Aus dem Institut/der Klinik für Geriatrie

der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Die funktionelle Charakterisierung genomweiter Assoziationssignale der Berliner Altersstudie II (BASE-II)

zur Erlangung des akademischen Grades

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1. Zusammenfassung

1.1. Abstract

Die interindividuellen Varianzen kognitiver Funktionen des Menschen (z.B. das episodische Gedächtnis und das Arbeitsgedächtnis) basieren unter anderem auf DNA-Sequenzvarianten. Genetische Assoziationsstudien haben bereits eine große Anzahl an Kandidatengenen entdeckt, dennoch können diese nur einen kleinen Teil der phänotypischen Unterschiede erklären. Diese Studie ist ein Teil einer fortlaufenden genomweiten Assoziationsstudie in Probanden der Berliner Altersstudie II (BASE-II), die sich der Identifizierung von Faktoren des „gesunden" vs. „ungesunden" Alterns verschrieben hat. In dieser Arbeit führten wir eine genomweite Untersuchung an 13 Eigenschaften hinsichtlich des episodischen Gedächtnisses und des Arbeitsgedächtnisses bei 1.318 BASE-II-Teilnehmern in einem Alter von 60 bis 80 Jahren durch. Diese Analysen konnten einige neue Einzelnukleotidpolymorphismen (SNPs) aufdecken, die mit der Gedächtnisleistung assoziiert sind, wie z.B. einem genomweiten Signal für den SNP rs9882688 ($P = 7{,}8 \times 10^{-9}$), welcher in einer potenziellen regulatorischen Region der microRNA (miRNA) hsa-miR-138-5p lokalisiert ist. *Expression quantitative trait locus* (eQTL)-Analysen, basierend auf Next-Generation RNA-Sequenzierungsdaten, konnten zeigen, dass dieser SNP eine signifikante Korrelation mit den Expressionslevel dieser miRNA in 309 menschlichen lymphoblastoiden Zelllinien ($P = 5 \times 10^{-4}$) vorweisen kann. Ein *in silico*-Algorithmus wies bei anderen, im GWAS hoch signifikanten Signalen einen weiteren SNP in der 3' untranslatierten Region (3'UTR) von *DCP1B* auf, dem eine Interaktion mit hsa-miR-138-5p (die mature Form von hsa-miR-138-1) vorhergesagt wurde. Dieses *in silico*-Ergebnis wurde durch *in vitro*-Experimente überprüft, wobei eine Bindung der miRNA an das 3'UTR-Reporterkonstrukt in zwei menschlichen Zelllinien demonstriert werden konnte (HEK293: $P = 0{,}0470$; SH-SY5Y: $P = 0{,}0866$). Durch das Erstellen eines Expressionsprofils von hsa-miR-138-5p und der *DCP1B* mRNA in menschlichem Gehirngewebe (post-mortem), konnte eine gleichzeitige Expression beider Moleküle im Hippocampus und Frontalen Kortex nachgewiesen werden, resultierend in einer Implikation der Interaktion zwischen der miRNA und deren möglichen Bindepartner *in vivo*. Zusammengefasst ist diese Studie durch die Kombination unvoreingenommener genomweiter Screenings, den *in silico*-Vorhersagen, den funktionellen *in vitro*-Assays und den Genexpressionsprofilen in der Lage die miRNA-138 als potenziellen molekularen Regulator der menschlichen Gedächtnisfunktionen zu identifizieren.

Absract

Genetic factors determine a substantial proportion of cognitive functions in humans, including processes related to episodic and working memory. While genetic association studies have proposed a number of candidate "memory genes" these currently explain only a minor fraction of the phenotypic variance. This work is part of an ongoing genome-wide association study (GWAS) in participants of the Berlin Aging Study II (BASE-II), a project aimed at deciphering factors related to "healthy" and "unhealthy" aging. Specifically, we performed genome-wide screening on 13 episodic and working memory traits in 1,318 BASE-II participants aged 60 years or older. These analyses highlighted a number of novel single nucleotide polymorphisms (SNPs) associated with memory performance, including one genome-wide significant marker (rs9882688, P-value = $7.8x10^{-9}$) located in a putative regulatory region of micro-RNA (miRNA) hsa-mir-138-1. Expression quantitative trait locus (eQTL) analyses on next-generation RNA-sequencing data revealed that genotypes at this SNP show a significant correlation with the expression levels of this miRNA in 309 human lymphoblastoid cell lines (P-value = $5x10^{-4}$). *In silico* modeling of other top-ranking GWAS signals pinpointed an additional memory-associated SNP in the 3' untranslated region (3'UTR) of *DCP1B* predicted to interfere with hsa-mir-138-5p (i.e. the mature form of hsa-mir-138-1) binding. These *in silico* predictions were confirmed *in vitro* by luciferase assays showing differential binding of this miRNA to 3'UTR reporter constructs in two human cell lines (HEK293: P-value = 0.0470; SH-SY5Y: P-value = 0.0866). Finally, expression profiling of hsa-mir-138-5p and *DCP1B* mRNA in human post-mortem brain tissue revealed that both molecules are expressed simultaneously in frontal cortex as well as hippocampus, suggesting that the proposed interaction between hsa-mir-138-5p and *DCP1B* may also take place *in vivo*. In summary, by combining unbiased genome-wide screening with extensive *in silico* modeling, *in vitro* functional assays, and gene expression profiling our study identified miRNA-138 as a potential molecular regulator of human memory function.

1.2. Einführung

Die Varianzen der interindividuellen Gedächtnisleistung des Menschen werden durch genetische, sowie nicht-genetische Faktoren entscheidend beeinflusst. Kognitive Eigenschaften werden zu großen Teilen vererbt und der genetische Einfluss nimmt über den Lebenszeitraum zu (McClearn *et al.* 1997). Verschiedene Kandidatengene mit einem Effekt auf das menschliche Gedächtnis sind bereits identifiziert worden (für eine aktuelle Übersicht: URL: http://www.genome.gov/gwastudies/), wie z.B. das Gen *WWC1* (WW and C2 containing 1, auch *KIBRA* für kidney and brain expressed protein; Chromosom 5q34; Papassotiropoulos *et al.* 2006) oder *CTNNBL1* (catenin, beta like 1). Ein Einzelnukleotidpolymorphismus (*single nucleotide polymorphism* [SNP]; rs17070145) in einem Intron des Gens *WWC1* zeigte in einer genomweiten Assoziationsstudie (GWAS) einen starken Einfluss auf das episodische Gedächtnis (Papassotiropoulos *et al.* 2006).

Weitere experimentelle Ansätze stützen die Annahme, dass die menschliche Gedächtnisleistung und andere kognitive Fähigkeiten durch microRNAs (miRNAs) beeinflusst sein könnten. MiRNAs sind kleine (18 – 24 nt), nicht-kodierende RNA-Moleküle, die in die post-transkriptionelle Regulierung der Proteinexpression involviert sind. Hierbei binden sie an die Boten-RNA (*messenger RNA* [mRNA]) und inhibieren dadurch direkt oder indirekt die mRNA-Translation. MiRNAs sind in Zellfunktionen wie Entwicklung, Differenzierung, Proliferation, Apoptose, und Metabolismus der Neuronen und anderen Zellen des menschlichen Organismus involviert (Satoh 2012). Mehr als 2.500 miRNAs (URL: http://mirbase.org/index.shtml) sind derzeit bekannt. Ein wichtiger Faktor der miRNA-vermittelten Genexpressionsregulierung ist die Bindungsaffinität zwischen der miRNA und ihrer Ziel-mRNA, die aufgrund der Sequenzkomplemetarität zwischen den interagierenden Molekülen stark beeinflusst ist (Peterson *et al.* 2014). Aus diesem Grund können natürlich auftretende DNA-Sequenzvarianten, wie die bereits erwähnten SNPs, innerhalb dieser Bindesequenzen durch Erhöhung, respektive Erniedrigung der Bindeaffinität interagieren.

Das Ziel dieser Arbeit besteht darin, gedächtnisassoziierter SNPs zu entdecken, die ihre Wirkung auf den Phänotyp durch einen Einfluss auf die miRNA-mRNA-Bindung ausüben. Hierbei kommt eine Methodik zum Einsatz, die bereits durch unsere Arbeitsgruppe beschrieben wurde (Lill *et al.* 2014). Mit Hilfe eines GWAS in Probanden der Berliner Altersstudie II (BASE-II) sind genetische Assoziationen mit dem Arbeitsgedächtnis, beziehungsweise dem episodischen Gedächtnis identifiziert worden. Diese assoziierten Signale wurden hinsichtlich ihrer Involvierung in die miRNA-vermittelte

Proteinexpressionsregulierung *in silico* untersucht, wobei ein bioinformatischer Vorhersagealgorithmus, entwickelt in unserer Arbeitsgruppe (Schilling 2011, Schilling 2013), eingesetzt worden ist. Die gedächtnisassoziierten SNPs, die einerseits einen prognostizierten Einfluss auf die miRNA-mRNA-Bindung vorweisen, wurden anschließend *in vitro* durch Renillaluciferase-Assays in zwei unterschiedlichen menschlichen Zelllinien validiert und im Anschluss ein Expressionsprofil der beteiligten RNA-Moleküle in menschlichen Gehirnautopsiegeweben von drei Erwachsenen, die nicht der BASE-II-Studie entstammen, erstellt.

1.3. Methodik

1.3.1. Genomweite Assoziationsstudie (GWAS) des Arbeits- und des episodischen Gedächtnisses

Probanden. Die Probanden des GWAS entstammen der BASE-II, einer multidisziplinären Studie, deren Aufgabe in der Untersuchung der Ursachen für „gesundes“ vs. „ungesundes“ Altern besteht. Der Fokus beruht hierbei auf den Schwerpunkten Genetik, Innere Medizin, Immunologie, Psychologie, Soziologie und Ökonomie (Bertram *et al.* 2013). Bis dato nehmen 2.200 Probanden aus dem Großraum Berlin an dieser Studie teil, die sich aus 1.600 älteren (60 – 80 Jahre, durchschn. 66,76 Jahre) und 600 jüngeren (20 – 35 Jahre, durchschn. 27,32 Jahre) Testpersonen zusammensetzt. Beide Gruppen enthalten die gleiche Anzahl an Frauen und Männer. Die hier präsentierten Analysen basieren auf der älteren Kohorte (n = 1.318), aufgrund der zum Analysezeitpunkt noch fehlenden Erhebungen aller implizierten Gedächtnistests oder Genotypisierungen. Eine Einverständniserklärung wurde von jedem Probanden unterzeichnet und die Ethikkommissionen der beteiligten Institutionen überprüften diese Studie.

Messung der Gedächtnisleistung. Diese Studie basiert auf 13 quantitativen Messungen (Tabelle 1) zur Erfassung der Leistungen des Arbeitsgedächtnisses (n = 2) und des episodischen Gedächtnisses (n = 11). Diese Tests wurden in der Abteilung Entwicklungspsychologie am Max-Planck-Institut für Bildungsforschung, respektive in der Forschungsgruppe Geriatrie der Charité – Universitätsmedizin Berlin durchgeführt.

Genotypisierung und genetische Assoziationsanalysen. Die DNA der Probanden wurde aus dem Blut unter Verwendung von Standardmethoden gewonnen. Die Genotypisierung erfolgte unter Einsatz des „Genome-Wide Human SNP Array 6.0“ (Fa. Affymetrix, Santa Clara, CA, USA) extern in den Laboren von Atlas Biolabs (Berlin, Deutschland), aufgrund der dort bestehenden technischen Voraussetzungen. Insgesamt sind 12.607.232 hoch qualitative SNPs für die genetische Assoziation mit den 13 Gedächtnistests der 1.318 Probanden (60 – 80 Jahre) inkludiert worden.

Tabelle 1: Zusammenfassung der durch die genomweite Assoziationsstudie erfassten kognitiven Messungen/ Eigenschaften

Domäne	**Test (Abk.)**	**Beschreibung**
WM	TCSWM	Der T-Score des spatialen Arbeitsgedächtnisses
WM	TFEUWC	Der T-Score des spatialen Arbeitsgedächtnisses kombiniert mit Tests zur Erfassung der frontalen exekutiven Kontrolle (hier: Wisconsin Card Sorting Test)
EM	CERAD_WL_learn	CERAD-Batterie: Wordliste Lernen
EM	CERAD_WL_recall	CERAD-Batterie: Wordliste Wiedergabe
EM	CERAD_WL_save	Verhältnis der gelernten Wörter vs. der wiedergegebenen Wörter (max. 100%)
EM	Item_Item	Item-Instruktion & Item-Test (Verhältnis der erfolgreichen minus der falschen Antworten)
EM	Item_Pair	Item-Instruktion & assoziativer Test (Verhältnis der erfolgreichen minus der falschen Antworten)
EM	Pair_Pair	Paar-Instruktionen & assoziativer Test (Verhältnis der erfolgreichen minus der falschen Antworten)
EM	SRFO	„Serial recall" (fortlaufende Wiedergabe): Genauigkeit der Wiedergabe (vorwärts)
EM	SRBK	„Serial recall" (fortlaufende Wiedergabe): Genauigkeit der Wiedergabe (rückwärts)
EM	SRFOBK	„Serial recall" (fortlaufende Wiedergabe): Kombination der Wiedergabe vorwärts und rückwärts
EM	Delayed_recall	Bilderkennung nach 2,5 Stunden (Verhältnis der erfolgreichen minus der falschen Antworten)
EM	Delayed_recall	Bilderkennung nach einer Woche (Verhältnis der erfolgreichen minus der falschen Antworten)

Abkürzungen: WM = Arbeitsgedächtnis (engl.: *working memory*); EM = episodisches Gedächtnis (engl.: *episodic memory*); CERAD = Consortium to Establish a Registry for Alzheimer´s Disease.

1.3.2. Die *in silico*-Vorhersagen der miRNA-mRNA-Bindung und potenzielle SNP-Effekte

Potenzielle SNP-Effekte auf die miRNA-mRNA-Interaktion wurden durch einen bioinformatischen Algorithmus, der in unserer Arbeitsgruppe entwickelt wurde (Schilling 2011, Schilling 2013), prognostiziert. Die möglichen miRNA-Bindestellen auf den 3'UTRs

aller bekannten protein-kodierender Transkripte (http://www.ensembl.org/biomart/martview) entstammen den Programmen miRanda v.3.38, TargetScan 5.09, PITA10 und mirBASE v18 (http://www.mirbase.org).

1.3.3. *In vitro*-Validierung gedächtnisassoziierter SNPs mit potenziellem Einfluss auf die miRNA-mRNA-Bindung

Zellkultur und Transfektion. Speziell angefertigte Reporterplasmide (pLightSwitch_3UTR) mit den erwünschten 3'UTR-Sequenzen sind zusammen mit den miRNA mimics durch SwitchGear Genomics (Menlo Park, CA, USA) wie folgt hergestellt worden: Die 3'UTR wurde in den Vektor stromabwärts des Renillaluciferasegens *RenSP* subkloniert. Die UTR-Sequenz mit dem Referenzallel des jeweiligen SNPs wurde als Template genutzt, um eine Punktmutation durch zielgerichtete Mutagenese zu erhalten. Die Kontrolle der jeweiligen Sequenzen erfolgte durch Sanger-Sequenzierung. Die 3'UTR-Konstrukte sind in naïven menschlichen Nierenzellen (HEK293) und menschlichen Neuroblastomazellen (SH-SY5Y) transfiziert worden, die in DMEM GlutaMax (Invitrogen, Darmstadt, Deutschland) mit 10% FBS (Biochrom, Berlin, Germany) für HEK293 oder 15% FBS für SH-SY5Y und 1% Penicillin/Streptomycin (Biochrom) unter Standardbedingungen (37°C, 5% CO_2) kultiviert wurden. Die Transfektion erfolgte in 96-well Platten (TPP, Trasadingen, Schweiz) bei einer Zellkonfluenz von etwa 50% unter der Verwendung von Dharmafect (ThermoScientific) und unter Einhaltung des gegebenen Protokolls. 50ng des 3'UTR-Konstrukts und 50nM der korrespondierenden miRNA mimic oder einer nicht-bindenden RNA-Negativkontrolle wurden in den Zellen ko-transfiziert. Nach 24 Stunden erfolgte ein Einfrieren der Zellen auf Trockeneis zur Verstärkung der Zelllyse und ein anschließender Auftauprozess auf Eis. Die LightSwitch Assay Reagenz (SwitchGear Genomics) ist unter Einhaltung der Herstellerangeben verwendet und mit dem gleichen Volumen an Zelllysat auf eine weiße 96-well Platte (Costar, Washington, D.C., USA) überführt worden. Die Endpunktmessung der Renillaluciferaseintensität ereignete sich an dem POLARStar Omega (BMG Labtech, Ortenburg, Deutschland) –Plattenlesegerät mit einer Integrationszeit von 3 s und 3.500 Gain/well. Für jedes unabhängige Experiment (n = 5 - 7) wurden sechs Replikate durchgeführt.

Statistische Auswertung der in vitro Experimente. Für die Analyse der Daten kam R, eine frei erhältliche Computersprache und eine Umgebung für statistische Programme und Grafiken, zum Einsatz (URL: http://www.r-project.org). Für jedes unabhängige Experiment

wurde der Mittelwert der Luciferaseintensität des 3'UTR-Reporterkonstrukts (mit Referenz- oder Alternativallel), ko-transfiziert mit der funktionellen miRNA durch den Basis-Mittelwert der Luciferaseaktivität des gleichen Reporters, ko-transfiziert mit der miRNA-Negativkontrolle, geteilt. Für die Erfassung der jeweiligen miRNA-Bindung, unabhängig des Allelstatus, wurde die Renillaluciferase-Expression mit der Basis-Expression unter Verwendung des Einstichproben-t-Tests verglichen und Änderungen in der Renillaluciferase-Expression der Konstrukte mit dem Referenz- oder Alternativallel mittels t-Test-Statistik zweier unabhängiger Proben ermittelt.

1.3.4. Die Analyse der microRNA- und mRNA-Expression in menschlichem Gehirngewebe

Gewebe und RNA-Extraktion. Das menschliche Gehirngewebe wurde post-mortem aus den Hippocampi, respektive Frontalen Kortizes dreier verstorbener Erwachsener gewonnen, die nicht der BASE-II-Studie angehören und bei denen keine neuropsychiatrische Krankheitsgeschichte detektiert wurde. Die Entnahme fand an der Charité – Universitätsmedizin Berlin statt. Anschließend sind die Gehirnproben in RNA*later*® (Applied Biosystems, Forster City, CA, USA) bei -20°C zur Vermeidung der RNA-Degradierung gelagert worden. Für die Extraktion der kleinen und der totalen RNA kam das miRVANA™ miRNA Isolation Kit (Life Technologies, Darmstadt, Deutschland) unter Einhaltung der Herstellerangaben zum Einsatz. Vor der Extraktion wurden die Gewebeproben durch den TissueLyser (QIAgen, Hilden, Deutschland) durch zweimaliges Schütteln in Lysis/Binding-Puffer für 2 min bei 20Hz homogenisiert.

Untersuchung der miRNA-Expressionslevel. Die Quantifizierung der miRNA umfasst zwei Schritte: (1) die Reverse Transkription (RT), wobei aus der RNA eine cDNA synthetisiert wird und (2) die quantitative PCR (qPCR). Spezifische Primer für die RT und die qPCR basieren auf vorgefertigten TaqMan® Small RNA Assays (Applied Biosystems). Für die RT wurde das TaqMan® MiRNA Reverse Transcription Kit (Applied Biosystems) unter Einhaltung der Herstellerangaben und 10ng RNA verwendet. Die qPCR erfolgte mittels TaqMan® Small RNA Assay unter Durchführung des beigelegten Protokolls. Die Realisierung und Visualisierung der Quantifizierung der miRNAs aus menschlichem Gehirngewebe geschah unter Inanspruchnahme des QuantStudio™ 12K Flex Real-Time PCR Systems (Applied Biosystems).

Untersuchung der mRNA Expression. Die RT erfolgte unter Verwendung des High Capacity RNA-to-cDNA Kits (Applied Biosystems) mit 0,2µg der totalen RNA und unter Einhaltung der Herstellerangaben in dem Thermo Cycler PTC-240 (MJ Research, Waltham, MA, USA). Die Expression der resultierenden *DCP1B* cDNA wurde durch eine PCR unter Verwendung der folgenden Primer durchgeführt: 5'-CCAGGGTCTCCTCACAACAT-3' (forward) und 5'-TCTTTTTCATGGCTGCTTGA-3' (reverse). Die Primer sind so entworfen worden, dass ein etwa 850bp langes cDNA-Amplikon und ein etwa 6,9kb langes gDNA-Amplikon entsteht. Für die Reaktion wurden 1,5µM jedes Primers, 60ng der cDNA oder gDNA als Kontrolle, 0,25mM dNTPs, 10mM $MgCl_2$, 30% Q solution (QIAgen) und 0,25U Taq-Polymerase in einem Endvolumen von 10µl eingesetzt. Die Reaktionen erfolgten in 96-well Platten in dem PTC-240 bei folgenden Parametern: 94°C (3 min), gefolgt von 40 Zyklen bei 94°C (45 s), 60,5°C (90 s), 72°C (60 s) und einem finalen Extensionsschritt bei 72°C (6 min). Die PCR Produkte wurden auf einem Ethidiumbromid-markierten 1%igen Agarosegel (Sigma Aldrich, Taufkirchen, Deutschland) mittels Elektrophorese visualisiert.

1.3.5. Expression quantitative trait locus (eQTL) Analysen der hsa-miR-138-5p unter Verwendung von Next-Generation Sequenzierdaten

eQTL-Analysen auf die potenzielle Rolle von rs9882688 auf die Expression von hsa-miR-138-5p wurden unter Verwendung der Next-Generation Sequenzierungsdaten kleiner RNAs, basierend auf einer lymphoblastoiden Zelllinie (LCL), durchgeführt. Für die verwendeten Analysen sind die normalisierten Expressionsdaten der hsa-miR-138-5p von vier Populationen europäischen Ursprungs der GEUVADIS-Projektdatenbank (URL: http://www.ebi.ac.uk/arrayexpress/files/E-GEUV-2/GD452.MirnaQuantCount.1.2N.50FN.samplename.resk10.txt) entnommen worden. Die Genotypen des SNP rs9882688 für diese Personen entstammen der 1000 Genomes Datenbank (URL: http://browser.1000genomes.org/index.html; Abecasis *et al.* 2010). Der finale Datensatz enthielt 333 europäisch stämmige Individuen mit deren Expressionsdaten von hsa-miR-138-5p und den Genotypen für rs9882688. Die Berechnung statistischer Analysen sind durch das Programm R realisiert worden. 24 Proben sind anschließend als Ausreißer deklariert, wodurch sich eine finale Probengröße von 309 ergab. Die folgenden eQTL-Analysen basierten auf einem additiven Modell unter Verwendung linearer Regression. Assoziationsergebnisse wurden auf Geschlecht und Population angepasst. Aufgrund der geringen Anzahl von G-Allelträgern (n = 2) sind diese aus weiteren Analysen ausgeschlossen. Die statistische Signifikanz dieser Untersuchungen wurde als zweiseitiger P-Wert angegeben.

1.4. Resultate

1.4.1. GWAS der episodischen und Arbeitsgedächtnisleistung im Menschen

Die GWAS-Analysen basieren auf den Ergebnissen von 13 Gedächtnistests (Tabelle 1) der 1.318 BASE-II-Probanden. Es wurden 28 Loci entdeckt, die mit den Testergebnissen assoziiert ($P \leq 1x10^{-6}$) waren. Keiner dieser SNPs ist in und um *WWC1* (*KIBRA*) oder *CTNNBL1* (Liu *et al.* 2014) lokalisiert. Dadurch wird ersichtlich, dass keiner der hier gemessenen Gedächtnistests signifikant durch DNA-Sequenzvarianten innerhalb dieser Gene beeinflusst wird. Drei Polymorphismen präsentierten P-Werte unter $1x10^{-7}$. Rs9882688 zeigte eine Assoziation ($P = 7,8x10^{-9}$ auf Chr. 3p21.32) mit dem Test „WL_Save“, rs1016365 ($P = 9,7x10^{-8}$ auf Chr. 8q13.3) mit dem Test „ItemItem“ und rs113948889 ($P = 9,9x10^{-8}$ auf Chr. 12p13.33) mit „TFEUWC“. Der SNP rs9882688 ist in keinem bekannten ORF (*open reading frame*) und liegt etwa 20kb stromaufwärts der hsa-miR-138-1, wodurch eine mögliche Lage in einem regulatorischen Element der miRNA vermutet wird. Rs1016365 ist etwa 6kb stromabwärts des Gens *EYA1* (Homo sapiens eyes absent homolog 1 [Drosophila]) lokalisiert, dessen Produkt zu der Familie der eye absent (EYA) -Proteine gehört. Rs113948889 befindet sich in einem Intron des Gens *DCP1B* (decapping mRNA 1B), welches eine wichtige Komponente in dem mRNA-Decapping-Komplex, einem Schlüsselfaktor der Regulierung des mRNA-Abbaus, darstellt.

1.4.2. *In silico*-Vorhersagen potenzieller SNP-Effekte auf die miRNA-mRNA-Bindung

Der bioinformatischer Algorithmus, entwickelt in unserer Arbeitsgruppe (Schilling 2011, Schilling 2013), konnte die potenzielle Rolle der gedächtnisassoziierten SNPs auf Chromosom 3, 8 und 12 und die sich mit ihnen in Linkage Disequilibrium (LD; $r^2 \geq 0,5$) befindlichen Varianten innerhalb von ±1Mb auf ihre potenzielle Rolle auf die miRNA-mRNA-Bindung vorhersagen. *In silico* konnte kein Effekt für die SNPs rs9882688 und rs1016365 prognostiziert werden. Alle „miRNA SNPs“ befinden sich in LD mit rs113948889 ($r^2 = 1$) und resultieren in einer möglichen Erhöhung der Proteinexpression bei Vorhandensein des Alternativallels. Der stärkste Effekt wurde für rs112215626 vorhergesagt, einem SNP in der Bindestelle der hsa-miR-4775 auf der 3’UTR von *DCP1B* (Abbildung 1a). Ein weiterer Effekt wurde für rs1044950 kalkuliert, der in der Bindestelle der hsa-miR-138-5p auf der 3’UTR von *DCP1B* lokalisiert ist (Abbildung 1b). Dieser SNP befindet sich ebenfalls in den kodierenden Regionen alternativer Transkripte dieses Gens und führt hier zu einer

Aminosäuresubstitution von Alanin zu Valin im Protein. Dieser Substitution konnte bisher kein signifikanter Einfluss auf die Funktion des Proteins vorhergesagt werden, wodurch die Annahme, dass die stärkere Funktion dieses SNPs in der Beeinflussung der miRNA-mRNA-Interaktion liegen könnte, Bestätigung findet.

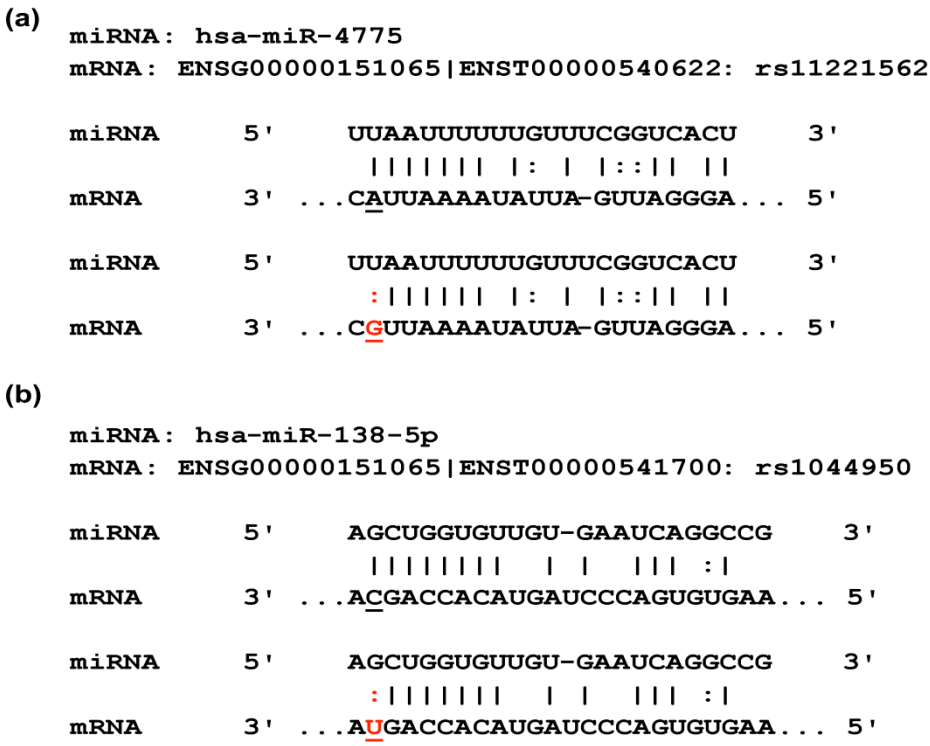

Abbildung 1: Prognostizierte miRNA-Bindestellen der *DCP1B*-Transkripte. *(a)* Rs112215626 ist in der Bindestelle von hsa-miR-4775 auf der *DCP1B* 3'UTR lokalisiert. *(b)* Rs1044950 ist in der Bindestelle von hsa-miR-138-5p auf der *DCP1B* 3'UTR lokalisiert. Den alternativen Allelen (rot markiert) beider SNPs wird ein Effekt auf die DCP1B-Proteinlevel vorhergesagt.

1.4.3. *In vitro*-Untersuchung gedächtnisassoziierter SNPs mit einem potenziellen Effekt auf die miRNA-mRNA-Bindung

Für diese Experimente wurden Renillaluciferase-Reporter-Assays in naïven HEK293- und SH-SY5Y-Zellen genutzt, wobei zwei unterschiedliche 3'UTR-Konstrukte (enthalten das Referenz- oder das Alternativallel) der DCP1B-Transkripte ENST00000540622 und ENST00000541700 zum Einsatz kamen, denen eine Bindung der hsa-miR-4775, respektive hsa-miR-138-5p vorhergesagt werden konnte. Eine Ko-Transfektion der Renilla-Konstrukte mit den hsa-miR-138-5p zeigte einen konsistenten, jedoch nur in den HEK293-Zellen signifikanten Effekt (n = 7; P = 0,0470; Abbildung 2a) auf die normalisierte Renillaluciferase-Expression. In beiden Zelllinien verursachte das Alternativ-(A)-Allel des SNPs rs1044950 im

Vergleich zum Referenz-(G)-Allel eine Erhöhung der Renillaluciferase-Expression um 11,8% (HEK293), respektive 10,5% (SH-SY5Y). Diese Resultate stimmen mit den in silico-Analysen überein, wobei die hsa-miR-138-5p stärker an die 3'UTR-Sequenz mit dem G-Allel, verglichen mit dem A-Allel, bindet. Die Reduzierung der Luciferase-Expression durch die Bindung der hsa-miR-4775 an die *DCP1B* 3'UTR konnte in HEK293-Zellen unabhängig des Genotyps des SNPs rs112215626 nachgewiesen werden, nicht jedoch in SH-SY5Y-Zellen (Abbildung 2b). Der Unterschied des Referenz-(T)-Allels und des Alternativ-(C)-Allels dieses SNPs auf die Bindung der hsa-miR-4775 ist weder in HEK293, noch in SH-SY5Y signifikant (Abbildung 2b). Zusammengefasst unterstützen diese Resultate die These, dass rs1044950 allel-spezifische Effekte auf die Expression der *DCP1B* 3'UTR-Konstrukte in Anwesenheit der hsa-miR-138-5p *in vitro* ausübt.

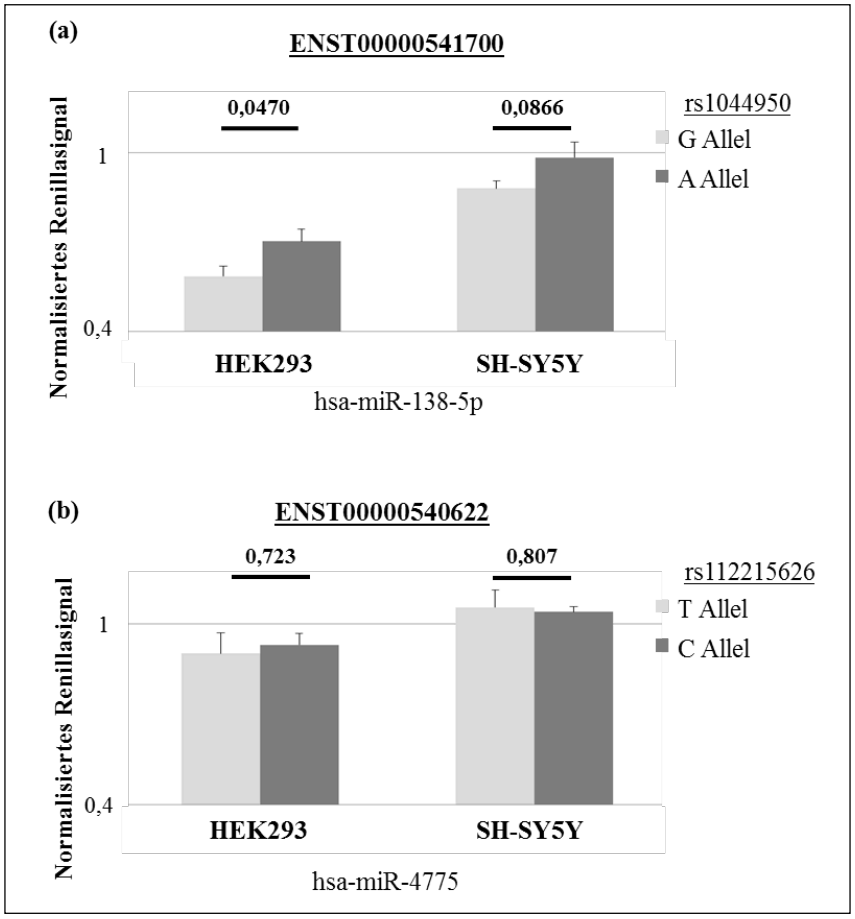

Abbildung 2: Die *in vitro*-Effekte von rs1044950 und rs112215262 auf die miRNA-mRNA-Bindung und die Genexpression. Die Balkendiagramme zeigen die normalisierte Renillaluciferase-Expression in Reporterkonstrukten, die die *DCP1B* 3'UTR und die korrespondierenden SNPs enthalten. Dargestellt sind die Mittelwerte der Renillaluciferasesignale und die Standardfehler in Relation zu der Intensität der Kontrolllucifraseintensitäten (Konstrukte ko-transfiziert mit einer miRNA-Negativkontrolle, entspricht der horizontalen Line): *(a)* Das Transkript ENST00000541700 weißt das Referenz- (G) oder das alternative (A) Allel für rs1044950 auf. Ko-Transfektion mit hsa-miR-138-5p erfolgte in HEK293 und SH-SY5Y. Der relative Mittelwert der Lumineszenz des Konstrukts mit dem G-, bzw. A-Allel liegt bei 0,585 (±0,0335), respektive 0,703 (±0,0417) in HEK293 und bei 0,880 (±0,0256) und 0,985 (±0,0513) in SH-SY5Y. *(b)* Das Transkript ENST00000540622 mit dem Referenz- (T) oder Alternativ- (C) Allel des SNPs rs112215626, wurde mit hsa-miR-4775 in HEK293 und SH-SY5Y ko-transfiziert. Der relative Mittelwert der Luciferaselumineszenz des Konstruktes mit dem T- vs. dem C-Allel liegt bei 0,903 (±0,0692) und 0,931 (±0,0382) bei HEK293 und bei 1,056 (±0,0582) und 1,041 (±0,0185) bei SH-SY5Y.

1.4.4. Die Expressionsanalyse von hsa-miR-138-5p und der *DCP1B* mRNA in menschlichem Gehirngewebe

Die miRNA hsa-miR-138-5p (Landgraf et al. 2007) und die Transkripte von *DCP1B* (URL: http://human.brain-map.org/) wurden bereits in menschlichem Gehirngewebe nachgewiesen. Es bestehen jedoch keine Angaben darüber, ob diese Expression (1) in gedächtnisrelevanten

Regionen (z.B. Hippocampus) und (2) zur gleichen Zeit *in vitro*, wie auch *in vivo* auftreten. Um diese Fragestellung zu beantworten, wurden die Hippocampi und die Frontalen Kortizes post-mortem von drei Probanden entnommen und auf ihre miRNA- und mRNA-Expressionsmuster untersucht. Die qPCR der hsa-miR-138-5p zeigte eine hohe Expression in den Hippocampi und Frontalen Kortizes aller Probanden (Abbildung 3a). Eine semi-quantitative PCR der *DCP1B* mRNA in den gleichen Gehirnproben zeigten weniger klare Ergebnisse (Abbildung 3b). Diese mRNA war lediglich in zwei Probanden nachweisbar und zeigte in den Frontalen Kortizes dieser Probanden höhere Expressionsmuster als in den Hippocampi der jeweils gleichen Personen. Unabhängig der interindividuellen und regionalen Unterschiede in den Expressionen lassen diese Experimente eine Ko-Expression der hsa-miR-138-5p und der *DCP1B* mRNA in menschlichen Hippocampi und Frontalen Kortizes erkennen. Aufgrund dieser Tatsache ist eine Interaktion beider RNAs auch *in vivo* zu implizieren.

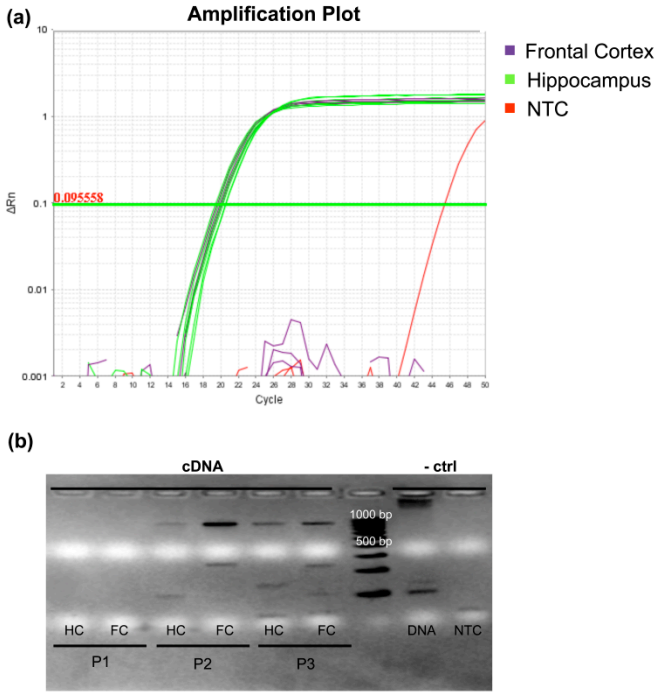

Abbildung 3: Expressionsprofile der hsa-miR-138-5p und *DCP1B* in Gehirnautopsiegeweben dreier verstorbener Probanden. *(a)* Ein Amplifikationsplot der qPCR-Experimente in drei Frontalen Kortizes (markiert in lila) und drei Hippocampi (markiert in grün). Hsa-miR-138-5p wurde in allen Geweben exprimiert (C_T ~19). Eine Negativkontrolle (NTC, markiert in rot)zeigte eine Amplifikation bei einem C_T ~45, während ein zweiter NTC keine Amplifikation aufwies. *(b)* Ethidiumbromid-markiertes 1%iges Agarosegel zeigte semi-quantitative Level der *DCP1B* cDNA (erwartet und visualisiert bei ~850 bp) in menschlichem (post-mortem) Gehirngewebe. Es wurde keine Expression in Proband 1 (P1) nachgewiesen. Die *DCP1B* cDNA-Level waren in den Frontalen Kortizes (FK) in Proband 2 und 3 (P2, P3) höher, als in den Hippocampi (HC). Ein gDNA-Amplikon war sichtbar bei den erwarteten 6,9 kb und keine Bande bei der NTC.

1.4.5. eQTL-Analysen der hsa-miR-138-5p unter Verwendung der Next-Generation Sequenzierdaten

Obwohl dem in dem GWAS führenden SNP (rs9882688) *in silico* kein Effekt auf die miRNA-mRNA-Interaktion vorhergesagt wurde, ist dieser Polymorphismus in einer potenziellen regulatorischen Region etwa 20kb stromaufwärts der primären miRNA hsa-miR-138-1 lokalisiert, deren funktionellen miRNAs (hsa-miR-138-5p und hsa-miR-138-1-3p) im Gehirn exprimiert werden (Landgraf *et al.* 2007). Hsa-miR-138-5p ist hierbei die gleiche miRNA, deren Bindung an die *DCP1B* 3'UTR durch rs1044950 *in silico* und *in vitro* beeinflusst wird. Daher besteht die Möglichkeit, dass rs9882688 durch seinen potenziellen Effekt auf die hsa-miR-138-5p-Expression ein Indikator für eine starke Involvierung dieser miRNA in die menschliche Gedächtnisleistung ist. Aus diesem Grund wurde eine eQTL-Analyse von 309 menschlichen LCLs durchgeführt, die einen signifikanten Effekt des SNPs auf die Expression der miRNA in diesen Zelllinien zeigte. Die Anwesenheit des Alternativ-(G)-Alles war in diesem Fall mit einer Erhöhung der Expression der hsa-miR-138-5p (n = 309; beta = 80,87; Standardfehler [SE] = 23,00; P = 0,000504) assoziiert, ebenso nach Ausschluss der zwei homozygoten G-Allelträgern (n = 307; beta = 77,35; SE = 25,56; P = 0,00270). Diese Resultate zeigen, dass der SNP, der eine Assoziation mit dem menschlichen episodischen Gedächtnis aufweist, eine signifikante Veränderung in der Expression der hsa-miR-138-5p in menschlichen Zelllinien ergeben.

1.5. Diskussion

In dieser Studie wurde die potenzielle Rolle gedächtnisassoziierter SNPs auf die miRNA-vermittelte post-transkriptionelle Regulierung der Genexpression untersucht. Die GWAS-Analysen von 13 Gedächtnistests (Tabelle 1) bei 1.318 Probanden im Alter von 60 bis 80 Jahren zeigten eine Reihe signifikanter Resultate, unter denen zwei SNPs eine potenzielle Involvierung in die Expression und Funktion der hsa-miR-138-5p aufwiesen, einer miRNA die bereits in der menschlichen Gehirnentwicklung und –funktion bekannt ist. Rs9882688 ist das führende GWAS-Signal dieser Studie und präsentierte einen signifikanten Einfluss auf die miRNA-Expression in LCLs. Zusätzlich zu diesem Resultat konnten wir nachweisen, dass rs1044950 (in LD mit einem gedächtnisassoziierten SNP) zu einer allelspezifischen Veränderung der Expression eines Reportergenkonstrukts, welches die *DCP1B* 3'UTR enthält und dem eine Bindestelle der hsa-miR-138-5p vorhergesagt wurde, in HEK293-Zellen führt. Ein Expressionsprofil der hsa-miR-138-5p und der *DCP1B* mRNA bestätigte die Annahme, dass beide RNAs zur gleichen Zeit in dem humanen (post-mortem) Gehirngewebe (Hippocampus und Frontaler Kortex) exprimiert wurden. Zusammengefasst konnten wir durch diese verschiedenen experimentellen Ansätze der hsa-miR-138-5p eine signifikante und bisher unerkannte Rolle in den Funktionen des menschlichen episodischen Gedächtnisses zuschreiben.

Die Daten dieser Studie stimmen mit verschiedenen anderen Projekten überein, bei denen miR-138 eine potenzielle Rolle in den Gehirnfunktionen der Säugetiere spielt. Als Beispiel zeigte Landgraf (Landgraf *et al.* 2007), dass miR-138 in dem Gehirn menschlicher Erwachsener nachweisbar ist. In dieser Studie agiert miR-138 wahrscheinlich durch die Beeinflussung der Aktivität von Antagonisten der Signalübertragung als negativer Regulator für die Größe dendritischer Dornfortsätze. Hsa-miR-138-5p ist laut dem *in silico*-Algorithmus in der Lage an viele weitere Zielgene zu binden. Ein guter Kandidat dieser miRNA ist das Gen *WWC1*, ein Gen mit einer wichtigen Rolle in der Funktion des Säugetiergedächtnisses (Zhang *et al.* 2014).

DCP1B kodiert für das Protein „decapping mRNA 1B", einer wichtigen Komponente des mRNA-Decappingkomplexes, einem Schlüsselfaktor im mRNA-Abbau. Das Decapping und der mRNA-Abbau finden in den P-Bodies (*processing bodies)* statt, die wiederum wichtige Orte für die miRNA-vermittelte mRNA-Degradierung sind. Diese Degradierung ist über die Reduktion des sogenannten DCP1:DCP2-Komplexes gehemmt (Behm-Ansmant *et al.* 2006).

Aus diesem Grund nehmen wir an, dass die Funktion von *DCP1B* und hsa-miR-138-5p innerhalb eines gleichen Reaktionsweges interagieren und so einen wichtigen Einfluss auf die Gedächtnisleistung ausüben könnten.

Obwohl wir in dieser Studie interessante Daten gesammelt haben, die die Hypothese unterstützen, dass hsa-miR-138-5p eine wichtige Rolle in der menschlichen Gedächtnisleistung spielt, zeigt diese Arbeit einige Einschränkungen. Die funktionellen Experimente, die die Rolle von rs1044950 auf die miRNA-mRNA-Bindung untersuchen, basieren auf *in vitro*-Experimenten unter Verwendung von Reporterkonstrukten. Auch wenn wir annehmen, dass diese Effekte auch *in vivo* zutreffen, sind keine direkten experimentellen Kontrollen dieser Hypothese vorhanden. Im Menschen ist eine gewisse Ebene an Untersuchungen nicht möglich, so können viele Experimente aufgrund bis dato fehlender Technologien nicht an lebendem Gehirngewebe durchgeführt werden. Dass die von uns angenommenen Effekte auch *in vivo* statt finden, müssen beide Bindepartner in dem gleichen Gewebe zur gleichen Zeit exprimiert sein. Dieser Fall trifft auf hsa-miR-138-5p und *DCP1B* zu, wie wir durch die RNA-Expressionsanalysen in den Hippocampi und Frontalen Kortizes beweisen konnten. Eine zweite Hypothese lautete, ob der gedächtnisassoziierte SNP rs9882688 in die Regulierung der Expression von hsa-miR-138-5p involviert ist, eine Annahme, die in peripheren LCLs bestätigt werden konnte. Dennoch bleibt unklar, ob dieser Effekt auch im menschlichen Gehirn auftritt. Durch eine Genotypisierung und durch das Erstellen eines miRNA-Expressionsprofiles in menschlichen Gehirnautopsiegeweben könnte diese Frage teilweise geklärt werden. Die eQTL- und die GWAS-Daten zeigen, dass das G-Allel in rs9882688 zu einer Erhöhung der hsa-miR-138-5p-Expression und zu einer Reduzierung der Gedächtnisleistung führt. Die Erhöhung der miRNA-Abundanz resultiert normalerweise in einer Erhöhung der mRNA-Repression und damit zu einer reduzierten Genexpression. Das A-Allel des SNPs rs1044950 ist mit einer schlechteren Gedächtnisfunktion assoziiert, während es zu einer Erniedrigung der miRNA-mRNA-Bindung *in vitro* führt. Diese Ergebnisse zeigten im Gegensatz zu den erwarten Effekten aus den eQTL-Ergebnissen mit rs9882688, eine erhöhte Zielgenexpression. Dieser gegensätzlich Effekt könnte auf die Involvierung von ceRNAs (*competing endogenous RNAs*) schließen, die aus Pseudogenen, langen nicht-kodierenden oder stabilen RNAs transkribiert werden (Salmena *et al.* 2011, Helwak *et al.* 2013) und die in einer möglichen Erhöhung der hsa-miR-138-5p bei A-Allelträgern des SNPs rs1044950 resultieren. Letztendlich sind die hier dargestellten GWAS-Resultate erste Ergebnisse, die in unabhängigen Datensätzen repliziert

werden müssen. Auch wenn in diesem Fall kleinere Effekte als die hier gezeigten, auftreten, sollten diese jedoch keine Effekte auf die funktionellen genetischen und Expressionsprofil-Resultate ausüben. Zusätzlich sollten in der Zukunft zu den eQTL-Analysen in menschlichen peripheren Zellen auch Zellen des Zentralnervensystems untersucht werden, um die regulatorische Funktion der hsa-miR-138-5p auf die endogene Expression des Gens *DCP1B* und anderer Gene (z.B. *WWC1*) auch *in vivo* nachweisen zu können.

Zusammengefasst ist durch die Kombination genomweiter Untersuchungen, zusammen mit *in silico*-Berechnungen, *in vitro*-Validierungen und dem Erstellen eines Genexpressionsprofiles die hsa-miR-138-5p als potenzielles regulatorisches Molekül der menschlichen Gedächtnisleistung identifiziert worden. Weitere Untersuchungen sind notwendig, um diese Ergebnisse *in vivo* erfassen zu können, und um andere Ziele und regulatorische Einheiten dieser miRNA zu finden, v.a. deren Verbindung zu der Gedächtnisleistung und anderen kognitiven Eigenschaften.

1.6. Literatur

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2. Anteilserklärung an den erfolgten Publikationen

Julia Schröder hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1:

Julia Schröder, Sara Ansaloni, Marcel Schilling, Tian Liu, Josefine Radke, Marian Jaedicke, Brit-Maren M. Schjeide, Andriy Mashychev, Christina Tegeler, Helena Radbruch, Goran Papenberg, Sandra Düzel, Ilja Demuth, Nina Bucholtz, Ulman Lindenberger, Shu-Chen Li, Elisabeth Steinhagen-Thiessen, Christina M. Lill, Lars Bertram (2014). MiRNA-138 is a potential regulator of memory performance in humans. Frontiers in Human Neuroscience. doi: 10.3389/fnhum.2014.00501

60 Prozent: DNA-Probenvorbereitung für die Genotypisierung, Design der UTR-Konstrukte, Kultivierung der Zelllinien, Ko-Transfektion der miRNAs und Reporterplasmide, Luciferase-Assay, Extraktion und Quantifizierung der mRNAs und miRNAs aus menschlichem Gehirngewebe.

Publikation 2:

Goran Papenberg, Shu-Chen Li, Irene E. Nagel, Wilfried Nietfeld, Brit-Maren Schjeide, Julia Schröder, Lars Bertram, Hauke R. Heekeren, Ulman Lindenberger, Lars Bäckman (2014). Dopamine and glutamate receptor genes interactively influence episodic memory in old age. Neurobiol Aging 35 (5), 1213 e3-8.

20 Prozent: DNA-Probenvorbereitung, Genotypisierung mittels TaqMan® Assay

Publikation 3:

Nicolas W. Schuck, Christian F. Doeller, Brit-Maren M. Schjeide, Julia Schröder, Peter A. Frensch, Lars Bertram, Shu-Chen Li (2013). Aging and KIBRA/WWC1 Genotype Affect Spatial Memory Processes in a Virtual Navigation Task. Hippocampus 23 (10), 919-30.

20 Prozent: DNA-Probenvorbereitung, Genotypisierung mittels TaqMan® Assay

Publikation 4:

Christina M. Lill, Marcel Schilling, Sara Ansaloni, **Julia Schröder**, Marian Jaedicke, Felix Luessi, Brit-Maren M. Schjeide, Andriy V. Mashychev, Christiane Graetz, Denis A.

Akkad, Lisa-Ann Gerdes, Antje Kroner, Paul Blaschke, Sabine Hoffjan, Alexander Winkelmann, Thomas Dörner, Peter Rieckmann, Elisabeth Steinhagen-Thiessen, Ulman Lindenberger, Andrew Chan, Hans-Peter Hartung, Orhan Aktas, Peter Lohse, Mathias Buttmann, Tania Kümpfel, Christian Kubisch, Uwe K. Zettl, Joerg T. Epplen, Frauke Zipp, Lars Bertram (2014). Assessment of miRNA-related SNP effects in the 3´untranslated region of the *IL22RA2* risk locus in multiple sclerosis. Neurogenetics 15 (2), 129-34

30 Prozent: Design der UTR-Konstrukte, Kultivierung der Zelllinien, Ko-Transfektion der miRNAs und Reporterplasmide, Luciferase-Assay

Publikation 5:

Shu-Chen Li, Goran Papenberg, Irene E. Nagel, Claudia Preuschhof, **Julia Schröder**, Wilfried Nietfeld, Lars Bertram, Hauke R. Heekeren, Ulman Lindenberger, Lars Bäckman "Aging magnifies the effects of dopamine transporter and D2 receptor genes on backward serial memory." Neurobiology of Aging. 2013; 34(1): 358 e351-310.

20 Prozent: DNA-Probenvorbereitung, Genotypisierung mittels TaqMan® Assay und MultiNA Kapillarelektrophorese

Unterschrift des Doktoranden/der Doktorandin

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(Dipl. Biologin Julia Schröder)

4. Publikationen

MicroRNA-138 is a potential regulator of memory performance in humans

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Keywords: genome-wide association study, GWAS, working memory, episodic memory, microRNA, hsa-mir-138-5p, *DCP1B*

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Abstract

Genetic factors underlie a substantial proportion of individual differences in cognitive functions in humans, including processes related to episodic and working memory. While genetic association studies have proposed several candidate "memory genes", these currently explain only a minor fraction of the phenotypic variance. Here, we performed genome-wide screening on 13 episodic and working memory phenotypes in 1,318 participants of the Berlin Aging Study II aged 60 years or older. The analyses highlight a number of novel single nucleotide polymorphisms (SNPs) associated with memory performance, including one located in a putative regulatory region of microRNA (miRNA) hsa-mir-138-5p (rs9882688, P-value = 7.8×10^{-9}). Expression quantitative trait locus analyses on next-generation RNA-sequencing data revealed that rs9882688 genotypes show a significant correlation with the expression levels of this miRNA in 309 human lymphoblastoid cell lines (P-value = 5×10^{-4}). *In silico* modeling of other top-ranking GWAS signals identified an additional memory-associated SNP in the 3' untranslated region (3'UTR) of *DCP1B*, a gene encoding a core component of the mRNA decapping complex in humans, predicted to interfere with hsa-mir-138-5p binding. This prediction was confirmed *in vitro* by luciferase assays showing differential binding of hsa-mir-138-5p to 3'UTR reporter constructs in two human cell lines (HEK293: P-value = 0.0470; SH-SY5Y: P-value = 0.0866). Finally, expression profiling of hsa-mir-138-5p and *DCP1B* mRNA in human post-mortem brain tissue revealed that both molecules are expressed simultaneously in frontal cortex and hippocampus, suggesting that the proposed interaction between hsa-mir-138-5p and *DCP1B* may also take place *in vivo*. In summary, by combining unbiased genome-wide screening with extensive *in silico* modeling, *in vitro* functional assays, and gene expression profiling, our study identified miRNA-138 as a potential molecular regulator of human memory function.

1. Introduction

Interindividual variations of memory performance in humans are regulated by genetic and non-genetic factors. Early estimates from twin studies suggest that approximately half of the phenotypic variance is attributable to heritable factors, while the remainder reflects shared and non-shared environmental factors (McClearn *et al.* 1997). These estimates have since received broad support from studies using different designs and analysis approaches (for recent review see Goldberg Hermo *et al.* 2014). For measures of general cognitive ability, the presumed genetic effects appear to increase across the lifespan, that is, from childhood to adulthood and late life (McClearn *et al.* 1997), suggesting that searching for genes in this domain may be most powerful in data sets of aged individuals (see also Lindenberger *et al*. 2008).

A number of candidate genes that may affect various aspects of memory performance in humans have been proposed to date (for review see Papassotiropoulos and de Quervain 2011). As is the case for many other genetically complex traits in humans, the most convincing of these have only recently emerged in the context of genome-wide association studies (GWAS; see NHGRI's "GWAS catalog" for an up-to-date overview; URL: http://www.genome.gov/gwastudies/; Welter *et al.* 2014). Among the most prominent findings are common polymorphisms (i.e. single nucleotide polymorphisms [SNPs]) in *WWC1* (WW and C2 containing 1, a.k.a. *KIBRA* for kidney and brain expressed protein; Papassotiropoulos *et al.* 2006) and *CTNNBL1* (catenin, beta like 1). *WWC1* is located on chromosome (chr) 5q34 and was identified nearly a decade ago in a GWAS on episodic memory in ~300 subjects in which the lead SNP (rs17070145) showed evidence for genome-wide significant association (Papassotiropoulos *et al.* 2006). Since the original study, a number of follow-up studies have been published, albeit with mixed results (Milnik *et al.* 2012). The other lead "memory gene", *CTNNBL1* (located on chr 20q11.23) was identified by the same group in a more recent GWAS (Papassotiropoulos and de Quervain 2011). Thus far, no reports have been published confirming this latter finding independently. In addition, a number of other candidate genes have been tested in non-GWAS association studies, some suggesting evidence for an increased effect sizes when comparing older vs. younger adults (Li *et al.* 2013; Papenberg *et al.* 2014).

A second – and thus far largely independent – line of genetic experiments suggests that memory performance, and likely a large number of other cognitive domains, may be influenced by the action of microRNAs (miRNAs). MiRNAs are short (i.e. typically between 18 and 24 nucleotide long), non-coding RNA molecules that are involved in regulating protein expression post-transcriptionally. This is achieved by binding to the target messenger-RNAs (mRNAs), thereby directly or indirectly interfering with mRNA translation. The last decade of research has shown that miRNAs are involved in a broad range of cellular functions, including the development, differentiation, proliferation, apoptosis, and metabolism of neurons and many other cell types in humans (Satoh 2012). Some estimates suggest that the expression of up to 50% of all human proteins may be affected by the action of one or more of the >2500 miRNAs currently believed to exist (URL: http://mirbase.org/index.shtml; Griffiths-Jones *et al.* 2006). One important factor determining the extent of miRNA-mediated expressional regulation is the binding affinity between miRNAs and their target mRNAs. This

is largely influenced by sequence complementarity between the respective interacting regions on both molecules (Peterson *et al.* 2014). Naturally occurring DNA sequence variants, e.g. trait associated SNPs, within the binding domains of either of these interactants can thus be expected to interfere with miRNA-to-mRNA binding either by decreasing (disruption of complementary sites) or increasing (creation of complementary sites) binding affinity.

In this study, we specifically searched for memory-associated DNA sequence variants predicted to affect miRNA-to-mRNA binding using a similar strategy as described previously by our group (Lill *et al.* 2014). Genetic associations, with measures of both working and episodic memory functions were assessed via genome-wide screening as part of an ongoing GWAS in participants of the Berlin Aging Study II (BASE-II). Associated SNPs were evaluated for their potential effects on miRNA-to-mRNA binding *in silico* using a bioinformatic prediction tool developed by our group. SNPs showing association with memory performance and predicted to directly alter miRNA-to-mRNA binding were further followed up using a range of *in vitro* experiments involving luciferase reporter assays in two human cell lines, as well as miRNA and mRNA expression profiling in human brain autopsy material from three adult individuals. Our analyses uncovered three memory-associated SNPs which potentially manifest their molecular effects by interfering with miRNA function. Intriguingly, two of these SNPs, by independent mechanisms, affect hsa-mir-138-1, a miRNA long known to be crucial in CNS development and function in mammals (Miska *et al.* 2004, Siegel *et al.* 2009) but hitherto not specifically linked to cognitive performance in humans.

2. Methods

2.1. Genome-wide association study (GWAS) of episodic and working memory performance in humans.

Participants. All GWAS participants were part of the Berlin Aging Study II (BASE-II), a multidisciplinary project investigating factors involved in 'healthy' vs. 'unhealthy' aging (Bertram *et al.* 2013). In addition to genetics, BASE-II covers a broad range of functional domains critical for understanding aging investigated in a multidisciplinary assessment protocol that includes measures from internal medicine, immunology, psychology, as well as sociology and economics. The behavioral test battery applied to each BASE-II participant includes an extensive coverage of cognitive abilities, including detailed assessments of working and episodic memory. At baseline, BASE-II includes 2,200 participants of Caucasian ancestry recruited from the greater Berlin area. The cohort is split into a subgroup of 1,600 older adults aged 60 to 80 years, mean 66.76 years at baseline, and 600 younger adults aged 20 to 35 years, mean 27.32 years. Both groups consist of equal numbers of males and females. The analyses presented here are limited to participants of the "old" stratum for whom genotype and cognitive data were available at the time of analysis (n = 1,318). Written consent was provided by all BASE-II individuals before participation. The study was approved by the institutional review boards of each relevant participating research unit prior to participant recruitment.

Assessment of memory performance. This study is based on 13 different quantitative measures (i.e., 2 and 11 respectively) of working memory (WM) and episodic memory (EM) were selected, assessed either at the Center for Lifespan Psychology at the Max Planck Institute for Human Development (MPIHD; n = 1,318 individuals with cognitive testing completed at time of analysis) or at the Charité Research Group on Geriatrics (CRGG; n = 961 with cognitive testing completed at time of analysis) at Charité University Hospital. At MPIHD, six participants from the same age group were tested simultaneously in two separate group sessions one week apart. In addition, we utilized test results from the CERAD Plus battery (Morris *et al.* 1989; Fillenbaum *et al.* 2008,), which was carried out individually to each participant at CRGG. See Supplementary Table 1 and Supplementary Methods for more detailed information on the type of WM and EM assessments used here.

Genotyping and genetic association analyses. Details on genotyping and analysis procedures can be found in the Supplementary Methods. In brief, DNA from all BASE-II participants was extracted from whole blood using standard procedures and then subjected to microarray-based SNP genotyping using the "Genome-Wide Human SNP Array 6.0" (Affymetrix, Inc.), followed by an extensive quality control and genome-wide imputation of unobserved genotypes using whole genome sequence data from the 1000 Genomes Project (Abecasis *et al.* 2010). Association analyses were carried out using the EM or WM variables as quantitative traits assuming an additive linear model, adjusted for age, sex, and years of education and to the first three principal components to account for potential population stratification. Association analyses were performed using SNPTEST v.1.3 (Marchini and Howie 2010), which accounts for uncertainty of imputed genotype calls via missing data likelihood tests. Overall, in this study we tested a total of 12,607,232 high-quality SNPs for

genetic association with the memory traits in 1,318 (MPIHD) and 961 (CRGG) subjects from the BASE-II subgroup aged 60-80 years.

2.2. *In silico* predictions of miRNA-to-mRNA binding and potential SNP effects.

To systematically assess the potential impact of SNP allele-status on miRNA-to-mRNA binding, we utilized a bioinformatic tool recently developed by our group described in detail elsewhere (Schilling 2011, Schilling 2013). In brief, this entailed a prediction of potential miRNA binding sites for 3'UTRs of all known protein-coding transcripts (downloaded from Ensembl Genes 71, http://www.ensembl.org/biomart/martview) using miRanda v.3.38, TargetScan 5.09 and PITA and v19 of the mirBASE database (http://www.mirbase.org). Only SNPs displaying strong linkage disequilibrium (LD; i.e. r^2 of 0.8; estimated from whole genome sequence data of the 1000 Genomes phase 1 CEU reference panel; Abecasis *et al.* 2010) with GWAS SNPs were considered further. For these SNPs, we finally estimated the potential effects on miRNA-to-mRNA binding using a modified version of the support vector regression (SVR) method developed by Betel (Betel *et al.* 2010).

2.3. *In vitro* assessment of SNPs predicted to affect miRNA-to-mRNA binding.

Cell culture and construct transfection. Experiments were performed as previously described (Lill *et al.* 2014). Custom made reporter plasmids (pLightSwitch_3UTR) containing the appropriate 3'UTR sequence and the miRNA mimics were purchased from SwitchGear Genomics (Menlo Park, CA, USA). The desired 3'UTRs were subcloned in the pLightSwitch_3UTR vector downstream of the *Renilla* luciferase gene. The UTR constructs containing the reference allele were used as a template to generate point mutations via site directed mutagenesis. All constructs were verified by Sanger sequencing. 3'UTR constructs were transfected into naïve human embryonic kidney (HEK293) and human neuroblastoma (SH-SY5Y) cells, which were cultured in DMEM GlutaMax (Invitrogen, Darmstadt, Germany) media with 10% FBS (Biochrom, Berlin, Germany) for HEK293 or 15% FBS for SH-SY5Y cells, with an additional 1% Penicillin/Streptomycin (Biochrom). The cells were grown in standard conditions (37°C, 5% CO_2). Transfection was carried out in 96-well plates (TPP, Trasadingen, Switzerland) at a cell confluency of about 50% using Dharmafect (ThermoScientific) following manufacturer´s instructions. 50ng of vector with the desired 3'UTR and 50nM of corresponding miRNA mimic or scrambled non-binding miRNA were co-transfected in the cells per well. The scrambled miRNA was used as a negative control in combination with all 3'UTR constructs and in all experiments. After 24 hours, HEK293 and SH-SY5Y cells were collected by freezing the culture plates directly on dry ice to enhance cell lysis. The plates were then thawed on ice and the resuspended cell lysates used for the luciferase assays. The LightSwitch Assay reagents (SwitchGear Genomics) were used following manufacturer´s instructions. Assay reagents mixed with the same volume of cell lysate were transferred to a white 96-well plate (Costar, Washington, D.C., USA). An end point read of *Renilla* luciferase intensity values was taken using the POLARStar Omega (BMG Labtech, Ortenburg, Germany) plate reader with three seconds integration time and 3,500 gain per well. Five to seven independent experiments per cell line and experimental condition were performed, using independent transfection mixes and/or different cell batches. For each independent experiment, six replicates were performed.

Statistical analysis of luciferase data. The analysis of the luciferase assay results were performed using R, an open-source language and environment for statistical computing and graphic (URL: http://www.r-project.org). We observed no outliers defined as deviating more than three standard deviations from the mean luciferase luminescence per experimental condition in each independent experiment. For each independent experiment, the mean luciferase activity of the 3'UTR reporter construct (i.e. containing either the reference or the alternative allele) co-transfected with a functional miRNA was divided by the baseline mean luciferase activity of the corresponding reporter construct co-transfected with the scrambled, non-targeting miRNA as negative control. To assess binding of the miRNAs to their predicted target sites irrespective of allele status, normalized luciferase activity of either 3'UTR reporter construct (i.e. either containing the reference or the alternative allele) co-transfected with the functional miRNA was compared to the control condition using the non-binding miRNA by one-sample t test (P-values reported for this analysis are one-tailed). Changes in *Renilla* gene expression levels in 3'UTR constructs containing the reference vs. alternative alleles were assessed based on the t test statistic for two independent samples (P-values reported for this analysis are two-tailed).

2.4. Analysis of expression levels of miRNA and mRNA molecules in human post-mortem brain tissue.

Tissue preparation and RNA extraction. Human brain tissue was collected post-mortem from hippocampi and frontal cortices from three deceased individuals without history of neuropsychiatric diseases at the Charité university hospital (Berlin, Germany). After collection, brain samples were stored (at -20°C) in RNA*later*® solution (Applied Biosystems, Forster City, CA, USA) to avoid degradation. We used the miRVANA™ miRNA Isolation Kit (Life Technologies, Darmstadt, Germany) to extract small and total RNAs from tissue samples following manufacturer´s instructions. Prior to extraction, all samples were homogenized using TissueLyser (QIAgen, Hilden, Germany) by shaking each sample twice in Lysis/Binding buffer for two minutes at 20Hz.

Assessment of miRNA expression levels. The quantification procedure comprises two steps: reverse transcription from RNA to cDNA followed by the amplification via quantitative PCR (qPCR). Specific primers for reverse transcription and qPCR were based on pre-made TaqMan® Small RNA Assays (Applied Biosystems). Reverse transcription was performed on 10ng RNA using the Taqman® MiRNA Reverse Transcription Kit according to the manufacturer's protocol (Applied Biosystems). The qPCR reaction was conducted using TaqMan® Small RNA Assays following the manufacturer's protocol (Applied Biosystems). In short this entailed: For reverse transcription 10ng of the extracted RNA was used in a reaction mix containing Reverse Transcription Buffer, 15mM dNTPs, 50U/µl MultiScribe™ Reverse Transcriptase, 20U/µl RNase Inhibitor, and Primer in a final volume of 15µl. The protocol in 384-well format for all reactions was as follows: 16°C for 30 min, 30 min at 42°C and a final step of 85°C for 5 min. The qPCR reaction was conducted in TaqMan® Universal PCR Master Mix II, TaqMan® Small RNA Assay (both Applied Biosystems) and 1.33µl of the RT-PCR product in a final volume of 20µl. The cycling protocol was: 10 min at 95°C, 15 sec at 95°C, 60 sec at 60°C for overall 50 cycles. The reactions were run and visualized on a QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems).

Assessment of mRNA expression levels. Reverse transcription was performed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems) on 0.2µg of total RNA following the manufacturer´s instructions. The reactions were run on the Thermo Cycler PTC-240 (MJ Research, Waltham, MA, USA). Expression of the resulting *DCP1B* cDNA was assessed via PCR primers 5'-CCAGGGTCTCCTCACAACAT-3' (forward) and 5'-TCTTTTTCATGGCTGCTTGA-3' (reverse). Primers were designed to lead to a ~850bp cDNA amplicon vs. a 6.9kb gDNA amplicon. PCR conditions were using 1.5µM of each primer, approximately 60ng of cDNA template, 0.25nM dNTPs, 10mM $MgCl_2$, 30% Q solution (QIAgen) and 0.25U Taq polymerase in a final volume of 10µl. Reactions were carried out in 96-well format on PTC-240 thermal cyclers at 94°C (3 min), followed by 40 cycles of 94°C (45 sec), 60.5°C (90 sec), and 72°C (60 sec), and a final extension step of 72°C (6 min). PCR amplicons were visualized by ethidium bromide stained gel electrophoresis in 1% agarose (Sigma Aldrich, Taufkirchen, Germany).

2.5. Expression quantitative trait locus (eQTL) analyses of hsa-mir-138-5p using next-generation sequencing data.

eQTL analyses on the potential role of rs9882688 in expression of hsa-miR-138-5p were performed using next-generation small RNA sequencing data of peripheral lymphoblastoid cell line (LCL) samples generated by the Genetic European Variation in Health and Disease (GEUVADIS) consortium (for a description of sequencing methods and data preparation see: Lappalainen *et al.* 2013). For the eQTL analyses here, we downloaded normalized expression data of hsa-miR-138-5p for four populations of European descent (i.e. Utah Residents with Northern and Western European Ancestry (CEU), Finns (FIN), British (GBR), Toscani (TSI)) as released by the GEUVADIS project database (URL: http://www.ebi.ac.uk/arrayexpress/files/E-GEUV-2/GD452.MirnaQuantCount.1.2N.50FN.samplename.resk10.txt). Subject-level genotypes for rs9882688 in the same individuals were obtained from the 1000 Genomes database (URL: http://browser.1000genomes.org/index.html; Abecasis *et al.* 2010). The resulting data set included 333 individuals of European descent with both hsa-miR-138-5p expression data and rs9882688 genotypes. Statistical analyses of these data were performed using R. MiRNA expression values that fell 1.5x the interquartile range (IQR) below the first quartile or 1.5x the IQR above the third quartile were defined as outliers and excluded from further analysis (24 samples). Expression levels of hsa-miR-138-5p showed a symmetrical, approximately normal distribution in the effective sample size of 309 individuals (as determined by the Shapiro-Wilk test implemented in R [P = 0.548] and quantile-quantile plotting, Suppl. Figure 1). Subsequent eQTL analyses on these 309 samples were based on an additive model using linear regression. Association results were adjusted for sex and population of origin. Due to the low frequency of homozygote carriers of the G allele (n = 2), sensitivity analyses were performed upon exclusion of those individuals. Statistical significance for these analyses is expressed as two-tailed P-values.

3. Results

3.1. GWAS of episodic and working memory performance in humans.

The GWAS analyses on 13 episodic and working memory traits in up to 1,318 individuals from the subgroup of BASE-II participants aged 60 years or older revealed 28 distinct genomic regions (or: loci) showing association P-values at $1x10^{-6}$ or below (Supplementary Table 2), indicating that at least some of these are genetically linked to human memory performance. Notably, these did not include SNPs in *WWC1* (*KIBRA*) or *CTNNBL1* (Liu *et al.* [under review]), suggesting that the analyzed traits are not significantly influenced by SNPs in these genes in our study population. The three most significant findings showed P-values at or below $1x10^{-7}$ and were observed with SNPs rs9882688 ($P = 7.8x10^{-9}$ on chr 3p21.32) for trait "WL_save" (part of the CERAD cognitive battery measuring the proportion of learned words vs. recalled words), with rs1016365 ($P = 9.7x10^{-8}$ on chr 8q13.3) for "ItemItem" (associative episodic memory task where probands were asked to learn and recall words irrespective of their pairing with other words), and with rs113948889 ($P = 9.9x10^{-8}$ on chr 12p13.33) for "TFEUWC" (a spatial working memory paradigm combined with tasks testing frontal executive control; see Supplementary Material for more information on these and all remaining traits). All of these signals were flanked by multiple additional SNPs showing P-values at or below $1x10^{-5}$ indicating that they do not reflect technical artifacts (see Supplementary Figure 2-4 for Manhattan and Q-Q plots for GWAS results of these three traits). Only the signal with rs9882688 on chr 3 surpassed the threshold for genome-wide significance (i.e. P-values at or below $5x10^{-8}$, a cutoff frequently used in the context of GWAS, see McCarthy *et al.* 2008). This SNP, which itself does not map to any known open reading frame, is located approx. 20kb upstream from the 5' end of miRNA hsa-mir-138-1 and, thus, potentially within active upstream regulatory elements of this miRNA. This interpretation is supported by the fact that rs9882688 is located within an ENCODE DNase I hypersensitivity cluster and within a known H3K27Ac mark, two features typically characterizing active regulatory elements (ENCODE Project Consortium *et al.* 2012). The second leading GWAS signal was elicited by SNP rs1016365, which is located approx. 6 kb 3' of *EYA1* (Homo sapiens eyes absent homolog 1 [Drosophila]) a member of the eyes absent (EYA) family of proteins. The third best associated SNP in our GWAS (rs113948889) maps to an intron of *DCP1B* (encoding decapping mRNA 1B), which is a core component of the mRNA decapping complex, a key factor in the regulation of mRNA decay. Focusing on these top GWAS findings, we next performed a number of different *in silico* and *in vitro* experiments to assess their potential role on miRNA function.

3.2. *In silico* predictions of miRNA-to-mRNA binding and potential SNP effects.

Using a bioinformatic tool recently developed in our group (Schilling 2011, Schilling 2013), we predicted the potential role of the GWAS SNPs on chr 3p21.32, 8q13.3, and 12p13.33 (and their proxies, i.e. other SNPs in strong LD [$r^2 \geq 0.5$] and mapping within ±1 Mb) on their potential to interfere with miRNA-to-mRNA binding. These analyses did not identify any such effects for the GWAS signals on chr 3 or 8, but several potentially relevant predictions for the memory associated SNPs on chr 12p13.33 (Table 1). All of the SNPs predicted to interfere with miRNA binding were proxies of rs113948889 with r^2 values of 1 and suggested a potential up-regulation of protein expression conferred by the respective non-reference

alleles. The strongest effect was estimated for rs112215626, predicted to interfere with the binding of hsa-miR-4775 to the 3'UTR of *DCP1B* mRNA (see miRNA-to-mRNA alignment in Figure 1a). The second strongest effect was estimated for rs1044950, which was predicted to interfere with the binding of hsa-miR-138-5p to the 3' UTR of another *DCP1B* transcript (Figure 1b). Interestingly, the same SNP is located within the coding sequence of alternative *DCP1B* transcripts where it is predicted to elicit an amino acid change from alanine to valine at the respective residues (i.e. Ala273Val, Ala249Val, Ala375Val; Supplementary Table 2). However, this missense substitution is not predicted to significantly alter protein function in any of the affected *DCP1B* transcripts (Supplementary Table 3) *in silico* based on estimates from PolyPhen2 (Adzhubei *et al.* 2010) or SIFT (URL: http://sift.bii.a-star.edu.sg/), indicating that the presumed effects on miRNA-to-mRNA binding may represent the overarching functional mechanism underlying the GWAS signal at this locus. Hence, we selected to follow-up the two leading potential *DCP1B* miRNA SNPs (i.e. rs112215626 and rs1044950) *in vitro*.

3.3. *In vitro* assessment of SNPs predicted to affect miRNA-to-mRNA binding.

We conducted luciferase reporter assays in naïve HEK293 cells and SH-SY5Y cells using two different 3'UTR constructs (containing either the reference or non-reference alleles) of *DCP1B* transcripts ENST00000540622 and ENST00000541700 predicted to bind hsa-mir-138-5p and hsa-mir-4775, respectively. In HEK293 cells, co-transfections of the resulting 3'UTR *Renilla* vectors and hsa-mir-138-5p showed significant reductions of luciferase luminescence in comparison to co-transfections with the non-binding miRNA control ($P_{one\text{-}tailed} \leq 0.000191$), confirming our predictions that this miRNA binds to the corresponding 3'UTR *in vitro* (Figure 2a). Analyses comparing luciferase luminescence with respect to allele status at rs1044950 revealed a consistent and significant increase in normalized luciferase expression in constructs containing the minor A-allele ($n = 7$, $P = 0.0470$; Figure 2a). While co-transfection of the same reporter constructs with hsa-mir-138-5p to SH-SY5Y cells generally showed similar effects pointing in the same direction, the difference in luciferase expression between constructs containing the G- vs. A-allele was not statistically significant ($n = 9$, $P = 0.0866$, Figure 2a). Thus, in both cell lines the non-reference (minor) A-allele of SNP rs1044950 increased luciferase expression as compared to the reference (major) G-allele by 11.8% (HEK293) and 10.5% (SH-SY5Y). These findings are in line with our *in silico* predictions, which suggested stronger binding of hsa-mir-138-5p in 3'UTR sequences containing the reference (G) allele compared to the alternative (A) allele. In contrast, we did not observe a significant reduction of *Renilla* expression upon co-transfecting either reporter construct containing *DCP1B* transcript ENST00000540622 with SNP rs112215626 and hsa-mir-4775 compared to the non-binding miRNA in control experiments in neither HEK293 nor SH-SY5Y cells ($n = 7$ and 6, respectively, $P_{one\text{-}tailed} \geq 0.05$, Figure 2b). This suggests that this miRNA, as opposed to hsa-mir-138-5p, does not bind to the corresponding *DCP1B* transcript, at least under these experimental conditions. In summary, the results of the luciferase reporter experiments suggest that SNP rs1044950 elicits allele-specific effects on the expression of constructs containing the corresponding *DCP1B* 3'UTR in the presence of hsa-mir-138-5p *in vitro*.

3.4. Analysis of expression levels of hsa-mir-138-5p and *DCP1B* mRNA in human post-mortem brain tissue.

While both hsa-mir-138-1 (Landgraf *et al.* 2007) and transcripts of *DCP1B* (URL: http://human.brain-map.org/) were previously shown to be expressed in both hippocampus and frontal cortex in humans, no data exist as to whether this expression actually occurs at the same time, a prerequisite condition for the interaction effects observed *in vitro* to also be relevant *in vivo*. To address this question, we obtained three different post-mortem hippocampus and frontal cortex specimen, which were profiled for both miRNA and mRNA expression patterns. qPCR of hsa-mir-138-5p revealed high expression of this miRNA in both hippocampal and frontal brain slices for all three individuals (Figure 3a). Hsa-mir-138-5p expression levels were comparable to those observed for hsa-miR-let-7b, a miRNA ubiquitously expressed in many human tissues including brain (and used here as positive control miRNA), in the same specimen (data not shown). Semi-quantitative PCR of *DCP1B* mRNA in the same brain samples revealed a more variable expression pattern (Figure 3b). Using this method *DCP1B* mRNA was only detectable in two of the three human brain samples (probands 2 and 3 in Figure 3b). Further, in both of these probands *DCP1B* mRNA expression levels were higher in frontal cortex as compared to hippocampus (owing to the semi-quantitative nature of the experiments, these differences could not be assessed for statistical significance). Regardless of the observed interindividual and regional expression differences, these experiments clearly demonstrate that both hsa-mir-138-5p and *DCP1B* mRNA are co-expressed simultaneously in both the hippocampi and frontal cortices in human brain, setting the temporal and spatial stage for an interaction of these two RNA molecules *in vivo*.

3.5. Expression quantitative trait locus (eQTL) analyses of hsa-mir-138-5p using next-generation sequencing data.

Although the lead GWAS signal (elicited by rs9882688) identified in this study was not predicted to interfere with miRNA-to-mRNA binding *in silico*, this SNP maps into a potential regulatory site 20kb upstream of miRNA hsa-mir-138-1. The primary transcript of this miRNA is processed into two different mature products hsa-mir-138-5p and hsa-mir-138-1-3p, which are both expressed in humans (Landgraf *et al.* 2007). Notably, hsa-mir-138-5p is the same miRNA whose binding to *DCP1B* is also potentially affected by the presence of SNP rs1044950, as suggested by the *in silico* and *in vitro* experiments outlined above. Thus, a potential effect of rs9882688 on the expression of hsa-mir-138-5p could be indicative of a more systematic involvement of this miRNA in human memory performance. To this end, we performed eQTL analyses on NGS-based small-RNA sequencing data in over 300 human lymphoblastoid cell line samples from the GEUVADIS project (Lappalainen et al. 2013). The results of these analyses revealed a significant dose-dependent effect of this SNP on hsa-mir-138-5p expression in these peripheral cell lines. Specifically, the presence of the minor G-allele of rs9882688 was associated with increased hsa-miR-138-5p expression levels (n = 309, beta = 80.87, standard error [SE] = 23.00, P-value = 0.000504, Figure 4). This finding was not solely driven by the two G-allele homozygotes in this dataset as evidenced by eQTL results after exclusion of these individuals (n = 307, beta = 77.35, SE = 25.56, P = 0.00270). These data suggest that the same SNP showing association with episodic memory

performance in humans, also significantly correlates with changes in hsa-mir-138-5p expression in human peripheral cell lines.

4. Discussion

We investigated the potential role of common DNA sequence variants associated with human memory performance in miRNA-mediated regulation of gene expression. A GWAS analysis on 13 memory traits in up to 1,318 individuals aged 60 years or older revealed a number of potential association signals in both working (rs113948889) and episodic memory (rs9882688 and rs1016365) domains. Among these were SNPs predicted to be involved in the expression and function of hsa-mir-138-5p, a miRNA known to be important in brain development and function. Specifically, genotypes at SNP rs9882688 (which represented the lead memory GWAS signal in this study) were shown to correlate significantly with the expression of this hsa-mir-138-5p in human LCLs using next-generation small-RNA sequencing data. In addition, we found that SNP rs1044950 (as proxy of another top GWAS signal) leads to allele-specific changes in the expression of reporter constructs containing the 3'UTR sequence of *DCP1B* transcripts predicted to contain binding sites for hsa-mir-138-5p in peripheral (HEK293, P = 0.047) and neuronal (SH-SY5Y, P = 0.0866) human cell lines. Finally, expression profiling of both hsa-mir-138-5p and *DCP1B* mRNA in post-mortem brain tissue revealed that both putative interactants are co-expressed in brain. In summary, in this study various lines of independent evidence converge on the notion that hsa-mir-138-5p may play a significant role in processes related to human episodic memory performance.

The data from our study are in line with previous research on the potential role of mir-138 in mammalian brain function. For instance, Miska (Miska *et al.* 2004) showed that mir-138 expression levels increase with age in the developing rat brain reaching their peak in juvenile and adult rats. Landgraf (Landgraf *et al.* 2007) later showed that mir-138 was also highly expressed in adult human brain samples, including frontal cortex and hippocampus. More recently, mir-138 was identified as part of a functional screen for dendritic miRNAs that regulate spine morphogenesis in rats (Siegel *et al.* 2009). In that study, mir-138 was found to act as a negative regulator of dendritic spine size possibly by tuning the activity of antagonistic signaling that regulate the actin cytoskeleton in spines. In a review on the same topic Schratt hypothesized that miR-138-related pathways might also contribute to long-lasting forms of synaptic plasticity [...]" (Schratt 2009). Our data, in which we observed multiple converging lines of genetic evidence suggesting a potential role of hsa-mir-138-5p in episodic memory performance, provide some first independent support of this hypothesis and extend it to humans. In addition to *DCP1B*, hsa-mir-138-5p is predicted to target a large number of different human transcripts *in silico* (ranging between a few hundreds to a few thousands depending on the prediction algorithm used). In the GWAS results generated here, genes containing hsa-mir-138-5p targets identified by three or more of the prediction algorithms show a significant ($P < 0.05$ based on 100,000 permutations) enrichment for memory-associated SNPs as compared to genes not targeted by this miRNA (Supplementary Table 4), further supporting the notion that hsa-mir-138-5p represents a potential molecular regulator of human memory function.

The gene *DCP1B* encodes "decapping mRNA 1B" which is a core component of the mRNA decapping complex, a key factor in the regulation of mRNA decay. Decapping and mRNA degradation takes places in P-bodies (processing bodies) (Kulkarni *et al.* 2010). Importantly, P-bodies have been suggested to be one of the predominant sites of miRNA-

mediated mRNA degradation, a process that is inhibited upon depletion of the decapping DCP1:DCP2 complex (Behm-Ansmant *et al.* 2006). Thus, *DCP1B* and mir-138 function within the same general pathway, i.e. the degradation of mRNAs bound by miRNAs, likely including those targeted by hsa-mir-138 itself.

While the novel experimental data generated here support the hypothesis that hsa-mir-138-5p plays an important role in physiological mechanisms involved in human memory performance, we note the following potential limitations of our findings. First and foremost, the functional genetic experiments implying a role of rs1044950 in interfering with miRNA-to-mRNA binding are based on *in vitro* experiments using reporter constructs. While it is tempting to speculate that these effects are also relevant *in vivo*, no direct experimental proof of this interpretation currently exists. In humans, however, this type of evidence is difficult to come by owing to the fact that molecular mechanisms in the living human brain cannot be monitored at sufficient resolution with current technologies. In order for the hypothesized effects to take place *in vivo*, both interactants need to be co-expressed in the same tissue at the same time. Our RNA-profiling experiments using human frontal cortex and hippocampus samples clearly demonstrate that this is the case for hsa-mir-138-5p and *DCP1B*. Second, another conclusion of this study is that SNP rs9882688 is involved in the regulation of hsa-mir-138-5p expression. While this could be shown in LCLs, it remains unclear whether these effects are also relevant in human brain. Third, linking the GWAS results for rs9882688 to this SNP's eQTL effects on hsa-mir-138-5p suggests that the same (i.e. G) allele increasing miRNA expression is associated with worse episodic memory performance (indicated by the negative beta-coefficient in Supplementary Table 1). Increased miRNA abundance is typically interpreted to lead to an increase in mRNA target repression and, as a consequence, reduced target gene expression. For SNP rs1044950, however, the minor (A) allele, associated with worse memory performance, leads to a decrease in miRNA-to-mRNA binding *in vitro*. This latter finding would suggest an increased target gene expression, i.e. an effect opposite to what can be expected from the eQTL results with rs9882688. One possible explanation for these different effect directions is the involvement of competing endogenous RNAs (ceRNAs). Such ceRNAs were recently proposed to exist, e.g. generated from transcribed pseudogenes, long noncoding RNAs, or stable RNAs (Salmena *et al.* 2011, Helwak *et al.* 2013). Here they could lead to increased levels of hsa-mir-138-5p in A-allele carriers of rs1044950, i.e. similar to what is observed for rs9882688-G. Finally, it needs to be emphasized that the GWAS results reported here represent preliminary findings that need to be replicated in independent data sets. However, even if these were to reveal smaller effect estimates than those reported here (e.g. as a result of the "winner's curse "; Kraft 2008), this should have no bearing on the functional genetic and expression profiling results of our study. In addition to generating independent genetic association data, future work will need to extend our eQTL findings to the CNS, confirm the regulatory role of hsa-mir-138-5p on endogenous expression of *DCP1B* and other target genes and their corresponding proteins (in particular those potentially involved in human memory function, such as *WWC1* [*KIBRA*]), and assess the role of this miRNA on their putative targets *in vivo*.

In summary, by combining unbiased genome-wide screening with extensive *in silico* modeling, *in vitro* functional assays, and gene expression profiling, our study identified hsa-

mir-138-5p as a potential molecular regulator of human memory function. Future work is needed to assess the relevance of these findings *in vivo* and to explore other regulators and targets of this highly abundant miRNA, in particular their connection to memory and other cognitive domains.

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Figures

Figure 1: Predicted miRNA binding sites in the *DCP1B* transcripts. *(a)* Rs112215626 is located in the seed region of hsa-miR-4775. The alternative (G) allele of rs112215626 (highlighted in red) may alter binding affinity of hsa-miR-4775 to the *DCP1B* 3'UTR and therefore alter protein levels. *(b)* Rs1044950 is located in the seed region of hsa-miR-138-5p. The alternative T allele of the rs1044950 (highlighted in red) may alter binding affinity of hsa-miR-138-5p to the *DCP1B* 3'UTR and subsequently alter protein levels.

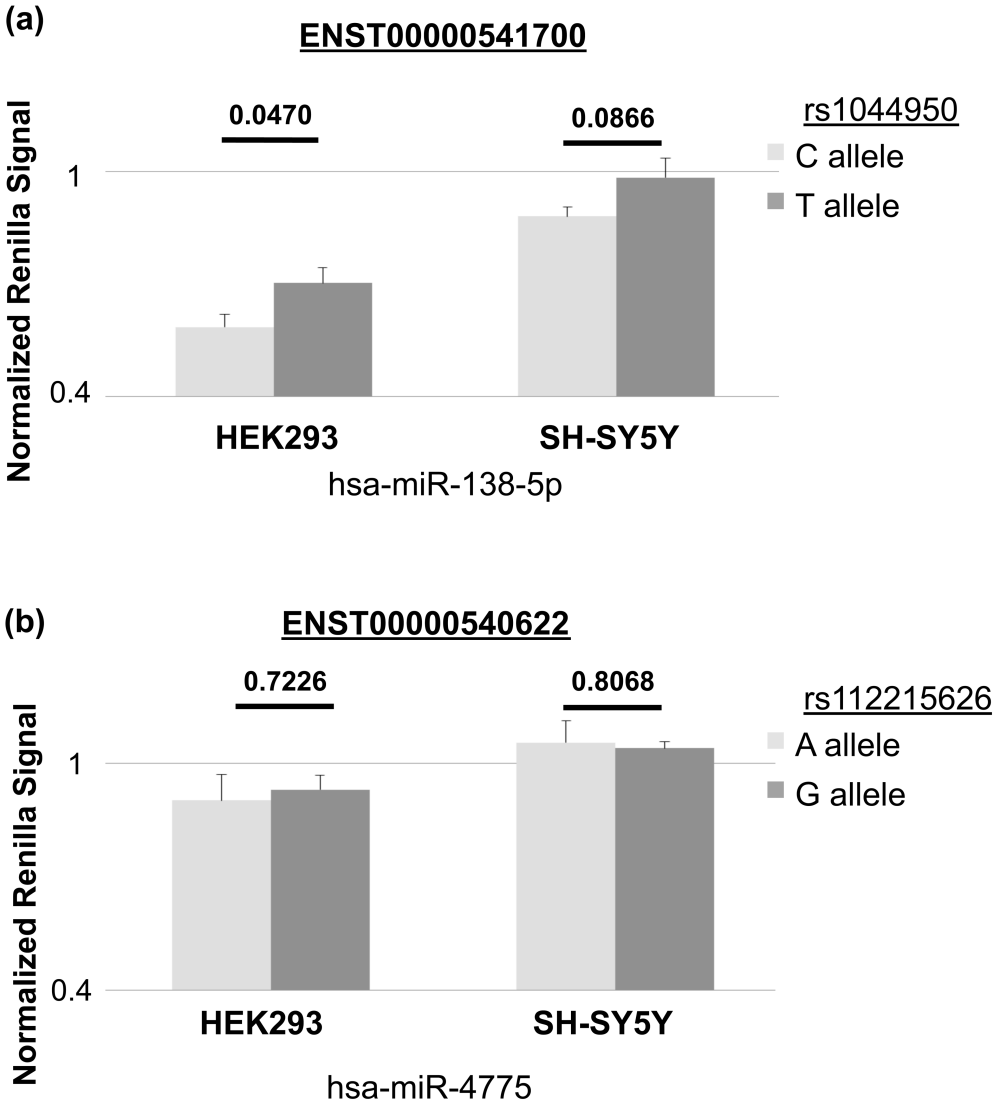

Figure 2: *In vitro* effects of rs1044950 and rs112215262 on miRNA-to-mRNA binding and gene expression. The bar charts show the normalized *Renilla* luciferase expression in constructs containing *DCP1B* 3'UTR and corresponding SNP alleles. Depicted are the mean *Renilla* luciferase intensities and the standard errors relative to the control luciferase intensities of the construct co-transfected with the non-targeting miRNA control (corresponding to the horizontal line): *(a)* for transcript ENST00000541700 containing the reference (G) or alternative (A) allele of rs1044950 and co-transfected with has-miR-138-5p into HEK293 and SH-SY5Y cells. The relative mean luciferase luminescence of the construct containing the G and the A allele was 0.585 (±0.0335) and 0.703 (±0.0417) in HEK293 cells, and 0.880 (±0.0256) and 0.985 (±0.0513) in SH-SY5Y cells. *(b)* for transcript ENST00000540622 containing the reference (T) or alternative (C) allele of rs112215626 and co-transfected with hsa-miR-4775 into HEK293 and SH-SY5Y cells. The relative mean luciferase luminescence of the construct containing T and the C allele was 0.903 (±0.0692) and 0.931 (±0.0382) in HEK293 cells, and 1.056 (±0.0582) and 1.041 (±0.0185) in SH-SY5Y cells.

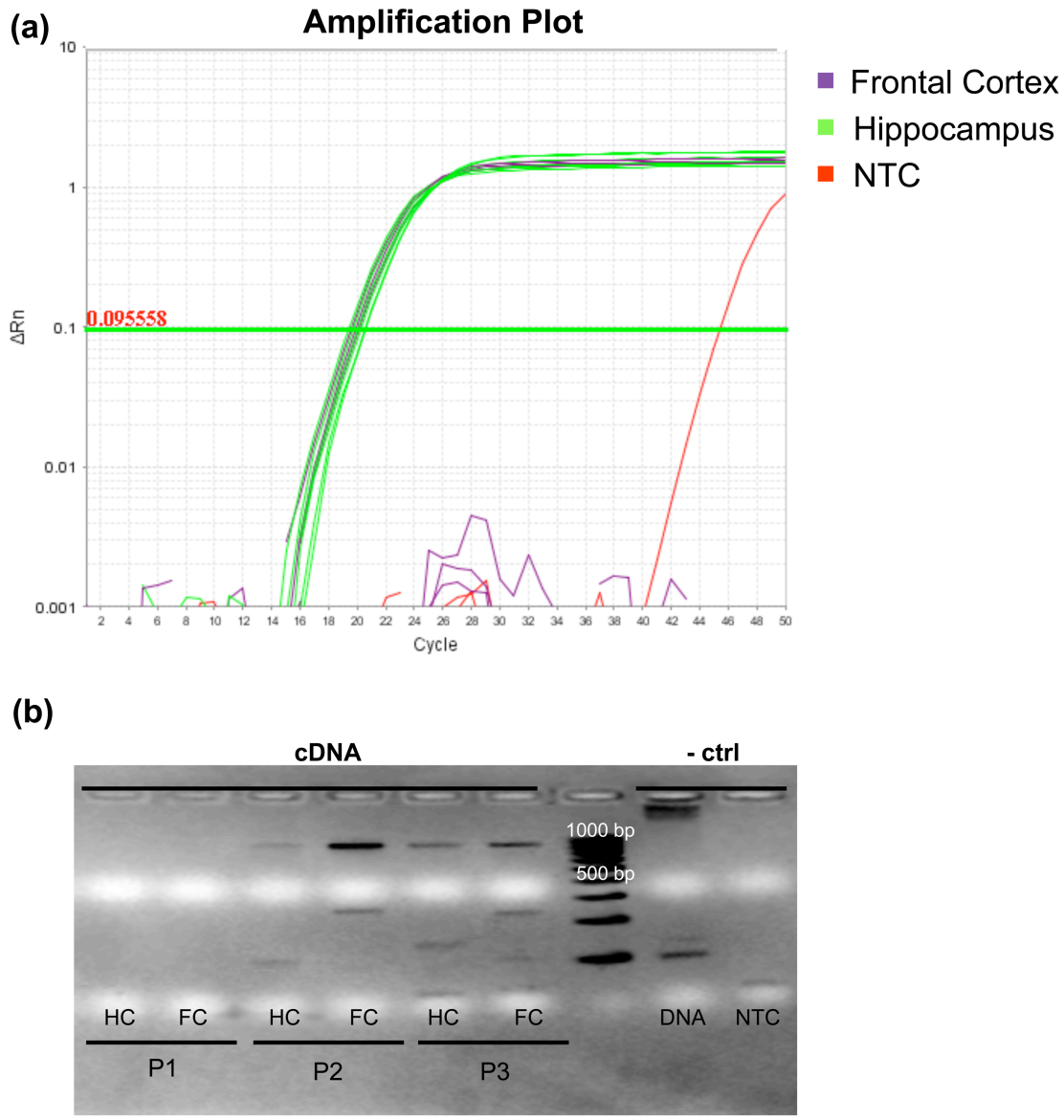

Figure 3: Expression profile of hsa-miR-138-5p and *DCP1B* in autopsy brain tissues of three deceased human probands. *(a)* Amplification plot of qPCR experiments in three frontal cortices (highlighted in purple) and three hippocampi (highlighted in green). Hsa-miR-138-5p was expressed in both frontal cortex (FC) and hippocampus (HC; C_T ~19). One of two NTCs (non template control, highlighted in red) showed amplification at C_T > 45, where the other one did not show any amplification. *(b)* Ethidium bromide stained gel electrophoresis in 1% agarose displays the semi-quantitative levels of *DCP1B* cDNA (expected and observed at ~850bp) in post-mortem human brain tissues. No expression was detected in proband 1 (P1). The *DCP1B* cDNA levels were higher in the FC, compared to HC of proband 2 (P2) and proband 3 (P3). A gDNA amplicon band (expected and observed at ~6.9kb) could be detected and no band in the NTC.

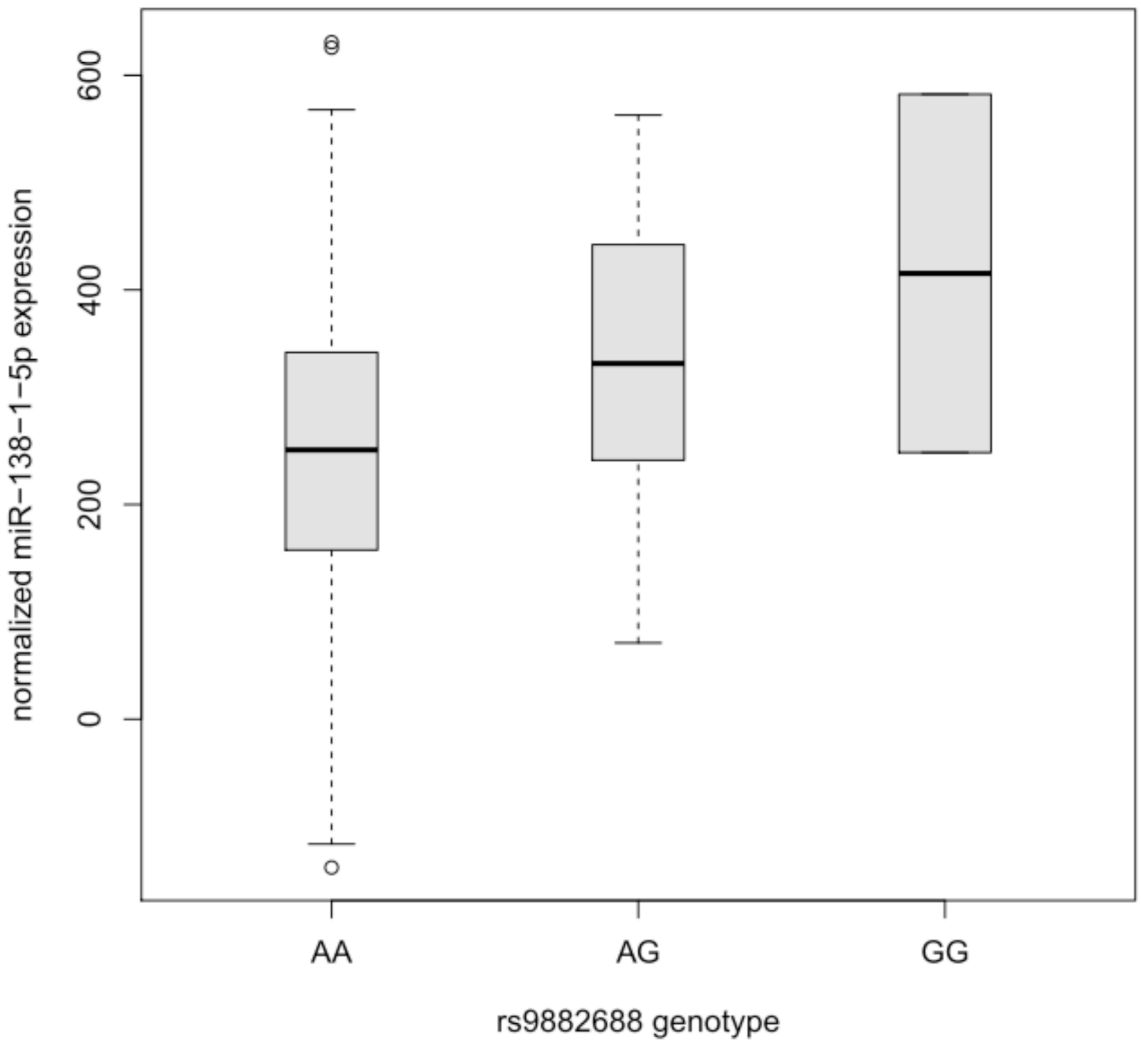

Figure 4: Box plot of the distribution of hsa-miR-138-1-5p expression levels in lymphoblastoid cell lines dependent on the rs9882688 genotype in 308 individuals of European descent. Horizontal lines represent median values, boxes represent interquartile ranges, and whiskers extend to 1.5x the interquartile range; values outside this range are depicted as circles. Carriers of the rs9882688 G allele showed an increase in hsa-miR-138-1-5p expression levels.

Table 1: SNPs in LD with memory-associated SNP rs113948889 and predicted to affect miRNA-to-mRNA binding *in silico*.

GWAS SNP	miRNA target site SNP	CHR	BP	Distance to GWAS SNP	miRNA target site SNP	LD R2	microRNA	Target gene	Target transcript
rs113948889	rs112215626	12	2,059,337	44,833	G/A	1	hsa-miR-4775	DCP1B	ENST00000540622
rs113948889	rs1044950	12	2,061,982	42,188	T/C	1	hsa-miR-138-5p	DCP1B	ENST00000541700
rs113948889	rs34730825	12	2,064,602	39,568	C/T	1	hsa-miR-3147	DCP1B	ENST00000543381
rs113948889	rs111963484	12	2,102,086	2,084	C/A	1	hsa-miR-4270	DCP1B	ENST00000535873
rs113948889	rs111963484	12	2,102,086	2,084	C/A	1	hsa-miR-4441	DCP1B	ENST00000535873
rs113948889	rs111963484	12	2,102,086	2,084	C/A	1	hsa-miR-505-5p	DCP1B	ENST00000535873
rs113948889	rs112637373	12	2,102,271	1,899	G/T	1	hsa-miR-2909	DCP1B	ENST00000535873
rs113948889	rs34730825	12	2,064,602	39,568	C/T	1	hsa-miR-92a-1-5p	DCP1B	ENST00000543381

Legend: Predicted miRNA target site SNPs in linkage disequilibrium with GWAS SNP rs113948889 listed in descending order of predicted effect on miRNA-to-mRNA binding. Minor alleles are listed first in "SNP allele" column. CHR = chromosome; BP = base-pair position on CHR; LD = linkage disequilibrium. Genomic annotations based on human genome (hg) build 19, transcript IDs according to ENSEMBL v.75. Shaded entries highlight SNPs assessed *in vitro*.

Supplementary Methods

Details on cognitive testing procedures assessing memory performance. For the genetic association analyses of this study, we used a total of 13 quantitative measures of episodic memory and working memory derived from: (a) the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) cognitive battery (Morris *et al.* 1989) on word list memory and word list recall; (b) forward and backward serial recall paradigms (Li *et al.* 2010, Li *et al.* 2013); (c) associative memory tasks that assessed item memory as well as item-pair recognition (Preuschhof *et al.* 2010); and (d) an image recognition test at retention intervals of 2.5 hours and one week (Papenberg *et al.* 2011). Below we describe all non-CERAD related procedures in more detail. Information on the CERAD cognitive test battery can be found in Morris *et al.* (1989) and URL: http://www.memoryclinic.ch/content/view/37/47/). See also Supplementary Table 1 for a summary of cognitive assessments.

1. Forward and backward serial recall task. For this task, participants were presented with six different lists of 12 words each. After the presentation of the last item in each list, the participants were asked to recall each word at its correct position. Word Lists 1 to 3 were recalled in forward order (from the first word to the last word of the list), whereas Word Lists 4 to 6 were recalled in backward order. Recall was self-paced. For each list, responses were scored using a strict serial-recall criterion: an accurate response required that both the identity of a word and its serial position were correct.

2. Item and pair associative episodic memory task. The item and pair associative episodic memory task (henceforth labeled as the "item-pair" task) had four conditions that were tested sequentially in one session. During an initial study phase, participants were visually presented with pairs of unrelated words and were instructed to study each pair under two conditions: either as two single words (item instruction) or as a pair of words (pair instruction). The study phase of each condition contained 30 pairs of semantically unrelated words. In the test phrase, subjects in one condition (item recognition) were asked whether they had seen the presented word during the study phase. Half of the words were old (target items), and the other half were new (distractor items). In the second condition (associative recognition), subjects had to decide whether a presented pair of words had been presented during the study phase. Half of the presented word pairs were old (target pairs), and the others were formed by recombining

words in the previously studied lists (rearranged distractor pairs). Taken together, by crossing over the two instruction conditions with the two test conditions, the task resulted in four conditions that assessed item memory (item–item and pair-item tests) and associative memory (pair-pair and item-pair tests). Recognition memory performance was measured as hits minus false alarms to minimize the effects of potential individual differences in response bias.

3. Image recognition memory with retention. Performance in the image recognition memory task was assessed at two retention intervals: 2.5 hours and one week. At the beginning of the first session, participants were presented with 48 complex, colored images of scenes of neutral emotional valence; all were derived from the International Affective Picture System (Lang *et al*. 1997). The images were encoded incidentally: during the study phase, participants were required to determine whether the scene was "indoor" or "outdoor"—there were 24 scenes in each category—without explicit requirement of memorization. During retrieval, participants viewed each image for 3 seconds and were asked to determine whether each scene had been presented ("old") or not presented ("new) during encoding. In each retrieval test, 24 unique old scenes and 24 unique new scenes (lures) were presented. Taking response bias into account, memory performance was measured as hits minus false alarms.

Genome-wide SNP genotyping, quality control and imputation procedures. DNA from all BASE-II participants was extracted from whole blood using standard procedures and then subjected to microarray-based SNP genotyping using the "Genome-Wide Human SNP Array 6.0" (Affymetrix, Inc.), according to the manufacturer's instructions. Prior to imputation (see below), we applied the following quality control (QC) filters which led to an exclusion of SNPs that 1) violated Hardy-Weinberg equilibrium (HWE) at $p \leq 1e{-}6$, and 2) showed call rate less than 98%. This filtering resulted in 829,344 autosomal SNPs in 1,946 BASE-II participants. Of these individuals, 214 were excluded from subsequent analysis because 1) they were missing information on "age" or "years of education" (used as co-variates in the linear regression model, see below), 2) showed call rates <95%, 3) showed evidence for sample duplication, relatedness or contamination, 4) showed inconsistencies between recorded and genotypic sex, 5) showed excessive heterozygosity, or 7) represented population outliers. The latter was determined using the EIGENSOFT program (Price *et al.* 2006). As all samples in our study were of self-reported Caucasian descent, we excluded ethnic outliers

using the “Eigenstrat” function in EIGENSOFT with iterative outlier removal. After the above filtering steps, principal components (PC) were computed again for the 1,732 remaining samples. Based on examination of the scree plot, the first three PCs were retained and used as covariates during the association analysis in order to adjust for potential residual population stratification. Genome-wide imputation of unobserved genotypes was carried out on the QC'ed data using the IMPUTE2 [v2.2.2] software (Marchini *et al.* 2007) based on precompiled "ALL 1000G phase1interim" reference panels from the IMPUTE website (June 2011 release). As suggested by Southam *et al.* (2011) ensure an appropriate level of quality for imputed SNPs, we also applied post-imputation QC filtering including only SNPs with IMPUTE-information thresholds larger than or equal to 0.8 and minor allele frequencies at or above 5%. After this post-imputation filtering, a total of 5,371,945 high-quality SNPs were retained for all subsequent association analyses.

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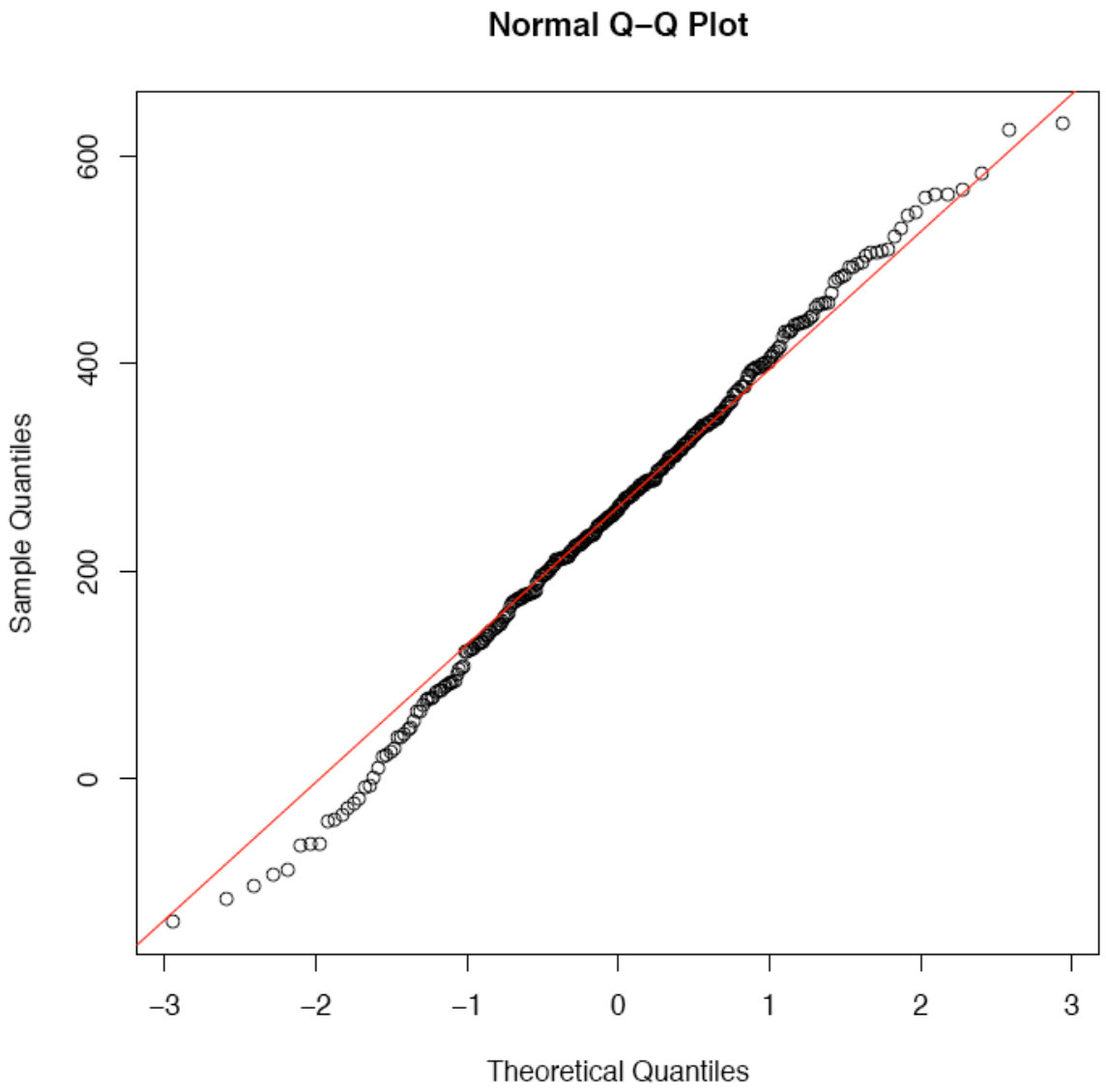
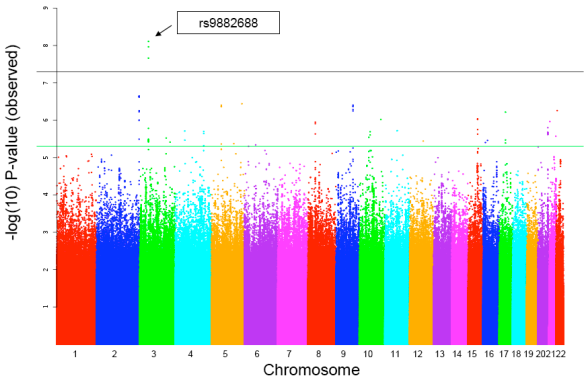
Supplement Figures

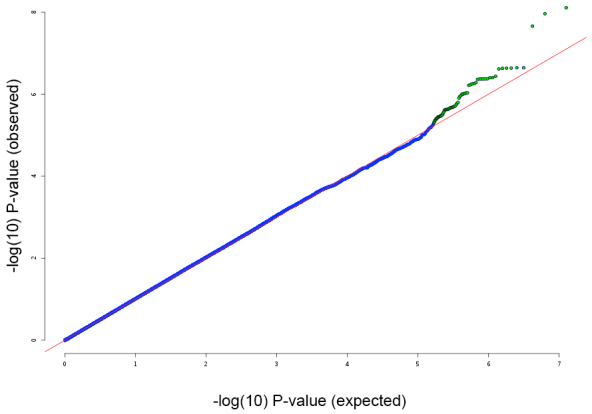


Supplement Figure 1: Quantile-quantile plot of the hsa-miR-138-1-5p expression levels in peripheral blood mononuclear cells across 308 individuals of European descent.

Suppl. Figure 2a: Manhattan plot of GWAS results for trait "WL_save"

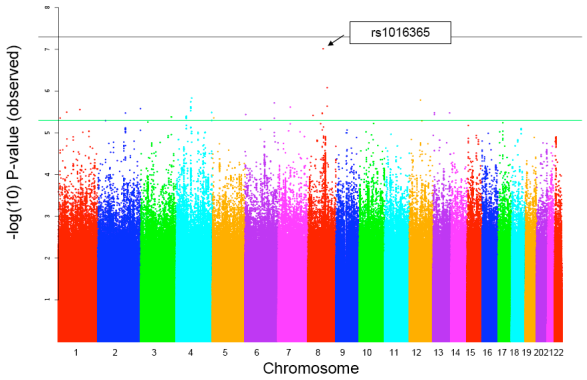


Suppl. Figure 2b: Q-Q plot of GWAS results for trait "WL_save" (lambda: 1.009)

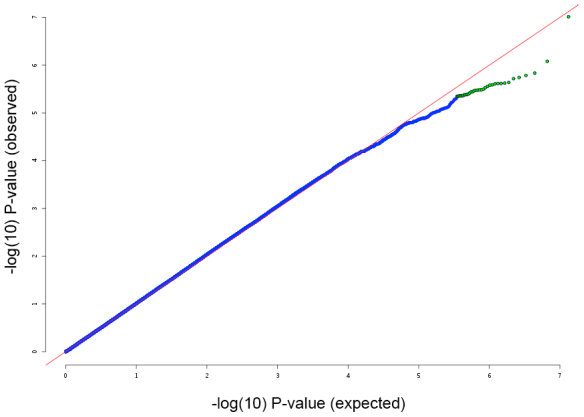


Supplement Figure 2: *(a)* Manhattan plot of GWAS results for trait "WL_Save". The SNP rs9882688 (P-value: 7.8 x 10^{-9}) on human chromosome 3 is located ~20kb upstream of hsa-miR-138-1. *(b)* Quantile-quantile plot of the GWAS results for trait "WL-save".

Suppl. Figure 3a: Manhattan plot of GWAS results for trait "ItemItem"

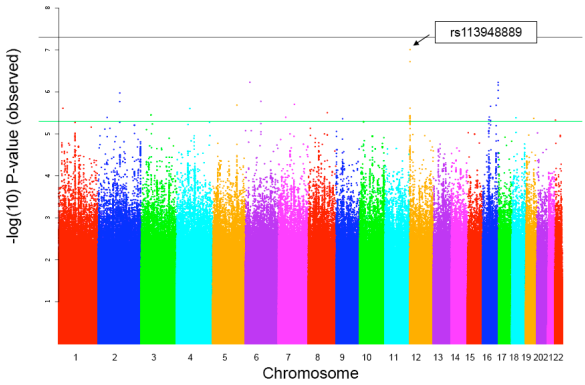


Suppl. Figure 3b: Q-Q plot of GWAS results for trait "ItemItem" (lambda: 1.009)

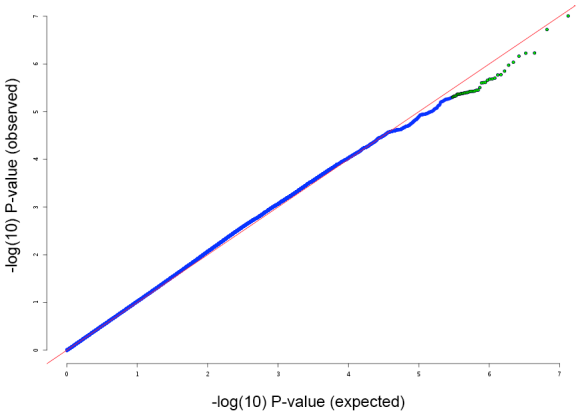


Supplement Figure 3: *(a)* Manhattan plot of GWAS results for trait "ItemItem". The SNP rs1016365 (P-value: **9.7 x 10^{-8}**) on human chromosome 8 is located ~6kb downstream of *EYA1* (**Homo sapiens eyes absent homolog 1 [Drosophila]**). *(b)* Quantile-quantile plot of the GWAS results for trait "ItemItem".

Suppl. Figure 4a: Manhattan plot of GWAS results for trait "TFEUWC"



Suppl. Figure 4b: Q-Q plot of GWAS results for trait "TFEUWC" (lambda: 1.027)



Supplement Figure 4: *(a)* Manhattan plot of GWAS results for trait "TFEUWC". The SNP rs113948889 (P-value: 9.9 x 10^{-8}) on human chromosome 12 is located in an intron of *DCP1B* (**encoding decapping mRNA 1B)**. *(b)* Quantile-quantile plot of the GWAS results for trait "TFEUWC".

Supplementary Table 1: Summary of cognitive measures / traits assessed by genome-wide association screening.

Domain	Test (abbrev)	Description	Reference for details
WM	TCSWM	T-score of spatial working memory	Nagel *et al.* (2008), Papenberg et al. 2011)
WM	TFEUWC	T-score of spatial working memory combined with tasks testing frontal executive control (here: Wisconsin Card Sorting Test)	Nagel *et al.* (2008)
EM	CERAD_WL_learn	CERAD battery: Word list learning	Guerrero-Berroa *et al.* (2014)
EM	CERAD_WL_recall	CERAD battery: Word list recall	Guerrero-Berroa *et al.* (2014)
EM	CERAD_WL_save	Proportion of learned words vs. recalled words (max. 100%)	Guerrero-Berroa *et al.* (2014)
EM	Item_Item	Item instruction & item test (proportion of hits minus false alarms)	Preuschhof *et al.* (2010)
EM	Item_Pair	Item instruction & associative test (proportion of hits minus false alarms)	Preuschhof *et al.* (2010)
EM	Pair_Pair	Pair instruction & associative test (proportion of hits minus false alarms)	Preuschhof *et al.* (2010)
EM	SRFO	Serial recall: accuracy of forward recall	Li *et al.* (2010), Papenberg *et al.* (2011), Li *et al.* (2012)
EM	SRBK	Serial recall: accuracy of backward recall	Li *et al.* (2010), Papenberg *et al.* (2011), Li *et al.* (2012)
EM	Delayed_recall	Image recognition after 2.5 hours (proportions of hits minus false alarms)	Papenberg *et al.* (2011)
EM	Delayed_recall	Image recognition after 1 week (proportions of hits minus false alarms)	Papenberg *et al.* (2011)

Abbreviations: WM = working memory; EM = episodic memory; CERAD = Consortium to Establish a Registry for Alzheimer's Disease.

Supplementary Table 2.
Results of GWAS analyses of 13 episodic and working memory traits in 1,318 individuals of BASE-II aged 60 or above.
Info: imputation information score as determined by IMPUTE2.2 (a score of "1" indicates that this SNP was genotyped directly)
MAF: minor allele frequency (here for allele_B)
beta: regression coefficient; SE: standard error
Trait: abbreviation of trait designation (See supplementary material for more details); Domain: EM = episodic memory, WM = working memory

SNP	CHR	BP	allele_A	allele_B	Info	MAF	P-value	beta	SE	Trait	Domain
rs9882688	3	44135356	A	G	0.66004	0.066827	7.77E-09	-0.61338	0.10376	WL_Save	EM
rs1016365	8	72280705	G	A	0.93044	0.35753	9.67E-08	-0.22841	0.042592	itemitem	EM
rs113948889	12	2104170	G	C	0.5194	0.037448	9.85E-08	-0.69077	0.12689	TFEUWC	WM
rs8110914	19	13434717	G	A	0.97064	0.20285	1.11E-07	-0.30298	0.056642	WL_Abruf	EM
7-53196334	7	53196334	C	T	0.62393	0.40619	1.14E-07	0.26082	0.048727	TCSRBK	EM
rs63560662	1	167506734	T	C	0.99201	0.38245	1.25E-07	0.24714	0.046423	WL_Lern	EM
5-79621077	5	79621077	A	T	0.78704	0.0054652	1.48E-07	1.6806	0.31229	WL_Lern	EM
rs4655090	1	23011945	C	T	0.84923	0.24484	1.48E-07	0.29015	0.0549	Fig_Sav	EM
rs10990641	9	106002821	G	C	0.98041	0.26868	1.50E-07	-0.27139	0.051282	WL_Lern	EM
rs11089441	22	30654464	G	T	0.911	0.30793	1.52E-07	-0.22353	0.042341	TCSRFOBK	EM
rs6761699	2	240736848	C	T	1	0.09001	2.27E-07	0.40453	0.07761	WL_Save	EM
rs9321270	6	131344887	C	A	0.98518	0.28702	2.90E-07	-0.21326	0.041351	pairpair	EM
11-71309373	11	71309373	C	T	0.65963	0.013155	3.34E-07	-0.95495	0.18243	pairpair	EM
rs2835657	21	38565064	T	C	0.99811	0.46762	3.60E-07	0.19745	0.038605	pairpair	EM
rs9511907	13	26313986	G	A	0.81026	0.2671	3.77E-07	-0.24528	0.047973	TCSRFO	EM
rs2822532	21	15650154	T	C	0.95952	0.062251	5.33E-07	-0.40776	0.08098	TCSRFOBK	EM
rs12969935	18	32725041	C	G	0.99726	0.10328	5.40E-07	-0.32571	0.064654	TCSWM	WM
rs13092345	3	162492109	A	T	0.9887	0.34313	5.61E-07	0.21192	0.04214	TCSRBK	EM
rs73062693	19	54680082	G	A	0.39675	0.03091	6.14E-07	0.83448	0.15886	itempair	EM
rs9756899	3	177112336	C	G	0.53287	0.25753	7.22E-07	0.34493	0.068266	Fig_abr	EM
rs10937682	4	5839601	G	A	0.95592	0.34329	9.83E-07	-0.24359	0.04941	Fig_abr	EM
rs7330783	13	82065791	C	T	0.93016	0.34114	1.04E-06	0.24022	0.048853	Fig_Sav	EM
rs76226947	12	40135628	G	A	0.93984	0.058721	1.14E-06	0.41973	0.085998	TCSWM	WM
rs1405376	4	72515164	C	A	0.99782	0.37033	1.81E-06	0.19509	0.040686	itemitem	EM
rs11645369	16	52950064	G	A	0.98773	0.24733	2.20E-06	0.21538	0.045279	TFEUWC	WM
rs10403211	19	43889542	T	C	0.52322	0.15612	5.70E-06	0.33299	0.072807	TCSWM	WM
rs114356618	2	207168168	T	C	0.66647	0.033748	6.18E-06	-0.60234	0.13169	TFEUWC	WM
9-90738260	9	90738260	C	A	0.50791	0.32505	7.05E-06	0.30939	0.067182	WL_Save	EM

Supplementary Table 3.

Summary of known transcripts for *DCP1B* [ENSEBL gene ID: ENSG00000151065] according to ENSEMBL v.71 (April 2013) and impact of SNP rs1044950.

Transcripts tested by luciferase reporter assay are shaded in grey. Note that the biotype for ENST00000541700 was reclassifed to "processed transcript" in v.72 (this classification is current until the day of this writing, i.e. v.75).
nc = non-coding; NMD = nonsense-mediated decay

Variation Name	CHR	BP	Ensembl Transcript ID	Biotype	Consequence to transcript	Residue change	SIFT prediction	SIFT score	PolyPhen prediction	PolyPhen score
rs1044950	12	2061982	ENST00000397173	protein_coding	missense_variant	Ala273Val	tolerated	0.41	benign	0.004
rs1044950	12	2061982	ENST00000540622	protein_coding	missense_variant	Ala249Val	tolerated	0.40	benign	0.048
rs1044950	12	2061982	ENST00000280665	protein_coding	missense_variant	Ala375Val	tolerated	0.56	benign	0.002
rs1044950	12	2061982	ENST00000541700	protein_coding	3_prime_UTR_variant	-	-	-	-	-
rs1044950	12	2061982	ENST00000536665	processed_transcript	nc_transcript_variant	-	-	-	-	-
rs1044950	12	2061982	ENST00000536665	processed_transcript	non_coding_exon_variant	-	-	-	-	-
rs1044950	12	2061982	ENST00000543381	nonsense_mediated_decay	NMD_transcript_variant	-	-	-	-	-
rs1044950	12	2061982	ENST00000543381	nonsense_mediated_decay	3_prime_UTR_variant	-	-	-	-	-

Supplementary Table 4.

Enrichment analysis of GWAS results for traits "TFEUWC" and "WL_Save" in the BASE-II data set.

Tabulation of GWAS results showing "significant" (i.e. P values at or lower than the cutoff indicated in first column) vs. "non-significant" (P-value > cutoff) P-values.
Genes estimated to contain at least one hsa-mir-138-5p target site predicted by at least three of the algorithms described in the methods were included in "target" group; genes w/o a target site predicted in any algorithm were included in "control" group. Genes were matched by genomic size based on longest transcript predicted to contain miRNA-138 site. SNPs were pruned based on r2 = 0.8 per gene.
P-value for enrichtment: based on 100,000 permutations in which "target" and "control" status of each SNP was resampled randomly.
Shaded rows highlight GWAS P-value cutoffs showing a significant enrichment in genes with predicted miRNA-138 site vs. those without.

	# SNPs in genes with miRNA-138 target sites		# SNPs in genes without miRNA-138 target sites			
GWAS P-value cutoff	**significant**	**non-significant**	**significant**	**non-significant**	**Chi square**	**P-value for enrichment**
0.0001	64	32271	3	31856	52.90334587	0.04084
0.0005	218	32117	43	31816	113.9032676	0.01686
0.0010	252	32083	78	31781	88.60683064	0.02549
0.0050	864	31471	931	30928	3.605219979	0.35887
0.0100	1760	30575	1792	30067	0.979849523	0.43692

Dopamine and glutamate receptor genes interactively influence episodic memory in old age

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Aging and KIBRA/WWC1 Genotype Affect Spatial Memory Processes in a Virtual Navigation Task

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Assessment of microRNA-related SNP effects in the 3′untranslated region of the IL22RA2 risk locus in multiple sclerosis

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Aging magnifies the effects of dopamine transporter and D2 receptor genes on backward serial memory

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4. Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

5. Publikationsliste

Julia Schröder, Sara Ansaloni, Marcel Schilling, Tian Liu, Josefine Radke, Marian Jaedicke, Brit-Maren M. Schjeide, Andriy Mashychev, Christina Tegeler, Helena Radbruch, Goran Papenberg, Sandra Düzel, Ilja Demuth, Frank Heppner, Nina Bucholtz, Ulman Lindenberger, Shu-Chen Li, Elisabeth Steinhagen-Thiessen, Christina M. Lill, Lars Bertram. (2014). MiRNA-138 affects memory performance in the elderly. Accepted by Frontiers in Human Neuroscience. doi: 10.3389/fnhum.2014.00501

Tian Liu, Shu-Chen Li, Goran Papenberg, **Julia Schröder**, Johannes T. Roehr, Wilfried Nietfeld, Ulman Lindenberger, Lars Bertram (2014). No Association between CTNNBL1 and Episodic Memory Performance. Accepted by Translational Psychiatry.

Christina M. Lill, Marcel Schilling, Sara Ansaloni, **Julia Schröder**, Marian Jaedicke, Felix Luessi, Brit-Maren M. Schjeide, Andriy V. Mashychev, Christiane Graetz, Denis A. Akkad, Lisa-Ann Gerdes, Antje Kroner, Paul Blaschke, Sabine Hoffjan, Alexander Winkelmann, Thomas Dörner, Peter Rieckmann, Elisabeth Steinhagen-Thiessen, Ulman Lindenberger, Andrew Chan, Hans-Peter Hartung, Orhan Aktas, Peter Lohse, Mathias Buttmann, Tania Kümpfel, Christian Kubisch, Uwe K. Zettl, Joerg T. Epplen, Frauke Zipp, Lars Bertram. Assessment of miRNA-related SNP effects in the 3´untranslated region of the *IL22RA2* risk locus in multiple sclerosis. Neurogenetics 2014

Nicolas W. Schuck, Peter A. Frensch, Brit-Maren M. Schjeide, **Julia Schröder**, Lars Bertram, Shu-Chen Li „Effects of aging and dopamine genotypes on the emergence of explicit memory during sequence learning.“ Neuropsychologia 2013; 51 (13), 2757-69.

Nicolas W. Schuck, Christian F. Doeller, Brit-Maren M. Schjeide, **Julia Schröder**, Peter A. Frensch, Lars Bertram, Shu-Chen Li „Aging and KIBRA polymorphism affect spatial memory processes in a virtual navigation task.“ Hippocampus 2013; 23 (10), 919-930.

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6. Eidesstattliche Versicherung

„Ich, Julia Schröder, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Die funktionelle Charakterisierung genomweiter Assoziationssignale der Berliner Altersstudie II (BASE-II)“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -www.**icmje**.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o.) und werden von mir verantwortet.

Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o.) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Berlin, 24.06.2014 _____

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