	voxel [mm]
3DT1	Munich Vision	11.4	4.4	-	1.2	0.9x0.9
	Munich Signa	22	6	-	1	0.9x0.9
	Paris	8.6	1.9	-	0.8	1.02x1.02
FLAIR	Munich Vision	4284	110	1428	5	0.98x0.98
	Munich Signa	8402	151	2002	5	0.94x0.94
	Paris	8402	161	2002	5.5	0.47x0.47

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 2: Characteristics of the study cohort (n=215)

Demographic Characteristics	
Age [years], mean (SD)	47.9 (10.7)
Education [years], mean (SD)	10.8 (3.3)
Female	127 (59.1%)
Clinical Features	
Symptomatic	197 (91.6%)
Prior clinically apparent stroke	128 (59.5%)
Years since first stroke, median (IQR)	4.11 (7.84%)
Migraine history	105 (48.8%)
History for depression	80 (37.2%)
Vascular Risk Factors	
Current smoker	49 (22.8%)
Smoking History	75 (34.9%)
Hypertension	43 (20.0%)
Hypercholesterolemia	72 (33.5%)
Diabetes	5 (2.33%)
Clinical Scores	
MDRS, median (IQR)	141 (7)
Modified Rankin scale, median (IQR)	0 (1)
Modified Rankin scale > 2	15 (7.0%)
NIHSS, median (IQR)	0 (1)
Barthel index, median (IQR)	100 (0)
Imaging Characteristics, mean (SD)	
Normalized LLV [%]	0.0225 (0.047)
Normalized WMHV [%]	6.75 (4.9)
BPF [%]	83.6 (5.3)

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).

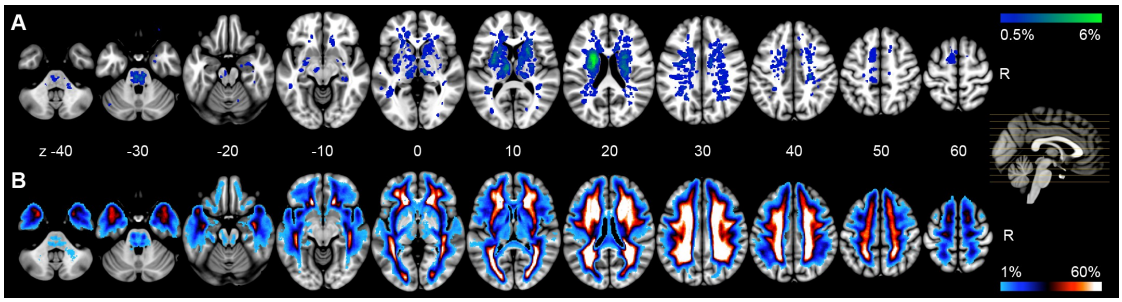

***Figure 1**: Lesion probability maps for lacunar lesions (LL) (**A**) and white matter hyperintensities (WMH) (**B**) in Montreal Neurological Institute 152 (MNI) standard space (N=215). The statistical maps are superimposed onto the MNI T1 template. R = right.*

The anatomical distribution of LL was similar between subgroups stratified for the normalized LL volume (figure 2).

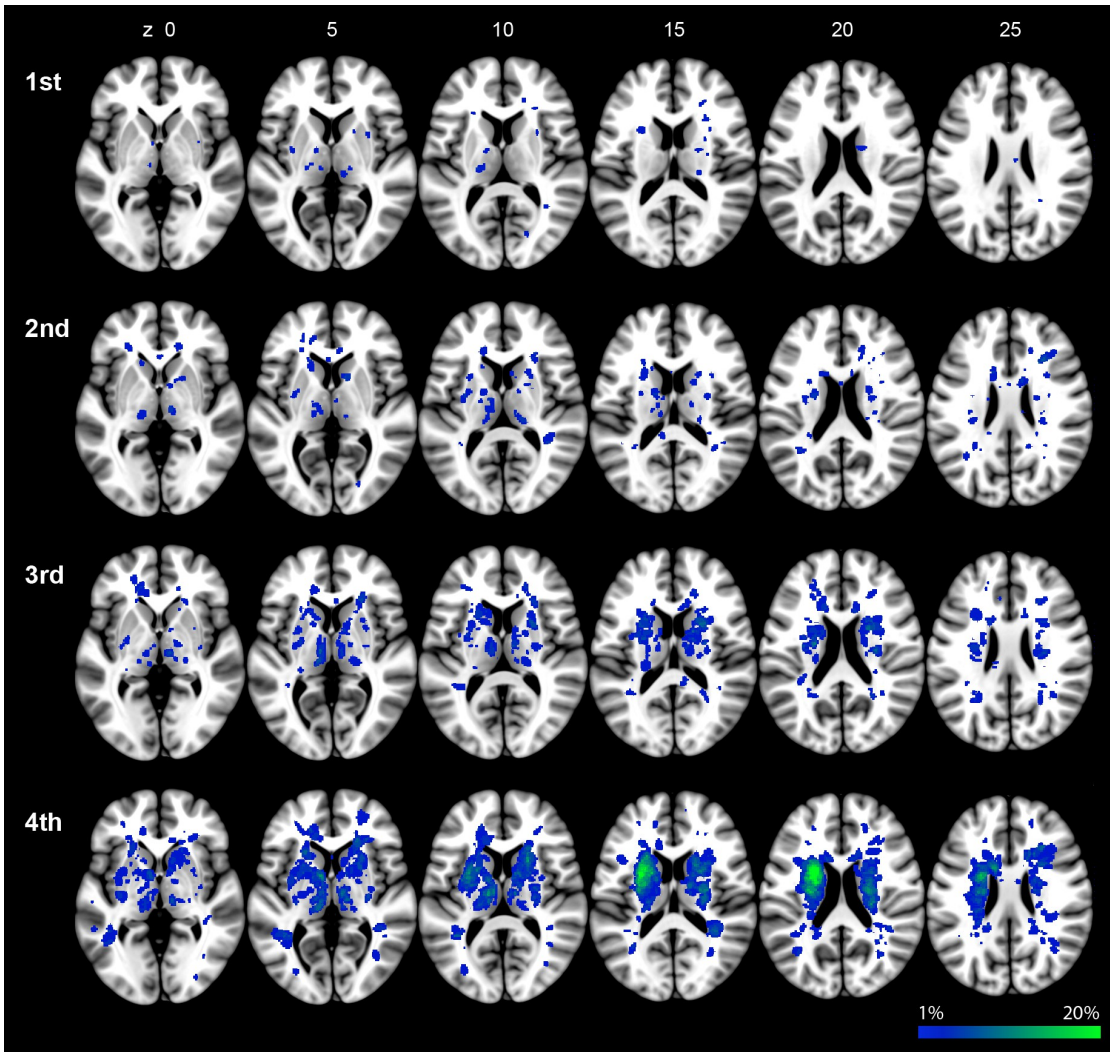

Figure 2: *Spatial distribution of LL in subgroups stratified for the global normalized LL volume (in quartils of patients with lesions, superimposed onto the MNI T1 template).*

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).

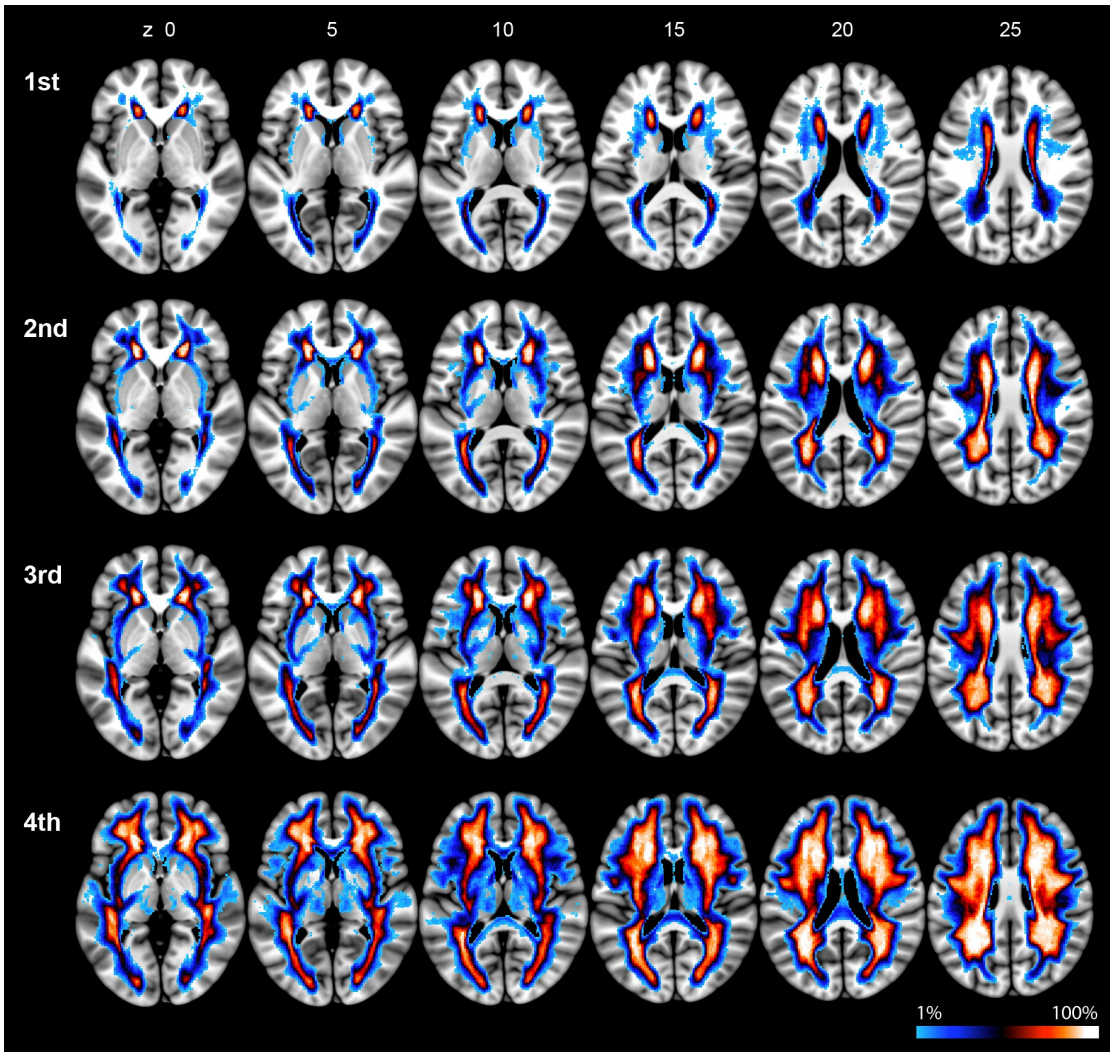

***Figure 3**: Spatial distribution of WMH in subgroups stratified for the global normalized WMH volume (in quartiles, superimposed onto the MNI T1 template).*

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).

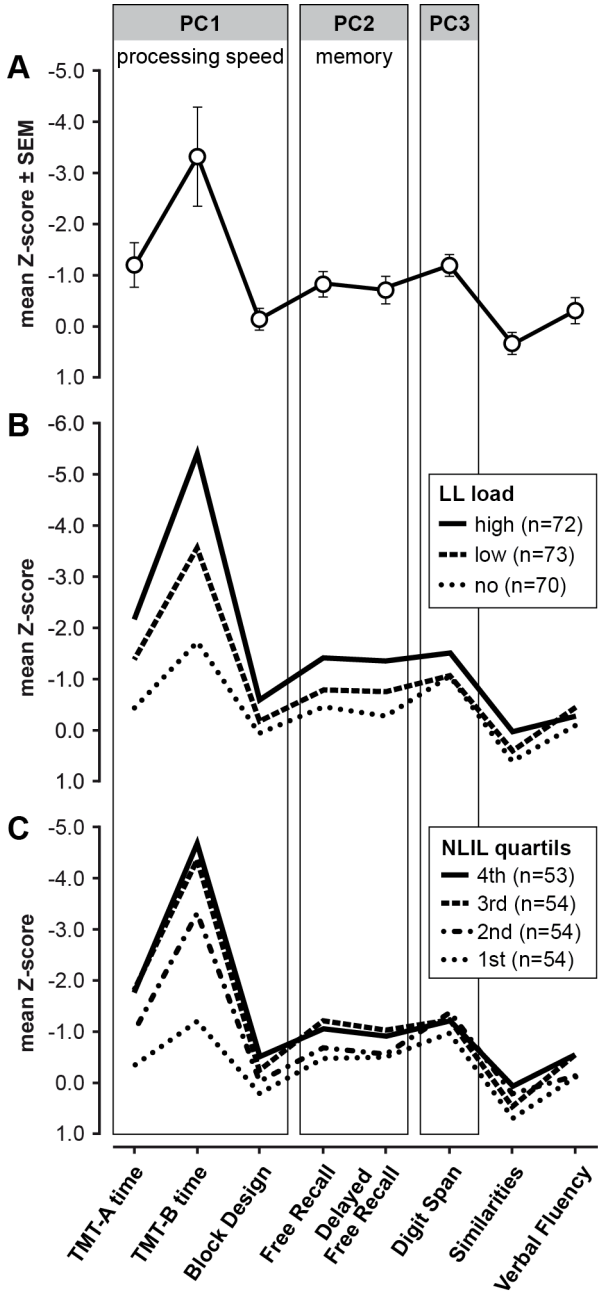

Figure 4: *A: Cognitive profile of the study cohort (N=215; TMT: trail making test). B: Profiles in subgroups stratified for the normalized LL volume (low = first and second quartile of patients with lesions; high = third and fourth quartile). C: Profiles in subgroups stratified for normalized WMH volume (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).

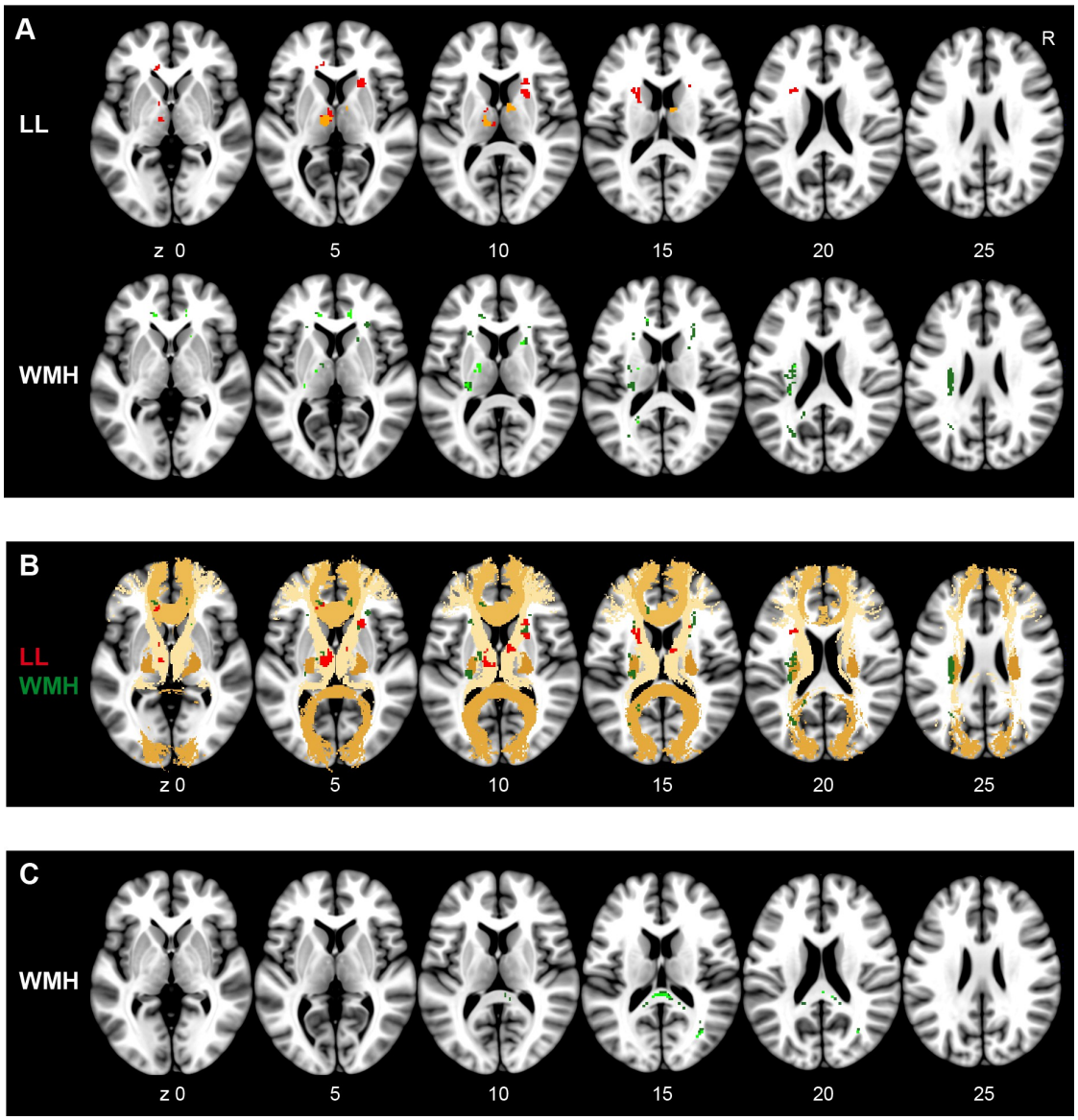

Figure 5: *Voxel-based lesion-symptom mapping results for processing speed (panels **A** and **B**) and memory (panel **C**) in MNI space. **A:** Significant clusters for processing speed after nonparametric mapping and correction for multiple testing. Orange (LL) and light green (WMH) indicate voxels 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.

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

A) Lacunar lesions

No.	MNI coordinates			Contributing lacunes (n)	Anatomical structure	side
	x	y	z			
1	-8	-14	5	9	thalamus	left
2	11	-4	9	13	thalamus	right
3	-20	9	16	21	internal capsule, anterior limb	left
4	22	11	11	15	internal capsule, anterior limb	right
5	-12	32	2	6	corpus callosum, genu	left

B) White matter hyperintensities

No.	MNI coordinates			Mean WMH Prevalence (%)	Anatomical structure	side
	x	y	z			
1	-7	-4	7	10	thalamus	left
2	-23	18	13	59	anterior corona radiata	left
3	24	17	10	47	anterior corona radiata	right
4	-14	35	10	19	corpus callosum, genu	left
5	11	36	4	24	corpus callosum, genu	right
6	-25	-63	22	54	occipital white matter	left
7	-24	-18	16	43	internal capsule, posterior limb	left

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).

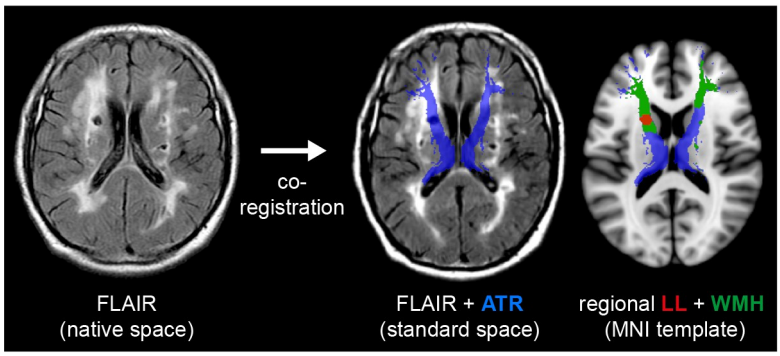

***Figure 6**: Segmentation of lesions projecting on the ATR: left: FLAIR image of a patient in native space (resliced according to MNI template); middle: same image after co-registration to MNI standard space with the ATR superimposed in half transparency; right: MNI T1 template illustrating the segmentation 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.

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)

Step	Variable	p	Standardized beta
1	LLV (ATR)	< 0.000	-0.277
2	WMHV (ATR)	< 0.000	-0.258
3	LLV (Fmin)	< 0.002	-0.195

Model B
Variables as in model A, with addition of BPF, age, sex and formal education (years)

Step	Variable	p	Standardized beta
1	BPF	< 0.000	0.267
2	LLV (ATR)	< 0.000	-0.265
3	WMHV (ATR)	< 0.001	-0.213

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

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

Auchus AP, Chen CP, Sodagar SN, Thong M, Sng EC. Single stroke dementia: insights from 12 cases in Singapore. J Neurol Sci. 2002 Nov 15;203-204:85-9.

Behrens TE, Johansen-Berg H, Woolrich MW, Smith SM, Wheeler-Kingshott CA, Boulby PA, et al. Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. Nat Neurosci. 2003 Jul;6(7):750-7.

Brett M, Leff AP, Rorden C, Ashburner J. Spatial normalization of brain images with focal lesions using cost function masking. Neuroimage. 2001 Aug 1;14(2):486-500.

Brickman AM, Siedlecki KL, Muraskin J, Manly JJ, Luchsinger JA, Yeung L-K, et al. White matter hyperintensities and cognition: Testing the reserve hypothesis. NBA. 2009 Nov 17:1-11.

Burton E, Ballard C, Stephens S, Kenny RA, Kalaria R, Barber R, et al. Hyperintensities and fronto-subcortical atrophy on MRI are substrates of mild cognitive deficits after stroke. Dement Geriatr Cogn Disord. 2003;16(2):113-8.

Caplan LR, Schmahmann JD, Kase CS, Feldmann E, Baquis G, Greenberg JP, et al. Caudate infarcts. Arch Neurol. 1990 Feb;47(2):133-43.

Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser M-G. Cadasil. Lancet Neurol. 2009 Jul 1;8(7):643-53.

Charlton RA, Morris RG, Nitkunan A, Markus HS. The cognitive profiles of CADASIL and sporadic small vessel disease. Neurology. 2006 May 23;66(10):1523-6.

Chui HC. Subcortical ischemic vascular dementia. Neurol Clin. 2007 Aug 1;25(3):717-40, vi.

Cummings JL. Vascular dementia and dementia of Alzheimer-type cognition. Arch Neurol. 1989 Oct 1;46(10):1046-7.

De Groot JC, De Leeuw F-E, Oudkerk M, Van Gijn J, Hofman A, Jolles J, et al. Periventricular cerebral white matter lesions predict rate of cognitive decline. Ann Neurol. 2002 Sep 1;52(3):335-41.

de Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, et al. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. Ann Neurol. 2000 Feb 1;47(2):145-51.

Debette S, Bombois S, Bruandet A, Delbeuck X, Lepoittevin S, Delmaire C, et al. Subcortical hyperintensities are associated with cognitive decline in patients with mild cognitive impairment. Stroke. 2007 Nov 1;38(11):2924-30.

Delano-Wood L, Abeles N, Sacco JM, Wierenga CE, Horne NR, Bozoki A. Regional white matter pathology in mild cognitive impairment: differential influence of lesion type on neuropsychological functioning. Stroke. 2008 Mar 1;39(3):794-9.

Dichgans M, Markus HS, Salloway S, Verkkoniemi A, Moline M, Wang Q, et al. Donepezil in patients with subcortical vascular cognitive impairment: a randomised double-blind trial in CADASIL. Lancet Neurol. 2008 Apr 1;7(4):310-8.

Doubal FN, MacLullich AMJ, Ferguson KJ, Dennis MS, Wardlaw JM. Enlarged perivascular spaces on MRI are a feature of cerebral small vessel disease. Stroke. 2010 Mar 1;41(3):450-4.

Enzinger C, Smith S, Fazekas F, Drevin G, Ropele S, Nichols T, et al. Lesion probability maps of white matter hyperintensities in elderly individuals: results of the Austrian stroke prevention study. J Neurol. 2006 Aug;253(8):1064-70.

Erkinjuntti T, Haltia M, Palo J, Sulkava R, Paetau A. Accuracy of the clinical diagnosis of vascular dementia: a prospective clinical and post-mortem neuropathological study. J Neurol Neurosurg Psychiatr. 1988 Aug 1;51(8):1037-44.

Gold G. Defining the neuropathological background of vascular and mixed dementia and comparison with magnetic resonance imaging findings. Front Neurol Neurosci. 2009;24:86-94.

Gold G, Kövari E, Herrmann FR, Canuto A, Hof PR, Michel J-P, et al. Cognitive consequences of thalamic, basal ganglia, and deep white matter lacunes in brain aging and dementia. Stroke. 2005 Jun 1;36(6):1184-8.

Hua K, Zhang J, Wakana S, Jiang H, Li X, Reich DS, et al. Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. Neuroimage. 2008 Jan 1;39(1):336-47.

Jacqmin M, Hervé D, Viswanathan A, Guichard J-P, During M, Dichgans M, et al. Confluent thalamic hyperintensities in CADASIL. Cerebrovasc Dis. 2010 Aug 1;30(3):308-13.

Jokinen H, Kalska H, Ylikoski R, Madureira S, Verdelho A, van der Flier WM, et al. Longitudinal cognitive decline in subcortical ischemic vascular disease--the LADIS Study. Cerebrovasc Dis. 2009;27(4):384-91.

Joutel A, Vahedi K, Corpechot C, Troesch A, Chabriat H, Vayssière C, et al. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. Lancet. 1997 Nov 22;350(9090):1511-5.

Jouvent E, Mangin J-F, Porcher R, Viswanathan A, O'Sullivan M, Guichard J-P, et al. Cortical changes in cerebral small vessel diseases: a 3D MRI study of cortical morphology in CADASIL. Brain. 2008 Aug 01;131(Pt 8):2201-8.

Jouvent E, Viswanathan A, Mangin J-F, O'Sullivan M, Guichard J-P, Gschwendtner A, et al. Brain atrophy is related to lacunar lesions and tissue microstructural changes in CADASIL. Stroke. 2007 Jun 1;38(6):1786-90.

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.

Kumral E, Evyapan D, Balkir K. Acute caudate vascular lesions. Stroke. 1999 Jan;30(1):100-8.

Kwee RM, Kwee TC. Virchow-Robin spaces at MR imaging. Radiographics. 2007 Jan 1;27(4):1071-86.

Liem MK, van der Grond J, Haan J, van den Boom R, Ferrari MD, Knaap YM, et al. Lacunar infarcts are the main correlate with cognitive dysfunction in CADASIL. Stroke. 2007 Mar 1;38(3):923-8.

Madureira S, Guerreiro M, Ferro JM. A follow-up study of cognitive impairment due to inferior capsular genu infarction. J Neurol. 1999 Sep;246(9):764-9.

Mega MS, Cummings JL. Frontal-subcortical circuits and neuropsychiatric disorders. J Neuropsychiatry Clin Neurosci. 1994 Fall;6(4):358-70.

Mendez MF, Adams NL, Lewandowski KS. Neurobehavioral changes associated with caudate lesions. Neurology. 1989 Mar;39(3):349-54.

Mungas D, Harvey D, Reed BR, Jagust WJ, DeCarli C, Beckett L, et al. Longitudinal volumetric MRI change and rate of cognitive decline. Neurology. 2005 Aug 23;65(4):565-71.

O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, et al. Vascular cognitive impairment. Lancet Neurol. 2003 Feb 1;2(2):89-98.

O'Brien JT, Wiseman R, Burton EJ, Barber B, Wesnes K, Saxby B, et al. Cognitive associations of subcortical white matter lesions in older people. Ann N Y Acad Sci. 2002 Nov 1;977:436-44.

O'Sullivan M, Barrick TR, Morris RG, Clark CA, Markus HS. Damage within a network of white matter regions underlies executive dysfunction in CADASIL. Neurology. 2005 Nov 22;65(10):1584-90.

O'Sullivan M, Singhal S, Charlton R, Markus HS. Diffusion tensor imaging of thalamus correlates with cognition in CADASIL without dementia. Neurology. 2004 Mar 9;62(5):702-7.

Opherk C. Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. Brain. 2004 Aug 19;127(11):2533-9.

Pérez-Iglesias R, Tordesillas-Gutiérrez D, Barker GJ, Mcguire PK, Roiz-Santiañez R, Mata I, et al. White matter defects in first episode psychosis patients: a voxelwise analysis of diffusion tensor imaging. Neuroimage. 2010 Jan 1;49(1):199-204.

Peters N, Opherk C, Bergmann T, Castro M, Herzog J, Dichgans M. Spectrum of mutations in biopsy-proven CADASIL: implications for diagnostic strategies. Arch Neurol. 2005 Jul 1;62(7):1091-4.

Peters N, Opherk C, Danek A, Ballard C, Herzog J, Dichgans M. The pattern of cognitive performance in CADASIL: a monogenic condition leading to subcortical ischemic vascular dementia. A J Psychiatry. 2005 Nov 1;162(11):2078-85.

Pohjasvaara T, Erkinjuntti T, Ylikoski R, Hietanen M, Vataja R, Kaste M. Clinical determinants of poststroke dementia. Stroke. 1998 Jan 1;29(1):75-81.

Prins ND, van Dijk EJ, den Heijer T, Vermeer SE, Jolles J, Koudstaal PJ, et al. Cerebral small-vessel disease and decline in information processing speed, executive function and memory. Brain. 2005 Sep 1;128(Pt 9):2034-41.

Román GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC. Subcortical ischaemic vascular dementia. Lancet Neurol. 2002 Nov 1;1(7):426-36.

Rorden C, Bonilha L, Nichols TE. Rank-order versus mean based statistics for neuroimaging. Neuroimage. 2007 May 1;35(4):1531-7.

Saczynski JS, Sigurdsson S, Jonsdottir MK, Eiriksdottir G, Jonsson PV, Garcia ME, et al. Cerebral infarcts and cognitive performance: importance of location and number of infarcts. Stroke. 2009 Mar;40(3):677-82.

Schneider JA, Boyle PA, Arvanitakis Z, Bienias JL, Bennett DA. Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. Ann Neurol. 2007 Jul;62(1):59-66.

Schulte T, Sullivan EV, Muller-Oehring EM, Adalsteinsson E, Pfefferbaum A. Corpus callosal microstructural integrity influences interhemispheric processing: a diffusion tensor imaging study. Cereb Cortex. 2005 Sep;15(9):1384-92.

Selnes OA, Vinters HV. Vascular cognitive impairment. Nat Clin Pract Neurol. 2006 Oct 1;2(10):538-47.

Smith CD, Snowdon DA, Wang H, Markesbery WR. White matter volumes and periventricular white matter hyperintensities in aging and dementia. Neurology. 2000 Feb 22;54(4):838-42.

Smith SM. Fast robust automated brain extraction. Hum Brain Mapp. 2002 Nov;17(3):143-55.

Smith SM, De Stefano N, Jenkinson M, Matthews PM. Normalized accurate measurement of longitudinal brain change. J Comput Assist Tomogr. 2001 May-Jun;25(3):466-75.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 2004;23 Suppl 1:S208-19.

Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage. 2002 Sep;17(1):479-89.

Szirmai I, Vastagh I, Szombathelyi E, Kamondi A. Strategic infarcts of the thalamus in vascular dementia. J Neurol Sci. 2002 Nov 15;203-204:91-7.

Tatemichi TK, Desmond DW, Paik M, Figueroa M, Gropen TI, Stern Y, et al. Clinical determinants of dementia related to stroke. Ann Neurol. 1993 Jun 1;33(6):568-75.

Tatemichi TK, Desmond DW, Prohovnik I. Strategic infarcts in vascular dementia. A clinical and brain imaging experience. Arzneimittelforschung. 1995 Mar;45(3A):371-85.

Tatemichi TK, Desmond DW, Prohovnik I, Cross DT, Gropen TI, Mohr JP, et al. Confusion and memory loss from capsular genu infarction: a thalamocortical disconnection syndrome? Neurology. 1992 Oct 1;42(10):1966-79.

Tekin S, Cummings JL. Frontal-subcortical neuronal circuits and clinical neuropsychiatry: an update. J Psychosom Res. 2002 Aug 1;53(2):647-54.

Tombaugh TN. Trail Making Test A and B: normative data stratified by age and education. Arch Clin Neuropsychol. 2004 Mar 1;19(2):203-14.

Troyer AK. Normative data for clustering and switching on verbal fluency tasks. J Clin Exp Neuropsychol. 2000 Jun 1;22(3):370-8.

Turken A, Whitfield-Gabrieli S, Bammer R, Baldo JV, Dronkers NF, Gabrieli JDE. Cognitive processing speed and the structure of white matter pathways: convergent evidence from normal variation and lesion studies. Neuroimage. 2008 Aug 15;42(2):1032-44.

Van der Linen M, Wijns C, Bruyer R, Ansay C, Seron X. Educationnal level and cued recall performance in older and young adults. Psychologica Belgica. 1993(33):37-47.

van Straaten ECW, Scheltens P, Knol DL, van Buchem MA, van Dijk EJ, Hofman PAM, et al. Operational definitions for the NINDS-AIREN criteria for vascular dementia: an interobserver study. Stroke. 2003 Aug 1;34(8):1907-12.

Vermeer SE, Longstreth WT, Koudstaal PJ. Silent brain infarcts: a systematic review. Lancet Neurol. 2007 Jul 1;6(7):611-9.

Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003 Mar 27;348(13):1215-22.

Viswanathan A, Godin O, Jouvent E, O'Sullivan M, Gschwendtner A, Peters N, et al. Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. Neurobiol Aging. 2010 Sep 1;31(9):1629-36.

Viswanathan A, Gschwendtner A, Guichard J-P, Buffon F, Cumurciuc R, O'Sullivan M, et al. Lacunar lesions are independently associated with disability and cognitive impairment in CADASIL. Neurology. 2007 Jul 10;69(2):172-9.

Viswanathan A, Guichard JP, Gschwendtner A, Buffon F, Cumurcuic R, Boutron C, et al. Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. Brain. 2006 Sep;129(Pt 9):2375-83.

Voineskos AN, Rajji TK, Lobaugh NJ, Miranda D, Shenton ME, Kennedy JL, et al. Age-related decline in white matter tract integrity and cognitive performance: A DTI tractography and structural equation modeling study. Neurobiol Aging. 2010 Apr 1.

Wechsler D. Wechsler Adult Intelligence Scale, German Version (WIE). Instructions Manual Pearson Assessment, The Psychological Corporation; 2006.

Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, et al. Bayesian analysis of neuroimaging data in FSL. Neuroimage. 2009 Mar;45(1 Suppl):S173-86.

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

Nikola Zieren[a,1] Marco Duering[a,1], Nils Peters[b], Sonia Reyes[c], Eric Jouvent[c], Dominique Hervé[c], Andreas Gschwendtner[a], Yvonne Mewald[a], Christian Opherk[a], Hugues Chabriat[c] and Martin Dichgans[a,*]

[a] Institute for Stroke and Dementia Research, Medical Centre, Ludwig-Maximilians-University, Marchioninistr. 15, 81377 Munich, Germany

[b] Department of Neurology, Medical Centre, Ludwig-Maximilians-University, Marchioninistr. 15, 81377 Munich, Germany

[c] Department of Neurology, AP-HP, Lariboisiere Hospital, 2 rue Ambroise Paree,
75010 Paris, France

[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.

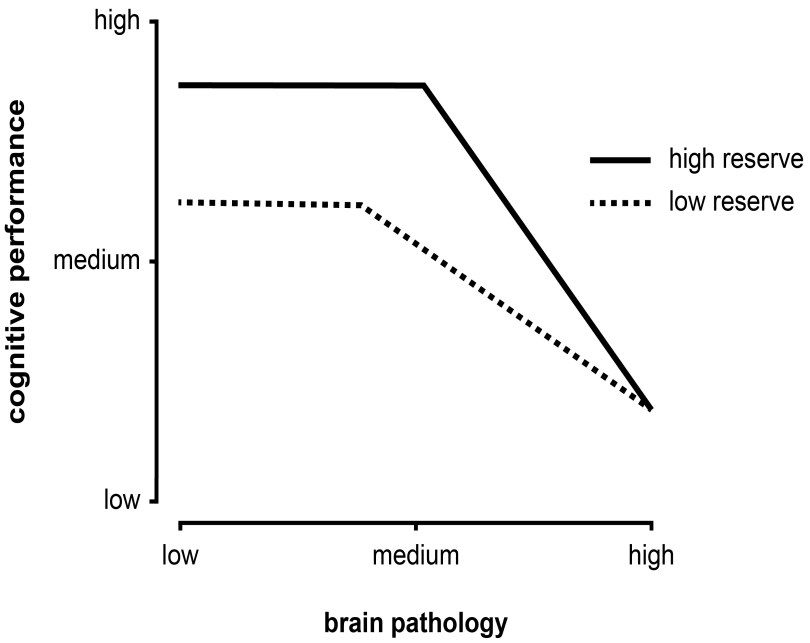

Figure 1: The cognitive reserve model (adapted from Stern, 2009)
The illustration shows the proposed slope of cognitive function with increasing brain pathology, depending on the level of cognitive reserve. Patients with higher cognitive reserve show better cognitive performance than patients with less 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 *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, *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.

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

		Education	
	Total (n=247)	**≤ 10 years (n=117)**	**> 10 years (n=130)**
Mean age [years] (range)	49.6 (24-75)	51.1 (24-75)	48.1 (27-72)
Female sex, n (%)	139 (56.3)	66 (56.4)	73 (56.2)
Clinical scores			
MDRS, median (IQR)	141 (8)	140 (10)	142 (4)
mRS, median (IQR)	0 (1)	0 (1)	0 (1)
MADRS, median (IQR)	7 (10)	7 (11)	7 (9)
Vascular risk factors			
Hypertension, n (%)	49 (19.8)	30 (25.6)	19 (14.6)
Hypercholesterolemia, n (%)	90 (36.4)	55 (47.0)	35 (26.9)
Smoking*, n (%)	138 (55.9)	63 (53.8)	75 (57.7)
Imaging characteristics			
BPF [%], median (IQR)	82.8 (6.79)	81.8 (6.44)	83.7 (6.704)
nLLV [%], median (IQR)	0.065 (0.0023)	0.095 (0.0031)	0.038 (0.0018)
nWMHV [%], median (IQR)	6.06 (7.38)	6.57 (7.13)	5.47 (7.84)

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

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

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.

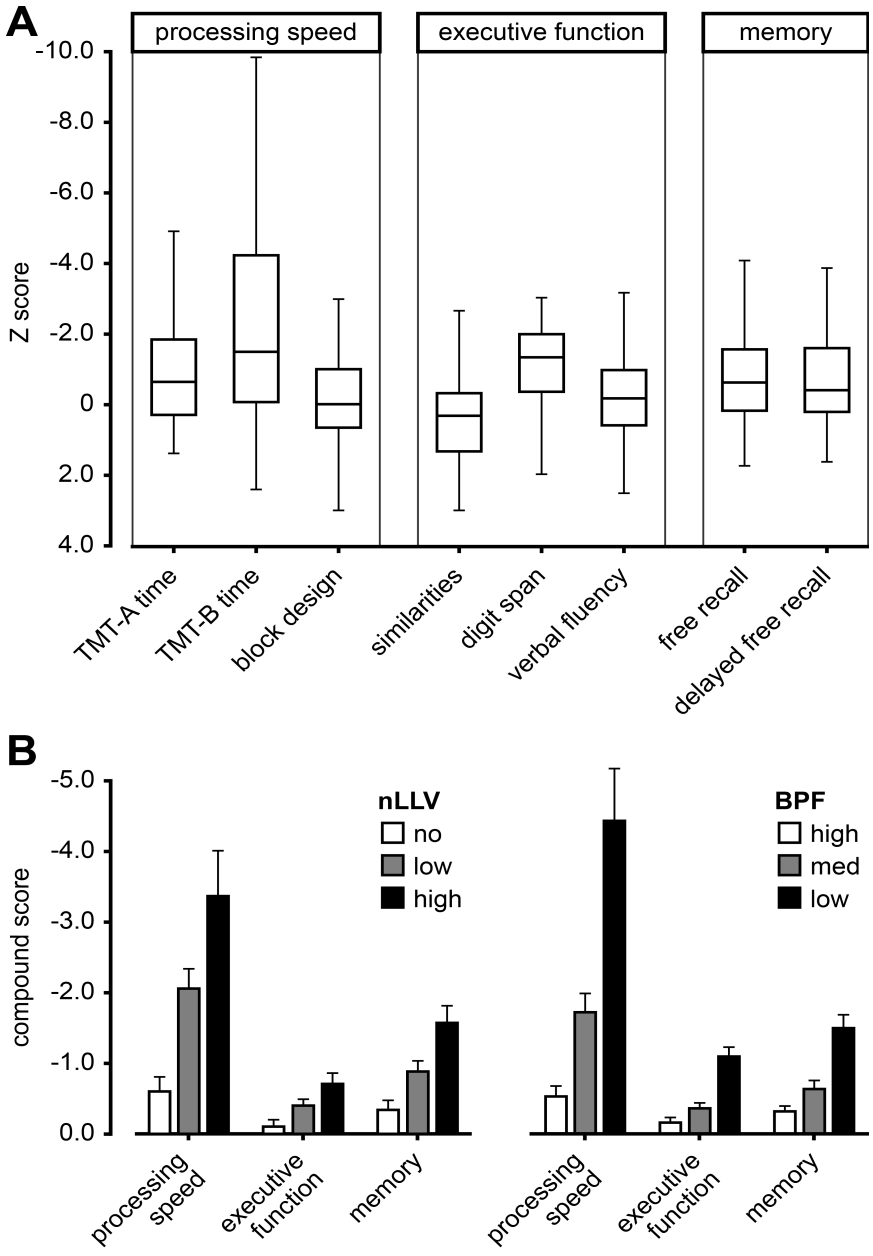

Figure 2: *Cognitive profiles of the patient sample*
(A) Profile of neuropsychological performance in the 247 patients expressed by Z scores with healthy controls as a reference (boxplots are shown without outliers). The three components derived by principal components analysis are depicted on top. The 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.

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.

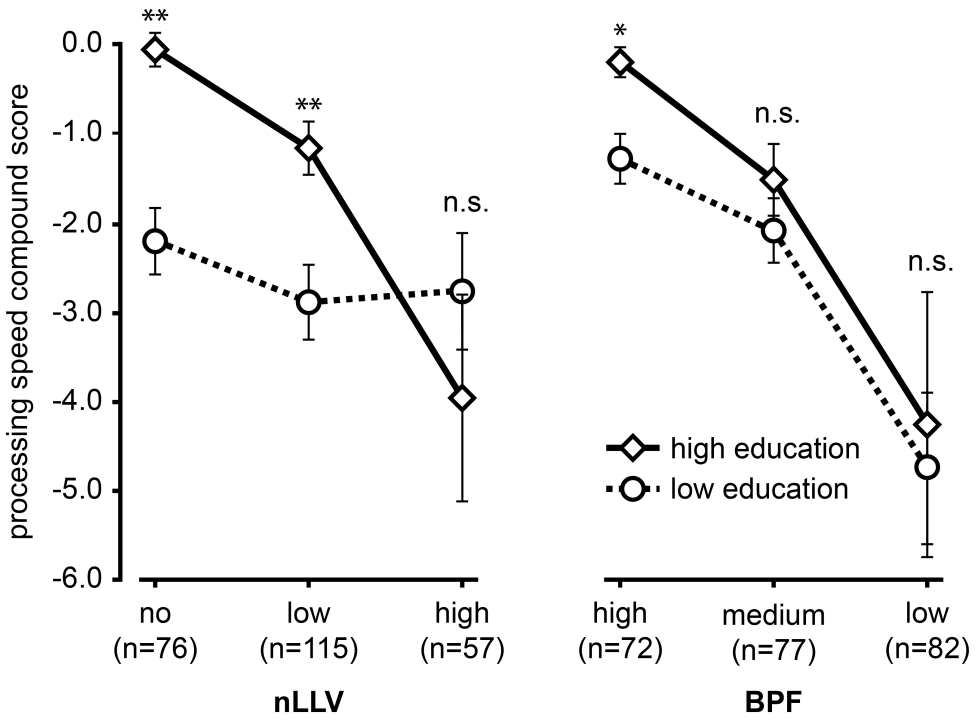

Figure 3. Effects of the interaction between educational level and pathology on processing speed and executive function
*Processing speed performance (mean score ± standard error) in the two educational groups at different levels of brain pathology. Patients with higher education perform better with 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 R^2=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.

3.5 References

Bennett, D.A., Wilson, R.S., Schneider, J.A., Evans, D.A., Mendes de Leon, C.F., Arnold, S.E., Barnes, L.L., Bienias, J.L. 2003. Education modifies the relation of AD pathology to level of cognitive function in older persons. Neurology 60(12), 1909-15.

Brickman, A.M., Siedlecki, K.L., Muraskin, J., Manly, J.J., Luchsinger, J.A., Yeung, L.K., Brown, T.R., Decarli, C., Stern, Y. 2009. White matter hyperintensities and cognition: Testing the reserve hypothesis. Neurobiol Aging.

Buckner, R.L. 2004. Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron 44(1), 195-208.

Chabriat, H., Joutel, A., Dichgans, M., Tournier-Lasserve, E., Bousser, M.G. 2009. Cadasil. Lancet Neurol 8(7), 643-53.

Dichgans, M. 2009. Cognition in CADASIL. Stroke 40(3 Suppl), S45-7.

Dichgans, M., Markus, H.S., Salloway, S., Verkkoniemi, A., Moline, M., Wang, Q., Posner, H., Chabriat, H.S. 2008. Donepezil in patients with subcortical vascular cognitive impairment: a randomised double-blind trial in CADASIL. Lancet Neurol 7(4), 310-8.

Duering, M., Zieren, N., Herve, D., Jouvent, E., Reyes, S., Peters, N., Pachai, C., Opherk, C., Chabriat, H., Dichgans, M. 2011. Strategic role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL. Brain 134(Pt 8), 2366-75.

Elkins, J.S., Longstreth, W.T., Jr., Manolio, T.A., Newman, A.B., Bhadelia, R.A., Johnston, S.C. 2006. Education and the cognitive decline associated with MRI-defined brain infarct. Neurology 67(3), 435-40.

Fritsch, T., McClendon, M.J., Smyth, K.A., Lerner, A.J., Friedland, R.P., Larsen, J.D. 2007. Cognitive functioning in healthy aging: the role of reserve and lifestyle factors early in life. Gerontologist 47(3), 307-22.

Heyman, A., Fillenbaum, G.G., Welsh-Bohmer, K.A., Gearing, M., Mirra, S.S., Mohs, R.C., Peterson, B.L., Pieper, C.F. 1998. Cerebral infarcts in patients with autopsy-proven Alzheimer's disease: CERAD, part XVIII. Consortium to Establish a Registry for Alzheimer's Disease. Neurology 51(1), 159-62.

Hsu, J.L., Chen, Y.L., Leu, J.G., Jaw, F.S., Lee, C.H., Tsai, Y.F., Hsu, C.Y., Bai, C.H., Leemans, A. 2012. Microstructural white matter abnormalities in type 2 diabetes mellitus: a diffusion tensor imaging study. Neuroimage 59(2), 1098-105.

Jagust, W. 2001. Untangling vascular dementia. Lancet 358(9299), 2097-8.

Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriat, H., Mouton, P., Alamowitch, S., Domenga, V., Cecillion, M., Marechal, E., Maciazek, J., Vayssiere, C., Cruaud, C., Cabanis, E.A., Ruchoux, M.M., Weissenbach, J., Bach, J.F., Bousser, M.G., Tournier-Lasserve, E. 1996. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 383(6602), 707-10.

Kemppainen, N.M., Aalto, S., Karrasch, M., Nagren, K., Savisto, N., Oikonen, V., Viitanen, M., Parkkola, R., Rinne, J.O. 2008. Cognitive reserve hypothesis: Pittsburgh Compound B and fluorodeoxyglucose positron emission tomography in relation to education in mild Alzheimer's disease. Ann Neurol 63(1), 112-8.

Kesler, S.R., Adams, H.F., Blasey, C.M., Bigler, E.D. 2003. Premorbid intellectual functioning, education, and brain size in traumatic brain injury: an investigation of the cognitive reserve hypothesis. Appl Neuropsychol 10(3), 153-62.

McGurn, B., Deary, I.J., Starr, J.M. 2008. Childhood cognitive ability and risk of late-onset Alzheimer and vascular dementia. Neurology 71(14), 1051-6.

Ngandu, T., von Strauss, E., Helkala, E.L., Winblad, B., Nissinen, A., Tuomilehto, J., Soininen, H., Kivipelto, M. 2007. Education and dementia: what lies behind the association? Neurology 69(14), 1442-50.

O'Brien, J.T., Erkinjuntti, T., Reisberg, B., Roman, G., Sawada, T., Pantoni, L., Bowler, J.V., Ballard, C., DeCarli, C., Gorelick, P.B., Rockwood, K., Burns, A., Gauthier, S., DeKosky, S.T. 2003. Vascular cognitive impairment. Lancet Neurol 2(2), 89-98.

O'Connor, D.W., Pollitt, P.A., Treasure, F.P. 1991. The influence of education and social class on the diagnosis of dementia in a community population. Psychol Med 21(1), 219-24.

Peters, N., Holtmannspötter, M., Opherk, C., Gschwendtner, A., Herzog, J., Samann, P., Dichgans, M. 2006. Brain volume changes in CADASIL: a serial MRI study in pure subcortical ischemic vascular disease. Neurology 66(10), 1517-22.

Peters, N., Opherk, C., Danek, A., Ballard, C., Herzog, J., Dichgans, M. 2005. The pattern of cognitive performance in CADASIL: a monogenic condition leading to subcortical ischemic vascular dementia. Am J Psychiatry 162(11), 2078-85.

Roe, C.M., Xiong, C., Miller, J.P., Morris, J.C. 2007. Education and Alzheimer disease without dementia: support for the cognitive reserve hypothesis. Neurology 68(3), 223-8.

Roman, G.C., Erkinjuntti, T., Wallin, A., Pantoni, L., Chui, H.C. 2002. Subcortical ischaemic vascular dementia. Lancet Neurol 1(7), 426-36.

Sarnacki, R. 1979. An Examination of Test-Wiseness In the Cognitive Test Domain. Review of Educational Research 49(2), 27.

Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E.,

Niazy, R.K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J.M., Matthews, P.M. 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23 Suppl 1, S208-19.

Smith, S.M., Zhang, Y., Jenkinson, M., Chen, J., Matthews, P.M., Federico, A., De Stefano, N. 2002. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage 17(1), 479-89.

Stern, Y. 2002. What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc 8(3), 448-60.

Stern, Y. 2009. Cognitive reserve. Neuropsychologia 47(10), 2015-28.

Stern, Y., Albert, S., Tang, M.X., Tsai, W.Y. 1999. Rate of memory decline in AD is related to education and occupation: cognitive reserve? Neurology 53(9), 1942-7.

Stern, Y., Habeck, C., Moeller, J., Scarmeas, N., Anderson, K.E., Hilton, H.J., Flynn, J., Sackeim, H., van Heertum, R. 2005. Brain networks associated with cognitive reserve in healthy young and old adults. Cereb Cortex 15(4), 394-402.

Stevens, T., Livingston, G., Kitchen, G., Manela, M., Walker, Z., Katona, C. 2002. Islington study of dementia subtypes in the community. Br J Psychiatry 180, 270-6.

Tewes, U. 2006. Wechsler Intelligenztest für Erwachsene (WIE). Instructions Manual Pearson Assessment, The Psychological Corporation.

Tombaugh, T.N. 2004. Trail Making Test A and B: normative data stratified by age and education. Arch Clin Neuropsychol 19(2), 203-14.

Troyer, A.K. 2000. Normative data for clustering and switching on verbal fluency tasks. J Clin Exp Neuropsychol 22(3), 370-8.

van der Linden M., B.R., Ansay C., Seron X. 1993. Educational level and cued recall performance in older and young adults. Psychologica Belgica 33, 37-47.

van Elderen, S.G., de Roos, A., de Craen, A.J., Westendorp, R.G., Blauw, G.J., Jukema, J.W., Bollen, E.L., Middelkoop, H.A., van Buchem, M.A., van der Grond, J. 2010. Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. Neurology 75(11), 997-1002.

Viswanathan, A., Godin, O., Jouvent, E., O'Sullivan, M., Gschwendtner, A., Peters, N., Duering, M., Guichard, J.P., Holtmannspötter, M., Dufouil, C., Pachai, C., Bousser, M.G., Dichgans, M., Chabriat, H. 2010. Impact of MRI markers in subcortical vascular dementia: a multi-modal analysis in CADASIL. Neurobiol Aging 31(9), 1629-36.

Viswanathan, A., Gschwendtner, A., Guichard, J.P., Buffon, F., Cumurciuc, R., O'Sullivan, M., Holtmannspötter, M., Pachai, C., Bousser, M.G., Dichgans, M., Chabriat, H. 2007. Lacunar lesions are independently associated with disability and cognitive impairment in CADASIL. Neurology 69(2), 172-9.

Viswanathan, A., Guichard, J.P., Gschwendtner, A., Buffon, F., Cumurcuic, R., Boutron, C., Vicaut, E., Holtmannspötter, M., Pachai, C., Bousser, M.G., Dichgans, M., Chabriat, H. 2006. Blood pressure and haemoglobin A1c are associated with microhaemorrhage in CADASIL: a two-centre cohort study. Brain 129(Pt 9), 2375-83.