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Identifikation genetischer und epigenetischer Faktoren, die die Entstehung und Progression der Parkinson- und Alzheimer-Krankheit beeinflussen

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AD	Alzheimer-Krankheit (Alzheimer‘s disease)
CI	Konfidenzintervall (confidence interval)
GBA	GBA-Gen (Glucocerebrosidase Gen)
GWAS	Genomweite Assoziationsstudie (Genome-wide association study)
HR	Hazard-Ratio
H&Y	Hoehn & Yahr
IQR	Interquartilsabstand (interquartile range)
mRNA	messengerRNA
miRNA	miRNA
MMST	Mini-Mental-Status-Test
NGS	next generation sequencing
PD	Parkinson-Krankheit (Parkinson‘s disease)
PRS	Polygenetischer Risikoscore
SNCA	α-Synuclein-Gen
SNP	Einzelbasenpaaraustausch (single-nucleotide polymorphism)
UPDRS-III	Unified Parkinson’s Disease Rating Scale part III

1 Einleitung

Die Parkinson-Krankheit (Parkinson's disease, PD) ist die zweithäufigste neurodegenerative Erkrankung nach der Alzheimer-Krankheit (Alzheimer‘s disease, AD) und betrifft etwa 1 % der Menschen über 60 Jahre. Die Inzidenz steigt mit zunehmendem Alter und ist bei Männern höher als bei Frauen. Da in den Industrienationen der Anteil älterer Menschen immer größer wird, erhöht sich auch die Zahl der Personen, die an der PD erkranken. Dies führt nicht nur zu mehr individuellem Leid, sondern auch zu einer zunehmenden sozio-ökonomischen Belastung.

Ätiologisch kann die PD in zwei Gruppen unterteilt werden, dabei unterscheidet man die monogene Form (~5-10 % aller Fälle) von der genetisch-komplexen Form (>90%). Das Hauptsymptom ist die Bradykinese, welches für die Diagnose einer PD in Kombination mit wenigstens einem weiteren Symptom wie Ruhetremor, Rigor und/oder posturale Instabilität auftreten muss. Diese zumindest am Anfang meist einseitig betonten motorischen Symptome sprechen bei der PD klassischerweise auf eine Dopamin-Ersatztherapie an und werden häufig von weiteren nicht-motorischen Symptomen begleitet. Dazu gehören beispielsweise vegetative Störungen (Hypotension, Obstipation, sexuelle Funktionsstörung), psychische Symptome (Depression, Angst, Demenz), Störungen des Schlafverhaltens, des Riechens und des Farbsehens. All diese Symptome haben einen Einfluss auf die Lebensqualität, wobei sich besonders die nicht-motorischen Symptome wie depressive Stimmung und Schlafstörungen negativ auf die Lebensqualität auswirken.

Pathophysiologisch ist die PD definiert als Untergang dopaminerger Neurone in der Substantia Nigra pars compacta und des Locus caeruleus. Außerdem findet man Proteinablagerungen, sogenannte Lewy-Körper, die vor allem aus Aggregationen des Proteins α-Synuclein (SNCA) bestehen. Dabei können nach Braak et al. sechs Stadien unterschieden werden, wobei die Medulla oblongata typischerweise zuerst befallen wird (Stadium 1) und sich die pathologischen Veränderungen dann über den Hirnstamm (Stadium 2) und das Mittelhirn inklusive Substantia Nigra (Stadium 3) bis zum zerebralen Kortex (Stadium 4-6) ausbreiten.

Für die klassischen monogenen Parkinsonformen konnten bisher ursächliche Veränderungen (Mutationen) in sechs Genen identifiziert werden. Diese sind *SNCA*, *LRRK2*, *VPS35*, *PRKN*, *PARK7* (auch bekannt als *DJ-1*) und *PINK1*. Im Gegensatz zu den monogenen Formen der PD, die auf Mutationen eines einzelnen Gens beruhen, geht man bei der genetisch-komplexen Form von einer multifaktoriellen Genese der Erkrankung aus. Es wird angenommen, dass hierbei genetische Varianten, Umweltfaktoren und möglicherweise auch epigenetische Prozesse und Interaktionen zwischen den einzelnen Faktoren einen Einfluss auf die Entstehung der PD haben. Man geht

heutzutage von ca. 90 Einzel-Nukleotidpolymorphismen (single-nucleotide polymorphism, SNPs) aus, die das Risiko für die genetisch-komplexe Form der PD erhöhen (Nalls et al. 2019). Neben diesen genetischen Einflussfaktoren gibt es zahlreiche Umwelt-/Lebensstilfaktoren, die mit der Entstehung der PD assoziiert sind. Wie in der Metaanalyse von Noyce et al. aus dem Jahr 2012 hervorgeht werden z. B. eine positive Familienanamnese (, die eine Kombination von genetischen und Umwelt-/Lebensstilfaktoren darstellt), Konstipation, affektive Störungen und Pestizidbelastungen mit der Erhöhung des Risikos einer späteren PD in Verbindung gebracht. Rauchen, Kaffee- und Alkoholkonsum hingegen werden mit einem niedrigeren PD-Risiko assoziiert. Bei den genannten Variablen handelt es sich nicht nur um Umwelt-/Lebensstilfaktoren, sondern auch um Frühsymptome (wie z. B. Konstipation, affektive Störungen) oder auch um Komorbiditäten. Prinzipiell kann aber durch solche Metaanalysen keine Aussage zu Ursache-Wirkungsbeziehungen getroffen werden. Die Kenntnis über diese Risiko- oder Prodromalfaktoren kann aber hilfreich sein, um die PD früher zu diagnostizieren.

Diese genetischen und umweltbedingten Risikofaktoren der PD können die Krankheitsentstehung allein nicht erklären. Daher ist es möglich, dass weitere Faktoren wie epigenetische Prozesse eine Rolle spielen. Unter Epigenetik versteht man all die Prozesse, die an der Regulation der Genexpression beteiligt sind, die aber nicht durch eine Veränderung der DNA-Sequenz selbst bestimmt werden. So könnte erklärt werden, dass dieselben Gene in unterschiedlichen Zellen unterschiedlich exprimiert werden. Zu den epigenetischen Mechanismen zählen beispielsweise DNA-Methylierung, Histonmodifikation sowie RNA-basierte Mechanismen. Zu den RNA-basierten Mechanismen gehört auch die Regulation der Genexpression und/oder Proteintranslation durch sog. micro-RNAs (miRNAs). MiRNAs sind kleine (~22 Basenpaare lange), nicht-kodierende RNAs, deren Funktion hauptsächlich die posttranskriptionelle Regulation der Genexpression ist. Durch das Binden der miRNAs an bestimmte messengerRNAs (mRNAs) kommt es entweder zum Abbau der mRNA oder zur Hemmung der Translation.

Im Gehirn scheinen miRNAs an allen neuronalen Prozessen beteiligt zu sein, inklusive der Entstehung neurodegenerativer Erkrankungen wie der PD und der AD. Beim Menschen gibt es nach derzeitigem Kenntnisstand mehr als 2000 verschiedene miRNAs. Dabei ist die Verteilung der verschiedenen miRNAs in unterschiedlichen Geweben sehr heterogen. Die Identifikation der miRNAs, die eine Bedeutung bei der Entstehung (und Progression) von Erkrankungen, insbesondere Erkrankungen des Gehirns wie etwa der PD oder der AD haben, stellt eine große Herausforderung dar. Einige Studien haben bereits differentiell exprimierte miRNAs in Parkinson-Patienten im Vergleich zu gesunden Kontrollen beschrieben, aber bisher sind die Ergebnisse der einzelnen Studien häufig nicht konsistent. Zum Teil liegt es sicherlich daran, dass einzelne Studien jeweils relativ kleine Stichproben untersucht haben und viele Studien unterschiedliche Gewebe,

z. B. Gewebe aus verschiedenen Hirnarealen oder aus dem Blut und seinen zellulären (und nicht-zellulären) Unterfraktionen, für die Analyse differentieller miRNA-Expression nutzten, was die Vergleichbarkeit der Ergebnisse erschwert. Mit wachsender Anzahl an Veröffentlichungen über differentiell exprimierte miRNAs bei Parkinson-Patienten und Kontrollen ist es schwierig einen Überblick zu behalten und zu entscheiden, welche miRNAs über mehrere Studien hinweg vergleichbare Ergebnisse zeigen. Es gibt einige *qualitative* Übersichtsarbeiten, die naturgemäß subjektiv geprägt sind. Sie beziehen sich jeweils auf Sammlungen unterschiedlicher Einzelstudien und heben aus diesem Grund zum großen Teil unterschiedliche miRNAs als relevant für die PD hervor. Eine weniger subjektive, systematischere und umfassendere Zusammenfassung und Interpretation der verfügbaren publizierten Daten ist möglich durch sog. *systematischen* Übersichtsarbeiten. Diese können durch Metaanalysen über die publizierten Daten der für in den systematischen Übersichtsarbeiten zusammengetragenen Artikeln auch eine *quantitative* Beurteilung der vorliegenden Evidenz für die Rolle der jeweiligen miRNAs ermöglichen.

Eine Metaanalyse ermöglicht die Quantifizierung der Evidenz für die Assoziation eines Faktors mit einem Phänotyp/ Ereignis (z. B. wie in diesem Fall die differentielle Expression einer miRNA in Erkrankten und Gesunden) mittels Zusammenfassung der Effekte einzelner Studienergebnisse in einem Summeneffekt. Dabei gibt es verschiedene Ansätze, wozu einerseits Effektgrößen-basierte Metaanalysen zählen. Dazu werden einheitliche Effektschätzer und entsprechende Varianzen benötigt, aus denen dann ein Gesamteffektschätzer als gewichteter Durchschnitt des Effekts der Einzelstudien gebildet wird. Je nach angewendetem Modell können die Studien unterschiedlich gewichtet werden. Beim „Fixed-Effect-Modell“ nach der „generic inverse variance Methode” ist das Gewicht jeder Studie die umgekehrte Varianz des Effektschätzers. So erhalten größere Studien typischerweise eine höhere Gewichtung als kleinere Studien. Beim „Fixed-Effect-Modell“ geht man davon aus, dass es nur *einen* globalen Effekt in allen Populationen gibt. Eine Voraussetzung für das Modell ist dementsprechend, dass Stichproben und Versuchsbedingungen vergleichbar sein sollten. Das „Random-Effects-Modell“ nach DerSimonian und Laird hat diese Voraussetzung nicht. Es geht von *mehreren* zugrundeliegenden wahren Effekten aus und berücksichtigt deshalb zusätzlich die Effektunterschiede zwischen den Populationen, die sog. Heterogenität zwischen den Studien, in der Gewichtung der Studien.

Andererseits kann man bei fehlenden Informationen zu Effektgrößen auch p-Wert-basierte Metaanalysen durchführen, die z. B. auf Stouffer`s Methode beruhen. Dabei werden p-Werte in Z-Werte umgeformt und die einzelnen Z-Werte unterschiedlicher Studien zu einer gewichteten Summe zusammengefasst, wobei die Gewichte proportional zur Wurzel der einzelnen Stichprobengrößen sind. Auch hier erhalten größere Studien durch die Gewichtung einen größeren Einfluss auf das Metaanalyseergebnis.

Indem man den kumulativen Effekt einzelner miRNAs mittels Metaanalysen bestimmt, kann man prinzipiell erkennen, welche miRNAs über mehrere Studien hinweg konstante und signifikante differentielle Expressionsunterschiede zeigen. Solche Ansätze wurden bei der PD (wie bei vielen anderen multifaktoriellen Erkrankungen) schon in anderen Zusammenhängen erfolgreich genutzt. Die Schwierigkeit bei Genexpressionsstudien besteht darin, dass sie sehr heterogen aufgebaut sind und dass Methoden und Studienergebnisse inkonsistent dargestellt werden. Hinzu kommt, dass verschiedene Gewebe in den Einzelstudien untersucht wurden.

So war es das Ziel der ersten Publikation dieser Dissertation (Schulz, Takousis et al. 2019), eine entsprechende Analysestrategie für die sehr heterogenen publizierten miRNA-Genexpressionsdaten zu entwickeln und diese auf das Feld der PD anzuwenden. Dies beinhaltete eine systematische Literatursuche, Extraktion relevanter Daten aus den Publikationen sowie die Wahl und Anwendung einer für die Genexpressionsdaten geeigneten Metaanalysemethode, um unter den über 1004 in der Literatur im Zusammenhang mit PD reportierten miRNAs diejenigen zu identifizieren, die bei PD-Patienten hochsignifikante und konsistente Effekte zeigen.

Auch bei der häufigsten neurodegenerativen Erkrankung, der AD, vermutet man, dass miRNAs eine wichtige Rolle bei der Krankheitsentstehung spielen. Die publizierte Literatur in Bezug auf eine differentielle Genexpression von miRNAs bei der AD ist im Vergleich zur PD erheblich umfangreicher und entsprechend noch unübersichtlicher. Insofern ist der von uns oben beschriebene Ansatz auch und gerade in diesem Feld essentiell, um für die AD relevante miRNAs zu erkennen. Aus diesem Grund war das Ziel der zweiten Publikation (Takousis et al. 2019) den oben erläuterten Ansatz der systematischen Metaanalyse von miRNA-Genexpressionsdaten auf die AD zu übertragen, d. h. alle bis dato veröffentlichten miRNA-Expressionsstudien für die AD zu identifizieren, die relevanten Daten aus den Publikationen zu exzerpieren und mit Hilfe von Metaanalysen alle konsistent und signifikant differentiell exprimierten miRNAs zu identifizieren.

Bei der Erforschung von Einflussfaktoren auf den Verlauf der PD ist bisher größtenteils auch die Rolle genetischer Faktoren ungeklärt. Fraglich ist, ob genetische Risikovarianten (Nalls et al. 2019) auch einen Einfluss auf den Krankheitsverlauf haben. Da die Symptomprogression bei der PD sehr heterogen ist, könnte die Entdeckung von beeinflussenden Faktoren des Krankheitsverlaufs zusätzliche Hinweise auf pathophysiologische Prozesse geben. Bisherige Untersuchungen zeigten z. B., dass einige dieser genetischen Varianten das Risiko für einen frühen Krankheitsbeginn erhöhen. Bis zum Beginn meiner Dissertationsarbeit hatte nur die Studie von Pihlstrøm et al. (siehe Referenz 9 in Paul, Schulz et al. 2018) den Einfluss eines aus 19 SNPs bestehenden sog. polygenetischen Risikoscores (PRS) auf den Krankheitsfortschritt (spezifisch auf die motorische Progression) und auf das Überleben untersucht, wobei ein hoher PRS mit einem schnelleren Krankheitsverlauf assoziiert

war, aber keinen Einfluss auf die Mortalität hatte. Der PRS eines Individuums wird aus der Summe aller seiner genetischen Risikoeffekte berechnet, wobei jedes einzelne Risikoallel an mit der Erkrankung assoziierten SNPs eine Gewichtung entsprechend seiner Effektgröße erhält.

Neben den typischen motorischen Symptomen der PD gibt es aber auch wie oben beschrieben zahlreiche nicht-motorische Symptome wie kognitive Störungen, die häufig (ca. 90 %) im Verlauf der PD auftreten. Bis zum Beginn unserer Studie gab es meines Wissens nach noch keine Publikation, die mittels des PRS den kumulativen genetischen Einfluss auf die kognitive Leistungsfähigkeit beschrieben hatte. Daher war es das Ziel der dritten Publikation (Paul, Schulz et al. 2018), anhand von Daten einer longitudinalen Studie erstmalig die Assoziation des PD-PRS mit kognitiver Leistungsfähigkeit im Verlauf zu untersuchen und den Zusammenhang dieses PRS auf die motorische Progression und Mortalität erneut zu überprüfen.

2 Zusammenfassungen der Publikationen

Die Grundlage dieser kumulativen Dissertation bilden folgende Publikationen (*geteilte Erstautorenschaft):

1. Schulz J*, Takousis P*, Wohlers I, Itua I, Dobricic V, Ruecker G, Binder H, Middleton L, Ioannidis JPA, Perneczky R, Bertram L, Lill CM. Meta-analyses identify differentially expressed miRNAs in Parkinson's disease. Ann Neurol. 2019 Jun;85(6):835-851.

2. Takousis P, Sadlon A, Schulz J, Wohlers I, Dobricic V, Middleton LT, Lill CM, Perneczky R, Bertram L. Differential expression of miRNAs in Alzheimer's disease brain, blood and cerebrospinal fluid. Alzheimers Dement. 2019 Sep 5. pii: S1552-5260(19)35119-2.

3. Paul KC*, Schulz J*, Bronstein JM, Lill CM, Ritz BR. Association of Polygenic Risk Score With Cognitive Decline and Motor Progression in Parkinson Disease. JAMA Neurol. 2018 Mar 1;75(3):360-366.

2.1 Publikation 1 (Schulz*, Takousis*, et al. Annals Neurol, Ann Neurol. 2019;85(6):835-851): Metaanalysen identifizieren differentiell exprimierte miRNAs in der Parkinson-Krankheit

Einleitung: Wie oben erläutert regulieren miRNAs die Genexpression. Die Veränderung der Genexpression ist ein wichtiger Parameter für die Entstehung von Erkrankungen, und es verdichten sich die Hinweise darauf, dass miRNAs dabei eine entscheidende Rolle spielen. Leider sind die Ergebnisse von miRNA-Expressionsstudien bei Parkinson-Patienten und Kontrollen nicht eindeutig, was u. a. an sehr kleinen Stichproben und unterschiedlich untersuchten Geweben der Einzelstudien liegt (s. o.). Das Ziel dieser Studie war es miRNAs zu identifizieren, die eine konstante differentielle Expression in der entsprechenden wissenschaftlichen Literatur zeigen.

Methoden: Es wurde eine systematische Literaturrecherche für miRNA-Expressionsstudien der PD durchgeführt, indem in der PubMed-Datenbank (http://www.pubmed.gov) mit bestimmten Kriterien nach relevanten Publikationen gesucht wurde (**Abbildung 1**). Die Einträge wurden auf Erfüllung der folgenden Einschlusskriterien überprüft (**Abbildung 1**): humane miRNA-Expressionsstudien von PD Fällen und Kontrollen, von Fachleuten geprüft (d. h. nach „peer review“), in Englisch, Angabe der p-Werte und Richtung des Expressionseffekts. Relevante Informationen aus den Publikationen wurden extrahiert und in eine projekt-gebundene Excel-basierte Datenbank aufgenommen. Dies beinhaltete folgende Charakteristika und Variablen: Name des Erstautors, Jahr der Veröffentlichung, PubMed-Identifikationsnummer (PMID), studien- und populationsspezifische Angaben wie Name der Population oder der Erhebungsort, Zahl der untersuchten Parkinson-Patienten und Kontrollen, Alters- und Geschlechtsverteilung in Patienten und Kontrollen, das untersuchte Gewebe, Methode der miRNA-Quantifizierung, miRNA-Identifikationsnummer, sowie die relevanten statistischen Variablen (p-Werte, Richtungen des Effekts, Effektgrößen und Varianzen).

Alle extrahierten Informationen wurden mindestens von einer zweiten, unabhängigen Person der Arbeitsgruppe unter Zuhilfenahme der originalen Publikationen qualitätskontrolliert, ggf. korrigiert und harmonisiert. So gab es z. B. für dieselben miRNAs in den Publikationen unterschiedliche Bezeichnungen, welche dann in der projekt-gebundenen Datenbank vereinheitlicht wurden. Da verschiedene Gewebe bzw. Körperflüssigkeiten unterschiedliche miRNA-Profile zeigen, wurden die miRNA-Daten entsprechend ihrer Probenherkunft in drei Gruppen stratifiziert und analysiert (s. u.): Daten generiert aus „Gehirnproben“, „Blutproben“ und „Liquorproben“. Innerhalb dieser Gruppen wurden intensive Qualitätskontrollen durchgeführt, z. B. wurden mehrfach in unterschiedlichen Studien verwendete Stichproben identifiziert und duplizierte Ergebnisse entsprechend

ausgeschlossen. Einige Studien untersuchten parallel unterschiedliche Hirnregionen derselben StudienteilnehmerInnen. Hier wurde entweder die größere Stichprobe (d. h. wenn die Stichprobe einer Gewebeprobe einer Hirnregion mehr als 30 % größer war als die Stichprobe einer Gewebeprobe einer anderen Hirnregion) oder das nach Braak-Klassifikation zuerst befallene Gewebe priorisiert. Um mögliche Verzerrungen durch diese Priorisierungsstrategie zu entdecken, wurde eine Sensitivitätsanalyse durchgeführt, indem geschaut wurde, ob sich die Ergebnisse durch Austauschen der priorisierten Gewebe änderten. Zudem wurden in einigen Publikationen mehrere p-Werte für dieselbe miRNA in derselben Stichprobe angegeben, da unterschiedliche experimentelle oder analytische Methoden verwendet worden waren. Zunächst wurde deshalb geprüft, ob eine Methodik der anderen überlegen war. Sofern dies nicht der Fall war, wählten wir einen konservativen Ansatz, indem der höchste (d. h. am wenigsten signifikante) p-Wert in die Analysen eingeschlossen wurde. Ein weiteres Problem stellten nicht exakt angegebene p-Werte dar (z. B. “p ≥0.05”), da für p-Wert-basierte Metaanalysen exakte p-Werte benötigt werden. Auch hier wählten wir einen konservativen Ansatz, indem die p-Werte wie folgt umgewandelt wurden: “p ≥0.05” und “p ≥0.01” wurden zu “p=0.5”, “p <0.05” zu “p=0.025”, “p <0.01” zu “p=0.005”, “p <0.001” zu “p=0.0005”, “p <0.0001” zu “p=0.00005” und “p <0.00001” zu “p=0.000005”. In drei Fällen wurde der p-Wert als „0.0000“ angegeben und wurde zu „0.00005“ umgewandelt. Des Weiteren gaben manche Publikationen die Effektgrößen nur graphisch an. Diese wurden deshalb mittels des Programms „Plot digitizer“ (http://plotdigitizer.sourceforge.net) quantifiziert.

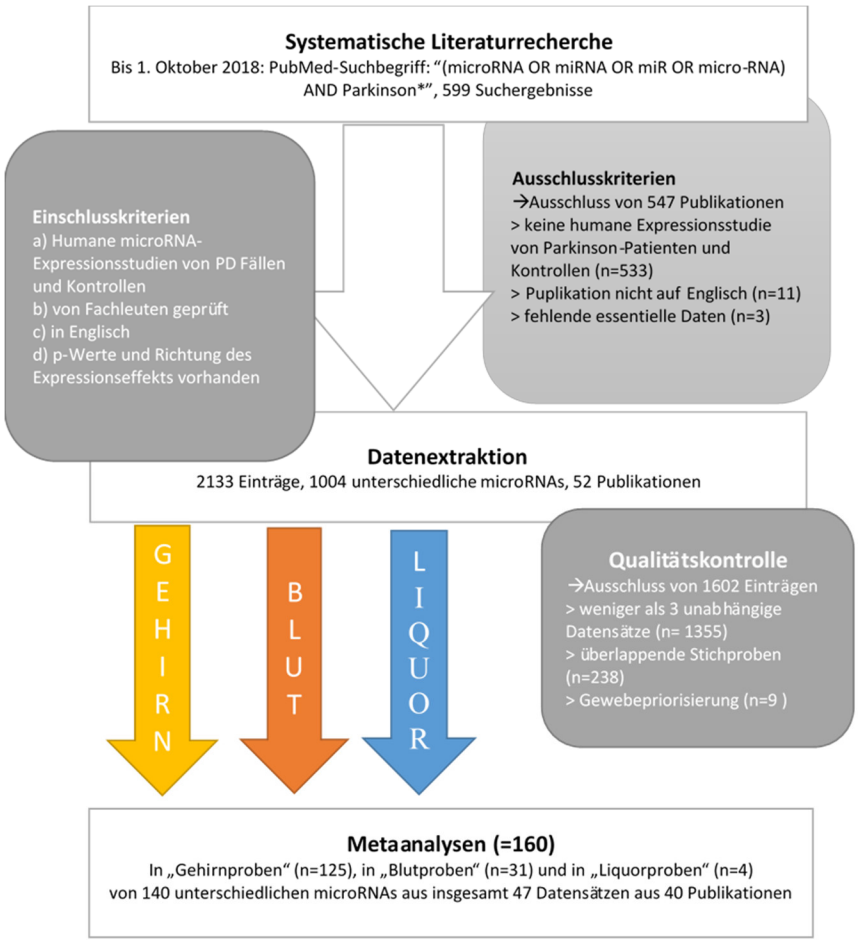
Das Vorliegen von Ergebnissen aus mindestens drei unabhängigen Datensätzen für eine miRNA wurde als Voraussetzung zur Durchführung der entsprechenden Metaanalyse definiert. Nur wenige Studien gaben Effektgrößen und Varianzen an und in diesen Fällen häufig in sehr heterogener Form. Aus diesem Grund vereinheitlichten wir die Effektgröße, indem als standardisierte Mittelwertdifferenz zwischen PD-Patienten und Kontrollprobanden „Hedges g“ berechnet wurde. Dies geschah je nach Datenlage entweder aus Mittelwerten, Mittelwertdifferenzen oder p-Werten, wobei in dieser Reihenfolge die Priorisierung erfolgte, falls eine Studie mehrere dieser Informationen bereithielt. Der Effektschätzer Hedges g wurde dann mittels des sog. „Fixed-Effect-Modells“ und des „Random-Effects-Modells“ (nach Der Simonian und Laird) metaanalysiert, wozu die Funktion *metagen* aus dem R-package ‘meta‘ (https://cran.rproject.org/web/packages/meta/meta.pdf) verwendet wurde. Falls Effektgrößen-basierte Metaanalysen nicht möglich waren, wurden p-Wert-basierte Metaanalysen nach Stouffer`s Methode durchgeführt. Dabei wurden die die p-Werte unter Beachtung der Richtung des Effekts in gerichtete Z-Werte konvertiert. Die einzelnen Z-Werte für eine miRNA aus unterschiedlichen Studien wurden dann zu einer gewichteten Summe zusammengefasst. Die Gewichte sind proportional zu der Wurzel aus der Stichprobengröße des jeweiligen Datensatzes.

Alle Metaanalysen wurden mithilfe eines durch mich erstellten Skripts in der R-Umgebung (https://www.r-project.org) durchgeführt.

Als studienweite Signifikanz wurde α=0.05 nach Bonferroni-Korrektur festgelegt. Diese Korrektur bezog sich auf alle 160 durchgeführten primären Metaanalysen (α=0.05/160=3.13×10^{-4}). Des Weiteren wurde die „Evidenz für eine Assoziation" der signifikanten Ergebnisse anhand der Heterogenität in „stark" oder „suggestiv" eingeteilt. Zur Bewertung der Heterogenität der Effektgrößen-basierten Metaanalysen wurde das Heterogenitätsmaß I^2 herangezogen, welches Heterogenität zwischen Studien darstellt, die über die Zufallswahrscheinlichkeit hinaus geht. Signifikante Ergebnisse in unserem Projekt mit niedriger oder moderater Heterogenität klassifizierten wir als „starke Evidenz zeigend". Sofern hohe Heterogenität vorlag, wurden die Forest-Plots erneut evaluiert. Wenn dort die Effektgrößen konsistent auf derselben Seite der Null waren, klassifizierten wir die Ergebnisse auch als „starke Evidenz zeigend", andernfalls als „suggestive Evidenz zeigend". Bei den p-Wert-basierten Analysen bewerteten wir Ergebnisse mit „starker" Evidenz, wenn > 80 % der Einzelstudien die gleiche Effektrichtung anzeigten. Alle anderen Ergebnisse wurden mit „suggestiver Evidenz" klassifiziert.

Zudem untersuchten wir, ob die hier signifikanten miRNAs der Gehirnproben an mRNAs binden, die von bekannten kausalen oder Risiko-PD-Genen stammen.

Abbildung 1 ***Flussdiagram der Literaturrecherche, Datenextraktion und Analyse der miRNA-Expressionsdaten bei PD***



Bei dieser Publikation war ich maßgeblich beteiligt an der Literaturrecherche, Datenextraktion, Qualitätskontrolle und Datenaufarbeitung. Ich führte die deskriptive Statistik und die Effektgrößen-basierten und p-Wert-basierten Metaanalysen eigenständig durch. Zudem führte ich die vergleichenden Analysen unserer Ergebnisse mit der bereits publizierten Literatur (Reviews, Abstracts, Originalarbeiten) eigenständig durch. Ich erstellte fast alle Grafiken und Tabellen (außer Supplementary Tables 5,6,7) der Publikation. Zusammen mit Frau Dr. Lill schrieb ich den ersten Manuskriptentwurf, integrierte die Verbesserungsvorschläge der Koautoren und führte Veränderungen an den Analysen und am Manuskript im Rahmen des Revisionsprozesses bei der wissenschaftlichen Zeitschrift *Annals of Neurology* durch.

Ergebnisse: In der Literaturrecherche wurden initial 599 wissenschaftliche Publikationen identifiziert. Nachfolgend wurden die Abstracts und ggf. die Volltexte dieser Veröffentlichungen in Bezug auf ihre Qualifikation für den Einschluss in unsere Studie geprüft. Dabei entsprachen 52 Publikationen den Einschlusskriterien. Da für die Metaanalysen mindestens 3 unabhängige Datensätze vorausgesetzt wurden, konnten letztlich 47 Datensätze aus 40 Publikationen nach der

Qualitätskontrolle in die Studie eingeschlossen werden (**Abbildung 1**). Die meisten Studien wurden ausgeschlossen, da sie keine humanen miRNA-Expressionsstudien von PD-Patienten und Kontrollen darstellten (**Abbildung 1**). Die Expressionsdaten wurden bei 11 der 47 Datensätze aus Gehirngewebe generiert, bei 32 Datensäten aus Blutproben und bei 4 aus Liquorproben. Nur eine Studie untersuchte gleichzeitig zwei unterschiedliche Materialien (Blut und Liquor). Insgesamt betrug die mediane Anzahl an Studienteilnehmern (Patienten und Kontrollen zusammengefasst) pro Datensatz über alle drei Strata hinweg 46 (Interquartilsabstand [IQR] 12-95, Spannweite 4-250). Die mediane Anzahl an Individuen der Gehirnproben betrug 11 (IQR 8-16, Spannweite 4-62), der Blutproben 81 (IQR 41-114, Spannweite 13-250) und der Liquorproben 93.5 (IQR 70-115, Spannweite 58-122).

Über die 52 eingeschlossenen Publikationen wurden Expressionsdaten von insgesamt 1004 verschiedenen miRNAs reportiert. Lediglich vier der initial 52 eingeschlossenen Studien (von diesen wurden Daten aus 40 Studien nach Qualitätskontrolle metaanalysiert, s. **Abbildung 1**) stellten Daten von mehr als 100 miRNAs zur Verfügung, die Mehrheit der Studien untersuchte nur wenige miRNAs (Median=3 IQR=1-5). Insgesamt wurden 160 primäre Metaanalysen durchgeführt, für die mindestens drei Datensätze vorlagen, wobei Daten wie oben bereits beschrieben nur innerhalb eines Stratums (Metaanalysen in Gehirnproben n=125, in Blutproben n= 31, in Liquorproben n=4) kombiniert wurden. Davon basierten 21 Metaanalysen auf Effektgrößen-Modellen. Insgesamt wurden 140 unterschiedliche miRNAs metaanalysiert, da siebzehn der 140 unterschiedlichen miRNAs sowohl in dem Stratum "Gehirnproben“ als auch „Blutproben“ metaanalysiert werden konnten, eine miRNA in den Strata „Gehirnproben“ und „Liquorproben“ und eine miRNA in allen drei Strata.

Die mediane Anzahl an Datensätzen, die in eine primäre Metaanalyse einflossen, betrug in allen Strata drei. Die mediane Stichprobengröße, die in die Metaanalysen eingeflossen war, war 88 (IQR 87-98) bei Gehirn-, 339 (IQR 267-596) bei Blut- und 309 (IQR 309-323,5) bei Liquorproben.

Drei der 125 miRNAs aus Gehirnproben, hsa-miR-497-5p, hsa-miR-132-3p und hsa-miR-133b, zeigten eine studienweit signifikante (α=3.13x10^{-4}) differentielle Expression zwischen Parkinson-Patienten und Kontrollen, wobei die Effekte aus den individuellen Studien für die jeweilige miRNA alle in die gleiche Richtung zeigten („starke“ Evidenz, **Tabelle 1**). Darüber hinaus gab es eine weitere studienweit signifikante miRNA, hsa-miR-628-5p, für die die vorliegende Evidenz aufgrund bestehender Heterogenität in den Effektrichtungen als „suggestiv“ klassifiziert wurde. Weitere 34 miRNAs zeigten lediglich nominale (d. h. p<0.05 unkorrigiert) Signifikanz (aufgeführt in den Supplementary Tables 2 und 3 der Originalarbeit (Schulz et al. 2019)). Basierend auf publizierten funktionellen Daten in der Datenbank miRTarBase (http://miRTarBase.mbc.nctu.edu.tw/) und in

Gehirn-spezifischen HITS-Clip Daten binden drei der vier aus Gehirnproben signifikanten miRNAs an mRNAs von etablierten kausalen PD-Genen bzw. PD-Risikogenen und regulieren so mutmaßlich die Expression der PD-Gene. So bindet z. B. hsa-miR-132-3p an die mRNA von *SNCA*.

Bei den miRNAs aus Blutproben waren 10 von 31 miRNAs studienweit signifikant differentiell exprimiert zwischen PD-Patienten und Kontrollen, wobei die Richtungen des Effekts fast alle in die gleiche Richtung zeigten („starke" Evidenz, **Tabelle 1**). Dabei ist bemerkenswert, dass alle Blut-miRNAs bei PD-Patienten vermindert exprimiert waren. Zusätzlich gab es drei weitere miRNAs, die als „suggestiv" klassifiziert wurden (**Tabelle 1**), und sieben die lediglich eine nominal signifikante Assoziation zeigten (Supplementary Tables 2 und 3 der Originalarbeit (Schulz et al. 2019)). Dagegen zeigte keine der vier metaanalysierten miRNAs aus Liquorproben signifikante differentielle Expression in unseren Metaanalysen. Interessanterweise fanden wir für hsa-miR-133b signifikante Ergebnisse sowohl bei den Analysen aus Gehirn-, als auch aus Blutproben.

Konklusion: Durch unsere systematische Arbeit wurden 17 miRNAs identifiziert, die über mehrere Studien hinweg einheitlich differentiell in PD-Patienten im Vergleich zu gesunden Kontrollen exprimiert waren. Davon zeigten 13 miRNAs nach unserer Heterogenitätsbewertung „starke Evidenz" für eine Assoziation mit PD.

Tabelle 1 ***Signifikante Metaanalyseergebnisse der differentiell exprimierten miRNAs zwischen Parkinson-Patienten und Kontrollen***

hsa-miR-	N Gesamt (Fälle, Kontrollen)	Datensatz-spezifische Expression	Ges. Expres-sion	P: FE | P	P: RE	I^2 (95% CI)	Evidenz
Gruppe "Gehirn"							
132-3p	84 (41,43)	-, -, -	runter	6.37E-05	n.a.	n.a.	stark
497-5p	119 (65,54)	+, +, +, +	hoch	1.35E-04	n.a.	n.a.	stark
133b	90 (45,45)	-, -, -| -	runter	2.50E-02| 1.90E-04	5.28E-02	86 (61;95)	stark
628-5p	88 (44,44)	-, +, +	hoch	1.67E-04	n.a.	n.a.	Sugg.
Gruppe "Blut"							
221-3p	596 (353,243)	-, -, -, -	runter	4.49E-35	9.04E-07	84 (60;94)	stark
214-3p	476 (260,216)	-, -, -	runter	2.00E-34	2.00E-34	0 (0;80)	stark
29c-3p	773 (436, 337) | 809 (444,365)	-, -, -, -, -, +,-|-	runter	2.87E-15 | 3.00E-12	2.00E-04	81 (61;90)	stark
29a-3p	711 (389, 322) | 1029 (554,475)	-, -, -, -, -, +, -,- |-,-,-	runter	2.80E-07 | 9.36E-12	2.70E-0.3	80 (62;90)	stark
19b-3p	371(223, 148) | 657 (369,288)	-, -, -, -, - | -, -	runter	2.71E-11 | 2.69E-10	2.13E-07	37 (0;77)	stark
193a-3p	326 (174, 152) | 632 (343,289)	-, -, - | -,-	runter	2.09E-12 | 3.04E-08	5.30E-03	87 (63;96)	stark
141-3p	476 (260,216)	-, -, -	runter	8.06E-07	n.a.	n.a.	stark
451a	145 (54,91)	-, -, -	runter	5.51E-06	3.00E-04	29 (0;93)	stark
146a-5p	411 (220,191)	-, -, -	runter	9.88E-06	n.a.	n.a.	stark
133b	433 (227,206)	-, -, -	runter	2.64E-04	n.a.	n.a.	stark
15b-5p	419 (254, 165) | 669 (392,277)	+, -, -, +, - | -	runter	3.18E-18 | 2.49E-12	2.90E-01	96 (93;98)	Sugg.
185-5p	346 (215, 131) | 646 (378,268)	-, -, + | -,-	runter	1.07E-08 | 4.84E-12	2.39E-01	97 (95;99)	Sugg.
181a-5p	406 (245, 161) | 696 (403,293)	+, -, -, -, + | -,-	runter	5.04E-05 | 2.21E-10	6.13E-01	96 (93;98)	Sugg.

Legende: Diese Tabelle zeigt studienweit-signifikante (alpha=17) Metaanalysenergebnisse basierend auf der p-Wert-basierten Methode, bzw. wenn möglich des Fixed-Effect-Models (FE) und Random-Effects-Models (RE). Wenn mehr Datensätze für p-Wert basierte Metaanalysen vorhanden waren, wurden diese als primäre Metaanalyse prioritisiert. „\|" wurde zur Trennung genutzt, um p-Wert-basierte Ergebnisse von Effektgrößen-basierten Ergebnissen zu trennen. N Anzahl. Ges. Expression: gesamte Expression über alle Metaanalysen. P: P-Wert. CI: Konfidenzinterval. I^2: Heterogenität in %, die über den Zufall hinaus geht. n.a.: keine Angabe

2.2 Publikation 2 (Takousis, et al. Alzheimers Dement. 2019 Sep 5. pii: S1552-5260(19)35119-2): Differentielle Expression von miRNAs im Gehirn, Blut und Liquor von Alzheimer-Patienten

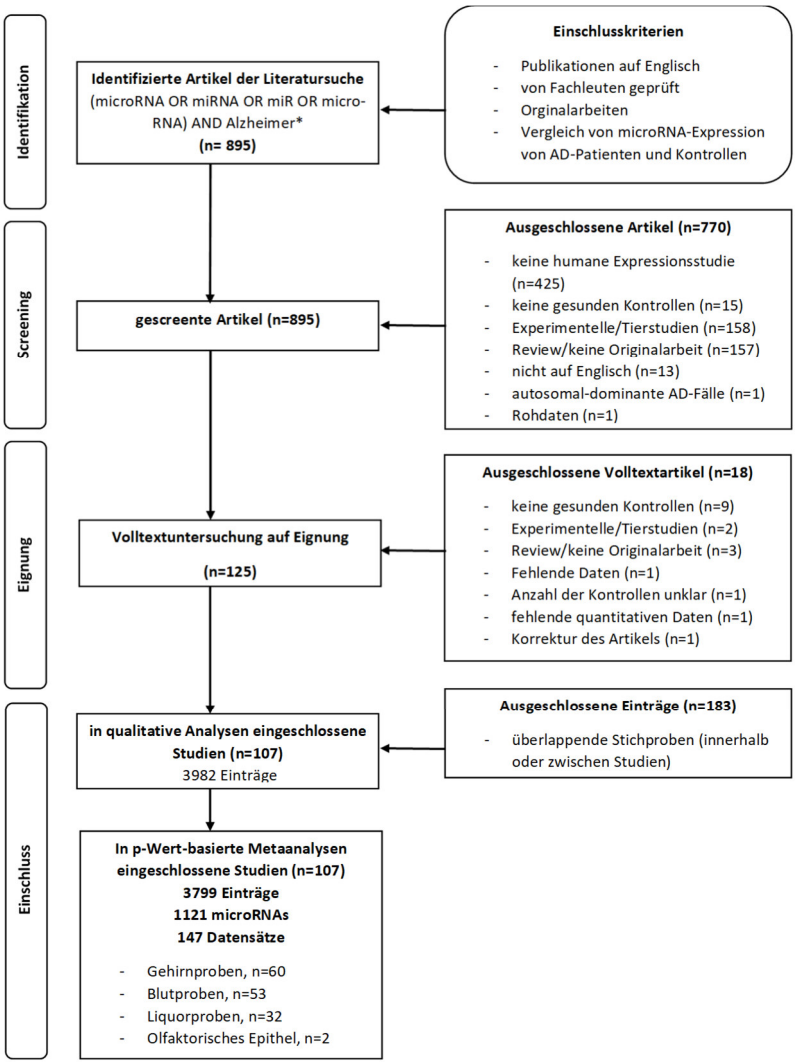
Einleitung: Auch bei der AD werden miRNAs in Krankheitsentstehung und -verlauf als vermutlich relevante Faktoren untersucht. Wie in dem PD-Feld berichten auch hier zahlreiche Studien mit überwiegend nicht übereinstimmenden, teilweise diskrepanten Ergebnissen über miRNAs, die bei AD-Patienten eine veränderte Expression im Vergleich zu gesunden Probanden zeigen, was die Interpretation dieser Arbeiten und deren Ergebnisse sehr erschwert. Ziel dieser Arbeit war aus publizierten Fall-Kontroll-Expressionsstudien die miRNAs zu identifizieren, die über Studien hinweg konsistente und signifikante Expressionsunterschiede zeigten.

Methoden: Da die Arbeit an Publikation 1 (Schulz et al. 2019) als methodische Vorlage für die Arbeit an diesem Projekt galt, sind die Methoden zum größten Teil vergleichbar. Aus diesem Grund wird hier nur kurz die Methodik dargestellt, insbesondere nehme ich Bezug auf Unterschiede zur Methodik aus Publikation 1. Analog zur Publikation 1 wurde eine systematische Literaturrecherche für miRNA-Expressionsstudien der AD in der Datenbank Pubmed (http://www.pubmed.gov) durchgeführt (**Abbildung 2**). Aus Veröffentlichungen, die die Einschlusskriterien erfüllten (Auflistung in **Abbildung 2**), wurden Daten in eine Excel-basierte projekt-gebundene Datenbank aufgenommen und auf Plausibilität und Qualität überprüft.

Wie für Publikation 1 wurden miRNAs zu denen mindestens drei unabhängige Datensätze pro Stratum (Gehirnproben, Blutproben, Liquorproben) vorlagen, metaanalysiert. Die Metaanalysen wurden wie unter Publikation 1 beschrieben mittels eines von mir verfassten, individuellen R-Skripts basierend auf Stouffer`s Methode durchgeführt (s. o.). Auf Effektgrößen-basierte Metaanalysen wurde in diesem Projekt verzichtet. Als studienweite statistische Signifikanzgrenze wurde ein Bonferroni-korrigiertes $\alpha = 0{,}05/ 461= 1{,}08x10^{-4}$ angenommen, basierend auf 461 durchgeführten Metaanalysen. Zudem wurden der „Evidenzgrad“ signifikanter miRNA-Ergebnisse aufgrund der Konsistenz der Effektrichtungen in den individuellen Studien eingeteilt (s. o.). Zudem wurde, wie für Publikation 1, untersucht, ob die mRNAs, an die die hier signifikanten miRNAs binden, von bekannten kausalen oder Risiko-AD-Genen stammen (s. o.).

Bei dieser Publikation war ich beteiligt an der Qualitätskontrolle und der Datenaufarbeitung der extrahierten Daten. Des Weiteren führte ich sämtliche p-Wert-basierte Metaanalysen mittels der für Publikation 1 erstellten Analyseskripte selbstständig durch und war an dem Verfassen des Manuskripts beteiligt.

Abbildung 2 Flussdiagram der Literaturrecherche, Datenextraktion und Analyse der miRNA-Expressionsdaten bei AD



Ergebnisse: Bei der systematischen Literaturrecherche wurden 895 Publikationen identifiziert, wovon 107 Veröffentlichungen die Einschlusskriterien erfüllten. Gründe für den Ausschluss von Publikationen waren z. B. das fehlende Vorliegen einer humanen Expressionsstudie, von gesunden Kontrollen oder Originalarbeiten. Nach der Qualitätskontrolle konnten Daten von 147 unabhängigen Datensätzen aus 107 Publikationen extrahiert werden und in die projekt-gebundene Datenbank aufgenommen werden. Dabei stammten 60 Datensätze von Gehirn-, 53 von Blut- und 32 von Liquorproben. Es gab zwei Datensätze, die von weiteren Geweben stammten, nämlich von der anterioren nasalen Septummukosa und dem Bulbus Olfactorius. Außerdem gab es 15 Publikationen, die miRNA-Expressionen in mehr als einem Stratum beschrieben.

Die mediane Stichprobengröße pro Datensatz lag bei 34,5 über alle Studien hinweg und bei 19, 68, 38 in den jeweiligen Probengruppen (d. h. Gehirn-, Blut- bzw. Liquorproben, respektive). Insgesamt wurden über 1121 unterschiedliche miRNAs über die Einzelstudien hinweg untersucht. Pro Studie

lag die mediane Anzahl an untersuchten miRNAs bei 4 (IQR 2-13). Es gab nur neun Studien, die Expressionsergebnisse von mehr als 100 miRNAs berichteten. Von allen eingeschlossenen miRNAs gab es 295, die wenigstens von drei verschiedenen Datensäten des gleichen Stratums untersucht worden waren. Letztlich konnten 461 individuelle Metaanalysen über alle drei Gruppen hinweg durchgeführt werden, wobei 260 auf Gehirnproben, 135 auf Blutproben und 66 auf Liquorproben basierten. Die mediane Zahl der Datensätzen, die in eine Metaanalyse einflossen, war 4 sowohl bei Gehirn- als auch Blutproben und 3 bei Liquorproben, wobei die Maximalwerte 19, 10 und 11 waren. Der Median der gesamten Stichprobengröße in den Metanalysen betrug 42.5 (IQR 23-85), 259 (IQR 195-318) und 164 (98-205) in Gehirnproben, Blutproben bzw. Liquorproben.

Fünfundzwanzig von 260 (10%) der metaanalysierten miRNAs aus Gehirnproben zeigten eine studienweit signifikante differentielle Expression in AD-Patienten und Kontrollen. Davon erhielten neun mircoRNAs aufgrund konsistenter Effektrichtungen die Klassifikation „starke“ Evidenz. Die drei signifikantesten miRNAs aus Gehirnproben waren hsa-miR-125b-5p, hsa-miR-501-3p und hsa-miR-138-5p. In Metaanalysen der Liquordaten zeigten alle fünf studienweit signifikant exprimierten miRNAs ebenfalls „starke“ Evidenz für eine Assoziation. Alle fünf dieser miRNAs waren in Patienten im Vergleich zu Gesunden geringer exprimiert. Fast ein Viertel (32/135) der aus Blutproben metaanalysierten miRNAs zeigten studienweit signifikante differentielle Expression zwischen AD-Patienten und Kontrollen, wobei 21 Assoziationen eine „starke“ Evidenz aufwiesen. Die signifikantesten miRNAs in Blutproben waren: hsa-miR-342-3p, hsa-miR-191-5p und hsa-let-7d-5p. Interessanterweise gab es fünf miRNAs, die signifikante differentielle Expression in Gehirn-, als auch in Blutproben zeigten. Dabei waren hsa-miR-181c-5p und hsa-miR-29c-3p sowohl im Gehirn als auch im Blut in Patienten vermindert exprimiert. Die anderen drei (hsa-miR-125b-5p, hsa-miR-146a-5p, hsa-miR-223-3p) waren in Gehirnproben von Patienten höher und bei den Blutproben niedriger exprimiert als bei Kontrollprobanden.

Unsere Untersuchung der mRNAs, an die die signifikanten miRNAs binden, zeigte, dass bekannte AD-Gene von einigen der hier signifikant differentiell exprimierten miRNAs reguliert werden. Dazu gehörten z. B. *APP*, *CCD6, CD2AP, CLU, FERMT2* und *PICALM*.

Konklusion: Insgesamt wurden in unserer Arbeit 57 signifikant differentiell exprimierte miRNAs identifiziert, wovon fünf in mehr als einem Gewebe-Stratum differentiell exprimiert waren. Dabei stammten mehr als die Hälfte der signifikanten miRNAs aus Blutproben. Diese miRNAs können z. B. als Ausgangspunkt für zukünftige Biomarkerstudien genutzt werden.

2.3 Publikation 3 (Kim*, Schulz*, et al. JAMA Neurol. 2018;75(3):360-366): Assoziation des polygenetischen Risiko-Scores mit kognitiver Leistungsfähigkeit und motorischer Progression bei der Parkinson-Krankheit in einer longitudinalen Studie

Einleitung: Große genomweite Assoziationsstudien (GWAS) haben zahlreiche genetische Varianten entdeckt, die inzwischen etablierte Risikofaktoren für eine PD darstellen. Bis dato war aber noch wenig über den Einfluss dieser Risikovarianten auf den Krankheitsverlauf der PD bekannt. Ziel dieser Arbeit war es, den kumulativen Effekt der etablierten Risikovarianten auf den Krankheitsprogress zu überprüfen.

Methoden: Dazu wurden Daten einer longitudinalen populationsbasierten Studie aus Kalifornien („Parkinson's Environment & Genes", PEG) verwendet, die Informationen über die motorische Progression, die Entwicklung der kognitiven Leistungsfähigkeit und das Überleben von Parkinson-Patienten erfasst hatte. Die kognitive Leistungsfähigkeit wurde mittels des neuropsychologischen Standardtests MMST (Mini-Mental-Status-Test) gemessen. Eine klinisch relevante Abnahme der kognitiven Leistungsfähigkeit wurde als Rückgang des initial gemessenen MMST um mindestens 4 Punkte definiert. Die motorische Progression wurde durch zwei etablierte Messmethoden erfasst, die UPDRS-III (Unified Parkinson's Disease Rating Scale part III)- und die H&Y (Hoehn & Yahr)-Skala, wobei ein Anstieg des UPDRS-III von 20 Punkten oder mehr über den Beobachtungszeitraum von 5 Jahren als schnelle motorische Progression gewertet wurde. Bei der H&Y Skala wurde das Erreichen des Stadiums 3 oder höher als schneller Verlauf definiert. In Bezug auf das Überleben der teilnehmenden PD-Patienten wurde kontinuierlich die Sterblichkeit überwacht, indem Personenstandsdaten kontrolliert, Todesanzeigen begutachtet und die Patienten und deren Familien aktiv nachverfolgt wurden.

Zu diesen phänotypischen Parametern waren 23 PD-Risikovarianten aus Speichel- oder Blutproben der PD-Patienten zuvor von der Arbeitsgruppe erfolgreich genotypisiert worden. Um den kumulativen genetischen Einfluss dieser Risikovarianten zu ermitteln, berechnete ich für jeden PD-Patienten einen gewichteten polygenetischen Risikoscore (PRS). Dazu wurde für jeden Patienten der entsprechende ß-Koeffizient (d. h. die log odds ratio) für eine Risikovariante mit der Anzahl der jeweiligen Risikoallele (0, 1 oder 2) multipliziert, die Summe dieser Produkte gebildet und anschließend mittels Z-Transformation standardisiert.

Um den Zusammenhang des gewichteten PRS mit den jeweiligen Verlaufs- und Überlebensereignissen (MMST, UPDRS-III, H&Y, das Überleben der Patienten) zu bestimmen,

wurde zur Schätzung der Hazard-Ratio (HR) die Cox-Regression (sog. „proportial hazards regression“) angewendet. Zur Signifikanz-Testung wurde der Wald X^2 Test verwendet. Für jedes Ereignis wurde ein eigenes Model berechnet und für Geschlecht und Alter bei Diagnose adjustiert. Außerdem wurde mittels Sensitivitätsanalysen untersucht, ob Ergebnisse unter Ausschluss genetischer Varianten des Glucocerebrosidase Gens *(GBA)* (rs35749011 und rs114138760) Bestand haben, da diese bereits als Einflussfaktoren auf die Krankheitsprogression in anderen Studien bekannt waren. In dieser Studie wurde keine Korrektur des Typ-1-Fehlers für multiples Testen durchgeführt, d. h. Signifikanz wurde definiert als $\alpha=0,05$.

Bei dieser Publikation war ich für die Qualitätskontrolle der Genotypisierung und Berechnung des PRS verantwortlich und beteiligte mich an der Auswertung der statistischen Analysen sowie dem Verfassen des zur Publikation eingereichten Manuskripts.

Ergebnisse: Von 285 Patienten waren 56 % männlich und hatten ein Durchschnittsalter von 69 Jahren. Im Schnitt wurden die PD-Patienten ca. 2 Jahre nach Erstdiagnose in die Studie aufgenommen und nach weiteren 5 Jahren wurde die Symptomprogression erneut untersucht. Über den Beobachtungszeitraum starben 58 % der Patienten. Im Mittel sank der MMST um 0,9 Punkte über 5 Jahre. Bei den motorischen Ereignisvariablen konnte erwartungsgemäß eine signifikante Korrelation festgestellt werden, wohingegen die kognitive Progression nur schwach mit den anderen Ereignissen korreliert war.

Tabelle 2 zeigt die Ergebnisse der Cox-Regression zwischen dem PRS und den einzelnen Verlaufsgrößen. Der PRS zeigte eine signifikante positive Assoziation mit der Abnahme kognitiver Leistungsfähigkeit (HR=1.44 (CI: 1.00, 2.07), p=0.049) sowie eine fast signifikante positive Assoziation mit motorischer Progression (UPDRS-III: HR=1.42 (CI: 1.00, 2.01), p=0.051; H&Y: HR=1.34 (CI: 1.00, 1.79), p=0.054). Ähnliche Ergebnisse wurden auch unter Ausschluss der *GBA*-Genvarianten beobachtet (**Tabelle 2**). Es konnte kein Zusammenhang zwischen dem PRS und dem Überleben der Patienten festgestellt werden (**Tabelle 2**).

Tabelle 2 Geschätzte HRs für klinische Verlaufsereignisse in PD und PRS

	Gesamter PRS		**PRS ex *GBA***	
Ereignisse	HR (95% CI)	p-Werte	HR (95% CI)	p-Werte
Überleben	1.08 (0.79, 1.47)	0.622	1.09 (0.78, 1.52)	0.632
Zeit bis Rückgang des MMST um 4-Punkte	1.44 (1.00, 2.07)	0.049	1.42 (0.98, 2.07)	0.068
Zeit bis Erhöhung des UPDRS-III um 20-Punkte	1.42 (1.00, 2.01)	0.051	1.50 (1.05, 2.14)	0.027
Zeit bis H & Y ≥3	1.34 (1.00, 1.79)	0.054	1.36 (1.01, 1.83)	0.045

HR=Hazard-Ratio, CI =Konfidenzintervall, PRS=Polygenetischer Risiko-Score, ex *GBA*= Exklusive der *GBA*-Varianten, MMST= Mini-Mental-Status-Test, UPDRS= Unified Parkinson's Disease Rating Scale part III, H&Y= Hoehn & Yahr Skala

Konklusion: Diese Studie ist die erste, die - anhand sorgfältig erhobener, longitudinaler Daten - einen kumulativen Einfluss genetischer Risikovarianten für die PD auf die kognitive Leistungsfähigkeit bei der Erkrankung untersucht und eine Assoziation identifiziert hat. Bis dato wurde die Abnahme der kognitiven Leistungsfähigkeit nur mit einzelnen Genen wie etwa *GBA* in Verbindung gebracht. Der MMST ist ein häufig eingesetzter Test, um kognitive Beeinträchtigung zu quantifizieren. Es gab bereits eine Studie, die einen PRS (mit 19 Risikovarianten) mit motorischer Progression anhand der H&Y-Skala in Verbindung gebracht hat. Diese Ergebnisse konnten mit unseren Daten bestätigt werden. Unsere Arbeit legt nahe, dass genetische PD-Suszeptibilitätsvarianten nicht nur das Risiko der PD modifizieren, sondern in ihrer Gesamtheit auch den weiteren Krankheitsverlauf beeinflussen könnten, wobei diese Ergebnisse in vergleichbaren Studien unabhängig validiert werden müssen.

3 Diskussion und Ausblick

In den ersten beiden Publikationen (Schulz, Takousis, et al. 2019; Takousis et al. 2019) dieser Dissertationsarbeit wurden zum ersten Mal im Bereich neurodegenerativer Erkrankungen systematische Metaanalysen über publizierte miRNA-Expressionsstudien unter Berücksichtigung der Besonderheiten von Genexpressionsdaten durchgeführt. Dabei konnten 28 differentiell exprimierte miRNAs im Gehirn und 42 im Blut von PD- und AD-Patienten im Vergleich zu gesunden Kontrollen identifiziert werden. Von diesen 70 miRNAs wurden 15 zuvor weder in Abstrakten der jeweiligen Einzelstudien hervorgehoben noch in qualitativen Übersichtsarbeiten erwähnt. Diese 15 miRNAs wurden also durch unsere Studie zum ersten Mal als relevant für die PD oder AD beschrieben.

Diese im Rahmen meiner Dissertation durchgeführten beiden Projektarbeiten (Schulz et al. 2019; Takousis et al. 2019) weisen folgende Limitationen auf: Durch z. T. sehr kleine Fallzahlen (kleinste Fallzahl mit n=4) der Individualstudien ist die Trennschärfe („Power“) bei einigen Metaanalysen nicht ausreichend und selbst Ergebnisse mit signifikanten Effekten, besonders mit vielen kleinen kombinierten Fallzahlen, müssen mit Vorsicht betrachtet werden. In den meisten Fällen konnte durch die Metaanalysen die Stichprobengröße im Vergleich jedoch erheblich vergrößert werden und eine für die Detektion moderater Effekte ausreichende Fallzahlgröße erlangt werden (mediane Stichprobengröße bei den Strata Gehirn/Blut/Liquor waren 88/339/309 bei PD und 42,5/259/164 bei AD), so dass Ergebnisse aus diesen Metaanalysen als aussagekräftig eingeordnet werden können. Trotzdem sollte für zukünftige mircoRNA-Expressionsstudien eine deutliche Erhöhung der Stichprobengröße ein zentrales Ziel sein.

Eine weitere Limitation beider Studien liegt darin, dass Aussagen zu Ursache-Wirkungsbeziehungen mit unseren Daten und Ergebnissen nicht möglich sind, da miRNA-Expressionslevel sich im zeitlichen Verlauf ändern können. Insofern bleibt bei Studien, die prävalente Patienten und Kontrollprobanden untersuchen bzw. wie in unserem Fall metaanalysieren unklar, ob die gefundenen Unterschiede zur Entstehung der Erkrankung beigetragen haben oder Folge der pathophysiologischen Vorgänge sind. Des Weiteren sind miRNA-Expressionslevel gewebe- bzw. sogar zellspezifisch. Die eingeschlossenen Studien untersuchten die miRNA-Expressionen nur auf Ebene einzelner Gewebe, aber nicht auf Zellebene. So ist es möglich, dass manche Expressionsunterschiede nicht auf eigentlichen Genexpressionsunterschieden in den Zellen selbst beruhen, sondern darauf, dass bestimmte Zellpopulationen im Gewebeverband untergegangen sind und es so zu einer unterschiedlichen Zellzusammensetzung in Fällen vs. Kontrollprobanden gekommen ist. Um heraus zu finden was auf Zellebene passiert, sind neue Technologien, die

einzelne Zellen untersuchen (z. B. laser capture microdissection und single cell sequencing) denkbar. Im Bereich der miRNA-Expressionsstudien waren derartige Daten bis dato allerdings noch zu rar, um sie in den Metaanalysen separat zu berücksichtigen.

Eine weitere Limitation ist dem Umstand geschuldet, dass wir in unseren Metaanalysen nur einen Bruchteil der microRNAs untersuchen konnten, die insgesamt im Blut oder Gehirn exprimiert werden. Unter den nicht berücksichtigten microRNAs könnten daher weitere relevante microRNAs mit differentieller Expression in der PD existieren. Dieser Umstand stellt eine Limitation unserer Studie dar, die durch die verfügbaren Daten im Feld selbst bedingt ist, nicht durch die Methodologie unserer Arbeit.

Weiterhin limitierend für unsere beiden Studien ist, dass die Einzelstudien in Bezug auf das Studiendesign und die -durchführung (d. h. Probenrekrutierung, Labormethodik, statistische Analysen und Darstellung der Ergebnisse) recht heterogen waren. Dies erklärt wahrscheinlich auch die Heterogenität einiger Ergebnisse. Leider war eine weitere Analyse des Einflusses der verschiedenen Faktoren auf die Effektschätzer (z. B. mittels stratifizierter Metaanalysen, Sensitivitätsanalysen oder Metaregressionsanalysen) aufgrund z. T. fehlender Informationen in den einzelnen Publikationen nicht möglich. Darüber hinaus muss man davon ausgehen, dass es auch zu Verzerrungen der Metaanalysenergebnisse durch "selektives Reportieren" oder andere Formen des Publikationsbias gekommen sein kann.

In Zukunft könnte diese Situation verbessert werden, indem publizierte Ergebnisse möglichst einheitlich dargestellt werden. Das bedeutet, dass *alle* Expressionsergebnisse wenigstens mit konkreter Fallzahl, Effektgrößen und entsprechenden Varianzen veröffentlicht werden sollten, um Effektgrößen-basierte Metaanalysen durchführen zu können. Zudem sind Angaben zu demographischen (Alter, Geschlecht), methodischen, diagnostischen und therapeutischen Einflussfaktoren essentiell, um die Ergebnisse der Studien in Bezug auf den möglichen Einfluss dieser Faktoren beurteilen zu können. Im Idealfall, um größtmögliche Transparenz und eine möglichst effektive Nachnutzung der einmal generierten Ressourcen zu erreichen, sollten die individuellen Rohwerte der Expressionsdaten und anonymisierten individuellen Informationen der Studienteilnehmer (Alter, Geschlecht, Therapie etc.) ebenfalls, z. B. als Anhang in den Publikationen zur Verfügung gestellt werden.

Die Relevanz, publizierte miRNA-Expressionsdaten in PD und AD quantitativ durch Metaanalysen zusammenzufassen, zeigt sich darin, dass wie oben aufgeführt einige der hier als signifikant und konsistent differentiell exprimiert identifizierten miRNAs in qualitativen miRNA-Reviews der letzten Jahre oder in den Abstrakten der in meine Analysen eingeschlossenen wissenschaftlichen Veröffentlichungen gar nicht erwähnt wurden. Der in diesem Projekt etablierte Ansatz zur

quantitativen Analyse von publizierten miRNA-Expressionsdaten kann als Grundlage für ähnliche Studien in anderen Erkrankungen dienen oder auf andere Genexpressionsdaten (z. B. in Bezug auf mRNAs oder sog. long non-coding RNAs) in PD, AD und anderen Erkrankungen ausgeweitet werden.

Da hier direkt noch keine Schlüsse zur pathophysiologischen Rolle der miRNAs aus Gehirngewebe geschlossen werden können, sind die von uns beschriebenen konsistent differentiell exprimierten miRNAs in PD und AD als plausible Kandidaten für zukünftige Studien zu verstehen, z. B. solche, die funktionelle Experimente *in vitro* oder *in vivo* durchführen, mit dem Ziel, ein besseres Verständnis der pathophysiologischen Zusammenhänge zu erlangen.

Ein großes Feld stellt die Entdeckung und Etablierung von Biomarkern zur Diagnosestellung oder Krankheitsvorhersage dar. Die hier hervorgehobenen miRNAs aus Blut- und Liquorproben können zunächst lediglich als „Klassifizierungsmarker" für prävalente PD-/AD-Fälle Verwendung finden, da nicht klar ist, ob schon vor Ausbruch der Erkrankungen Expressionsunterschiede bestanden haben. Mithilfe von longitudinalen Studien könnte man aber auch Veränderungen der Expressionsunterschiede über die Zeit beurteilen und so möglicherweise basierend auf unseren Studienergebnissen auch Identifikationsmarker zur Früherkennung etablieren. Des Weiteren könnten die von uns beschriebenen miRNAs in Bezug auf die Möglichkeiten der Ausnutzung in therapeutischen Ansätzen berücksichtigt werden.

Aus der dritten Publikation (Paul, Schulz et al. 2018) dieser Dissertation geht hervor, dass eine Assoziation zwischen dem kumulativen Effekt der bekannten genetischen PD-Risikofaktoren und der zunehmenden kognitiven Einschränkung bei der PD vorliegt. Zudem konnte ein in Vorläuferstudien bereits berichteter Zusammenhang zwischen diesem kumulativen genetischen Effekt und der motorischen Progression bei der PD bestätigt werden. Weiterhin wurde kein Zusammenhang zwischen dem genetischen Risikoscore und der Krankheitsmortalität festgestellt.

Die vielleicht größte Limitation dieser Studie ist die für komplex-genetische Studien verhältnismäßig kleine Stichprobengröße, was die Notwendigkeit der Replikation unserer Ergebnisse unterstreicht. Nach Publikation unseres Papiers haben Iwaki und Mitarbeiter bis zu 4307 Patienten in Bezug auf die Assoziation des PRS mit verschiedenen Progressionsmarkern analysiert (Iwaki et al. 2019). Darunter analysierten sie auch motorische (u.a. UPDRS-III, H & Y) und kognitive (u.a. MMST) Progressionsmarker in einem Teil der eingeschlossenen Patienten und fanden keinen Effekt des PRS in Bezug auf den motorischen und kognitiven Krankheitsfortschritt. Diese fehlende Replikation unserer Ergebnisse kann verschiedene Ursachen haben: 1) Typ-1-Fehler unserer Studie, d. h. unsere Ergebnisse in Bezug auf die motorische und kognitive Progression der PD stellen einen falsch-positiven Befund dar. Aufgrund unterschiedlicher Gepflogenheiten in verschiedenen

Forschungsfeldern der PD wurde in dieser Studie keine Korrektur für multiples Testen (wie in den beiden anderen hier angeführten Publikationen) durchgeführt. Generell zeigen die p-Werte unserer Studie nur eine moderate Evidenz für eine Ablehnung der jeweiligen Null-Hypothese. Insofern kann zum jetzigen Zeitpunkt nicht ausgeschlossen werden, dass es sich bei den Analyseergebnissen zumindest teilweise um falsch-positive Assoziationen handelt. 2) Zudem kann ein Typ-2-Fehler in den Analysen von Iwaki et al vorliegen, d. h. deren PRS-Ergebnisse in Bezug auf die motorische und kognitive Progression der PD stellen einen falsch-negativen Befund dar. Zu beachten ist, dass Iwaki et al sehr viele verschiedene Studien in ihren Analysen subsumierten. Es ist möglich, dass es durch fehlende Harmonisierung einzelner Studien und z. B. nicht-standardisierte Messverfahren zu einem falsch-negativen Ergebnis gekommen ist. 3) Weiterhin definierten wir PD-Progressionsmarker anders und nutzten unterschiedliche statistische Modelle. Iwaki et al definierten Veränderungen bei der motorischen Progression und zunehmenden kognitiven Einschränkung als kontinuierliche Variablen und nutzten lineare Regressionsmodelle, um die Assoziation zu berechnen, während wir das Cox-Regressionsmodell mit dichotomen Variablen angewendet haben, da wir nicht von einem linearen Zusammenhang in Bezug auf die Krankheitsprogression ausgehen konnten und wollten. Zudem passten Iwaki et al ihre Regressionsanalysen neben Alter und Geschlecht teilweise noch an weitere Faktoren an, wie z. B. Alter zu Erkrankungsbeginn und Familiengeschichte. Wir adjustierten hingegen nur für Geschlecht und Alter, da wir die anderen Variablen nicht als Störvariablen („Counfounder“) klassifizierten. Diese methodischen Unterschiede können zu Unterschieden in den Analyseergebnissen führen.

Eine weitere Limitation unserer Studie besteht darin, dass die 23 in unserer Studie berücksichtigten genetischen Risikovarianten nicht das Abbild aller inzwischen bekannten Risiko-SNPs für die PD darstellen. Hintergrund hierfür ist, dass unsere Daten aufgrund früherer, d.h. aus dem Jahr 2014 (s. Referenz 5 in Paul et al), PD-GWAS Ergebnisse publiziert wurden. Darüber hinaus konnten aus technischen Gründen nicht alle der damals bekannten Risikovarianten genotypisiert werden (es fehlten 3 von 26 SNPs). In der Folge wurden in einer größeren GWAS aus dem Jahr 2019 weitere PD-Risikovarianten identifiziert (Nalls et al. 2019), die in unserer Studie nicht berücksichtigt werden konnten. Die Anzahl der etablierten Risikovarianten für die PD liegt inzwischen bei 90 SNPs in 78 genetischen Regionen (Nalls et al. 2019). Wenn es einen zugrundeliegenden kumulativen Effekt des genetischen Risikoprofils auf die Krankheitsprogression führt, hätte diese inkomplette Berücksichtigung der genetischen Varianten in unserem Modell jedoch am ehesten zu einer Verzerrung hin zu einem Nulleffekt geführt (sog. „bias towards the null“), was hier nicht der Fall war.

Der Verlust von Teilnehmern im Verlauf der prospektiven Studie aufgrund von Tod, anderer Krankheit oder anderen Faktoren („Loss of follow-up“) ist eine weitere Limitation unserer Studie,

da dies zu einem Selektionsbias gerade in einem longitudinalen Design führen kann, der auch bei sorgfältiger Studienplanung und -durchführung nie vollkommen ausgeschlossen werden kann.

Zuletzt können die Testinstrumente zur Bestimmung der motorischen und kognitiven Progression nur mit einer gewissen Genauigkeit das tatsächliche klinische Bild wiedergeben. Zudem kann es durch die durch Anwendung der Cox-Regression notwendige dichotome Einteilung zu einer großen phänotypischen Bandbreite in den Gruppen gekommen sein und auch die Zuteilung möglicherweise durch Testungenauigkeit teilweise inkorrekt erfolgt sein (sog. „Misklassifikation"). Allerdings wurde in unserer Studienplanung darauf geachtet, anhand von Ergebnissen aus publizierten Studien bezüglich der Validität der Testinstrumente das klinisch sinnvollste Messinstrument und eine relevante und für Störfaktoren möglichst robuste dichotome Klassifikation zu finden.

Zusammenfassend legt unsere Studie trotz der oben aufgeführten Limitationen nahe, dass nicht nur die Krankheitsentstehung, sondern auch der Krankheitsverlauf polygenetisch bedingt ist. Zu einer individuellen „Vorhersage" in Bezug auf den zu erwartenden Krankheitsverlauf oder eine „personalisierte Therapie" eignen sich die in unserer Studie beschriebenen Assoziationen, sofern sie echt sind, aufgrund der kleinen Effekte (allein) jedoch nicht.

Die Ergebnisse der drei Publikationen können in ihrer weiterentwickelten Form als hilfreiche Grundlage betrachtet werden, um exaktere Vorhersagemodelle der AD und PD zu entwickeln. Solche Modelle könnten z. B. genetische Faktoren, Umwelt-/Lebensstilfaktoren und epigenetische Faktoren kombinieren und so den kumulativen Einfluss auf die Krankheitsentstehung oder den Krankheitsverlauf zu bestimmen. Je nach Vorhersagekraft solcher Modelle sind großangelegte Präventionsprogramme denkbar oder der Einsatz neuer Therapien, die nur in einem frühen Krankheitsstadium wirken. Zudem könnte man den Krankheitsverlauf besser einteilen und Patienten mit schneller fortschreitendem Krankheitsverlauf besser über ihre Situation aufklären.

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5 Anhang

Die Anhänge zu den einzelnen Publikationen finden frei zugänglich im Internet unter folgenden Seiten:

Publikation 1:

https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.25490

Publikation 2:

https://alz-journals.onlinelibrary.wiley.com/doi/abs/10.1016/j.jalz.2019.06.4952

Publikation 3:

https://jamanetwork.com/journals/jamaneurology/fullarticle/2669922

5.1 Publikation 1: Schulz J*, Takousis P*, Wohlers I, Itua I, Dobricic V, Ruecker G, Binder H, Middleton L, Ioannidis JPA, Perneczky R, Bertram L, Lill CM. Meta-analyses identify differentially expressed miRNAs in Parkinson's disease. Ann Neurol. 2019;85(6):835-851.

RESEARCH ARTICLE

Meta-Analyses Identify Differentially Expressed microRNAs in Parkinson's Disease

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Objective: MicroRNA (miRNA)-mediated (dys)regulation of gene expression has been implicated in Parkinson's disease (PD), although results of miRNA expression studies remain inconclusive. We aimed to identify miRNAs that show consistent differential expression across all published expression studies in PD.
Methods: We performed a systematic literature search on miRNA expression studies in PD and extracted data from eligible publications. After stratification for brain, blood, and cerebrospinal fluid (CSF)-derived specimen, we performed meta-analyses across miRNAs assessed in three or more independent data sets. Meta-analyses were performed using effect-size– and *p*-value–based methods, as applicable.
Results: After screening 599 publications, we identified 47 data sets eligible for meta-analysis. On these, we performed 160 meta-analyses on miRNAs quantified in brain (n = 125), blood (n = 31), or CSF (n = 4). Twenty-one meta-analyses were performed using effect sizes. We identified 13 significantly (Bonferroni-adjusted $\alpha = 3.13 \times 10^{-4}$) differentially expressed miRNAs in brain (n = 3) and blood (n = 10) with consistent effect directions across studies. The most compelling findings were with hsa-miR-132-3p ($p = 6.37 \times 10^{-5}$), hsa-miR-497-5p ($p = 1.35 \times 10^{-4}$), and hsa-miR-133b ($p = 1.90 \times 10^{-4}$) in brain and with hsa-miR-221-3p ($p = 4.49 \times 10^{-35}$), hsa-miR-214-3p ($p = 2.00 \times 10^{-34}$), and hsa-miR-29c-3p ($p = 3.00 \times 10^{-12}$) in blood. No significant signals were found in CSF. Analyses of genome-wide association study data for target genes of brain miRNAs showed significant association ($\alpha = 9.40 \times 10^{-5}$) of genetic variants in nine loci.
Interpretation: We identified several miRNAs that showed highly significant differential expression in PD. Future studies may assess the possible role of the identified brain miRNAs in pathogenesis and disease progression as well as the potential of the top blood miRNAs as biomarkers for diagnosis, progression, or prediction of PD.

ANN NEUROL 2019;85:835–851

Parkinson's disease (PD) is the second-most common neurodegenerative disease affecting 1% of people over the age of 60. The increasing incidence of PD in industrialized, aging populations constitutes a growing socioeconomic burden.[1] Idiopathic PD results from a combination of multiple genetic[2–4] and environmental/lifestyle factors.[5,6] However, the currently known risk factors only explain a small fraction of the phenotypic variance of PD. Likewise, PD

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progression and its response to therapy represent multifactorial processes that are only poorly understood.[6]

It is likely that epigenetic mechanisms contribute to PD development and progression.[6,7] Epigenetics refers to regulatory mechanisms of gene expression that are not mediated by the DNA sequence itself, but by chemical or allosteric DNA modifications or by the action of regulatory noncoding RNAs. microRNAs (miRNAs) are small noncoding RNAs that serve as posttranscriptional regulators of gene expression. They bind to messenger RNA (mRNA) and promote their degradation and/or decrease their translation.[8] In brain, miRNAs appear to play a role in essentially all processes related to neuronal function, including the development of neurodegenerative disorders such as PD.[9–11] The prominent role that miRNAs may play for the integrity of the central nervous system is exemplified by experiments inducing a selective depletion of Dicer, the enzyme that cleaves precursor forms of miRNAs (pre-miRNAs) into mature miRNAs. Depletion of this protein in midbrain dopaminergic neurons in mice leads to neurodegeneration and locomotor symptoms mimicking PD.[12] However, identifying specific miRNAs playing important roles in PD development and progression remains a challenge. In humans, several studies have reported on differential miRNA expression in PD patients compared to controls, but results have been inconclusive. This is, in part, attributed to the fact that sample sizes tend to be comparatively small and that studies often analyze different tissues or biological fluids (Tables 1–3). As a consequence, it has become exceedingly difficult to interpret the often discrepant results.

One way to address this challenge is to assess the cumulative evidence for differential miRNA expression, for example, by systematic meta-analyses combining all available published expression data in the field. Such approaches demonstrated their value in the context of genetic associations and environmental risk factors in several multifactorial diseases, including PD (eg, previous works[3,5]). For gene expression studies, combining published data by meta-analysis is a particularly challenging task because of the nonstandardized fashion that data are reported across publications. The aim of this study was to overcome these difficulties and identify consistently differentially expressed miRNAs in PD based on published evidence. To this end, we performed a systematic literature search to identify all relevant miRNA expression studies comparing idiopathic PD versus control subjects and extracted data from all eligible articles using a standardized protocol optimized for the extraction of expression data. Finally, we applied *p*-value–based meta-analyses in order to identify miRNAs that are consistently differentially expressed in PD.

Materials and Methods

Literature Search and Eligibility Criteria

The workflow and data collection procedures applied in this study (Fig 1) are similar to those for genetic association studies developed earlier by our group,[3,13] adapted to the characteristics of gene expression studies. A systematic literature search for miRNA expression studies in PD was performed using PubMed (http://www.pubmed.gov), applying the search term "(microRNA OR miRNA OR miR* OR micro-RNA) AND Parkinson*". Citations were assessed for eligibility using the title, abstract, or full text, as necessary. Only articles in English and published in peer-reviewed journals (last PubMed search date: October 1, 2018) were considered. Original studies comparing the expression of miRNAs in patients with clinical and/or neuropathological diagnosis of PD and unaffected controls were included. Studies were included irrespective of patient treatment status. miRNA expression studies on monogenic PD or PD families were excluded, as well as studies examining only patients with PD with dementia. A summary of eligible studies can be found in Tables 1–3.

Data Extraction

Details extracted for each eligible study consisted of the first author name, year of publication, and the PubMed identifier, along with key study- and population-specific details such as population and city of origin, number of idiopathic PD patients, number of controls, source of specimen (ie, brain, blood, and/or cerebrospinal fluid [CSF], and a more specific description for each specimen type, eg, substantia nigra, frontal cortex, amygdala, etc, or whole blood, serum, peripheral blood mononuclear cells [PBMCs], etc), experimental method(s) used, identifiers of the miRNAs, their expression in samples of PD patients versus controls (ie, up- or downregulation or no difference), and corresponding *p* values. Where available, effect size estimates (means and standard deviations [used as provided or calculated from 95% confidence intervals {CIs} or standard errors), mean differences and corresponding measures of variance, and/or details on the applied test statistics were extracted. Some of the effect-size data were extracted from results displayed in figures by using a specialized data capture program ("Plot digitizer"; http://plotdigitizer.sourceforge.net). All extracted data were double checked by an independent member of our group against the original publications.

For quality control (QC), we assessed reported miRNAs for their inclusion in miRBase (v21; http://www.mirbase.org). miRNA names corresponding to expired entries, nonhuman miRNAs, or RNA sequences not listed in miRBase were excluded from the analysis. miRNAs reported in the included studies were aligned to mature miRNA sequences according to miRBase. The same mature miRNA sequence reported with different miRNA names in different publications (applicable to 10 of 2,133 entries) was subsumed under one common identifier. This concerned miRNAs hsa-miR-199a-3p/hsa-miR-199b-3p, hsa-miR-365a-3p/hsa-miR-365b-3p, and hsa-miR-517a-3p/hsa-miR-517b-3p.

TABLE 1. Overview of Published miRNA Studies in Brain Specimens of PD Patients and Controls

Study	Country	N	Subspecimen	miRs	miRs MA	Featured miRs
Kim, 2007[12]	USA	3/3	Midbrain (cerebral cortex, cerebellum)[a]	1	1	133b
Sethi, 2009[34]	USA	4/6	Temporal cortex	4	4	—
Minones-Moyano, 2011[35]	Spain	14/21	Frontal cortex (SN, amygdala, cerebellum)[b]	2	2	34c-5p, 34b-3p
Cho, 2013[36]	USA	15/11	Frontal cortex (striatum)[b]	1	n.a.[c]	205-5p
Alvarez-Erviti, 2013[37]	Spain	6/5	SN: (amygdala)[a]	7	4	—
Kim, 2014[38]	USA	8/8	SN-DA neurons	1	n.a.[c]	126-3p
Schlaudraff, 2014[39]	Germany	5/8	Midbrain (DA neurons)	1	1	—
Villar- Menéndez, 2014[40]	Spain	6/7	Striatum	1	n.a.[c]	34b-3p
Cardo, 2014[41]	UK	8[e]/4[e]	SN	484	123	198, 135b-5p, 485-5p, 548d-3p
Briggs, 2015[42]	USA	8/8	SN: DA neurons	157	1	—
Pantano, 2015[43]	Spain	7/7	Amygdala	125	98	—
Hoss, 2016[44]	USA	29/33	Frontal cortex	892	123	10b-5p
Nair, 2016[45]	USA	12/12	Striatum	13	n.a.[c,d]	—
Wake, 2016[46]	USA	29/36	Frontal cortex	3	n.a.[d]	—
Tatura, 2016[47]	Germany	22/10	Anterior cingulate gyrus	41	29	144-3p, 199b-5p, 221-3p, 488-3p, 544a
McMillan, 2017[48]	UK	6/5	SN	1	1	7-5p

Legend: PD = idiopathic Parkinson's disease; N = number of PD patients/controls; Subspecimen = the tissues provided in brackets represent brain regions that have not been included in the meta-analysis because of tissue prioritization (see superscribed letters for details and see Materials and Methods); miRs = number of miRNAs for which test statistics, that is, *p* values and directions of effect, were provided in the article; miRs MA = number of miRNAs meta-analyzed in our study; featured miRs = indicates miRNAs highlighted as relevant for PD in the abstract of the respective publication; SN = substantia nigra; DA neurons = dopaminergic neurons; n.a. = not applicable (miRNA data not included because of population overlap or other reasons; see Materials and Methods);
[a]tissue prioritization according to Braak;
[b]tissue prioritization based on higher sample size;
[c]sample overlap (as reason for exclusion of data from meta-analysis);
[d]lack of 3 independent data sets for the miRNAs reported in this study (as reason for exclusion of data from meta-analysis);
[e]The effective sample size differs across individually tested miRNAs, numbers listed here represent the maximum effective sample size.

Data Cleaning and Reformatting

Data were analyzed after stratification for specimens derived from brain, blood, and CSF. Potential sample overlaps, that is, investigations of the same miRNA in identical or overlapping data sets of the same specimen type (ie, brain, blood, or CSF), for instance in two different publications, were systematically assessed in each stratum. Overlap was determined based on the origin and descriptions of the data sets, overlapping co-authors, and/or references to previous studies. In case of sample overlap, only the data entry from the largest data set was retained for further analysis. In some data sets (n = 3), miRNAs were assessed in more than one brain tissue in the same (or largely overlapping) individuals. Here, we chose only one brain tissue for inclusion in the meta-analysis. The first selection criterion was sample size, that is, if the number of analyzed samples was substantially (ie, at least 30%) larger in one brain tissue versus the other, we retained the larger sample and excluded the other. Otherwise, the prioritization on which brain tissue to include was based on the PD Braak staging[14] in order to maximize power (ie, assuming that brain regions affected earlier in the disease course will show more pronounced effects); that is, the tissue from the region affected earliest in the disease process was selected for inclusion. To assess potential bias introduced by this "prioritization" strategy, we performed sensitivity analyses by including data from "lower priority" regions instead. For the other strata (blood-derived tissue and CSF), only

TABLE 2A. Overview of Published miRNA Studies in Blood Specimens of PD Patients and Controls Published Until 2016

Study	Country	N	Subspecimen	miRs	miRs MA	Featured miRs
Margis, 2011[49]	Brazil	8/8	Whole blood	6	3	1-3p, 22-3p, 29a-3p
Martins, 2011[50]	Portugal	19/13	PBMCs	21	6	—
Cardo, 2013[51]	Spain	31/25	Plasma	7	4	331-5p
Soreq, 2013[52]	Israel	7/6	Serum	15	2	—
Khoo, 2012 [53]	Germany	42/30	Plasma	3	3	450b-3p, 626, 505-3p, 1826
Botta-Orfila, 2014[54]	Spain	1010	Serum	14	9	29a-3p, 29c-3p, 19a-3p, 19b-3p
	Spain	20/20	Serum	14	9	
	Spain	65/65	Serum	4	4	
Burgos, 2014[22]	USA	50/62	Serum	5	1	
Vallelunga, 2014[55]	Italy	31/30	Serum	8	3	339-5p, 223-5p, 324-3p, 24-3p, 30c-5p, 148b-3p
Zhao, 2014[56]	China	46/46	Serum	1	1	133b
Serafin, 2014[57]	Italy	38/38	PBMCs	2	n.a.[c]	30b-5p, 29a-3p
Alieva, 2015[58]	Russia	20/24	Lymphocytes	5	n.a.[c,d]	129-5p, 7-5p, 132-3p, 9-5p, 9-3p
Serafin, 2015[59]	Italy	36/36	PBMCs	5	1	103a-3p, 30b-5p, 29a-3p
Fernández-S., 2015[60]	Spain	8/28	Serum	3	3	19b-3p
Takahashi, 2015[61]	Japan	30/47	Plasma	6	n.a.[d]	—
Dong, 2016[62]	China	30/30	Serum	12	5	141-3p, 214-3p, 146b-5p, 193a-3p
	China	92/74	Serum	4	3	
Ding, 2016[63]	China	45/36	Serum	15	9	195-5p, 185-5p, 15b-5p, 221-3p, 181a-5p
	China	61/55	Serum	5	5	
Yılmaz, 2016[64]	Turkey	102/102	Whole blood	5	n.a.[d]	335-3p, 561-3p, 579-3p
Chen, 2016 [65]	China	24/61	PBMCs	4	1	—
Cosín-Tomás, 2016[66]	Spain	20/21	Plasma	4	1	—
Ma, 2016[67]	China	138/112	Serum	16	15	29c, 146a-5p, 214, 221

Legend:
PBMCs = peripheral blood mononuclear cells. n.a. = not applicable (miRNA data not included because of population overlap or other reasons; see Materials and Methods);
[c]sample overlap (as reason for exclusion of data from meta-analysis);
[d]lack of 3 independent data sets for the miRNAs reported in this study (as reason for exclusion of data from meta-analysis).

one specimen subtype was assessed per study; thus, prioritization was not applicable.

If a study reported several p values for the same miRNA in the same samples based on different experimental or analytical methods (eg, microarray versus real-time quantitative polymerase chain reaction, different normalization approaches), we reassessed whether one method was preferential to the other based on the information provided in the publication (eg, higher accuracy/reliability), and only the most accurate result was included. If no decision could be reached, we chose a conservative approach and retained the largest p value. For p values reported with a reference to a predefined significance threshold only (applicable to data from 19 of 40 publications and a total of 121 of 2,133 data entries), we used the following conservative conversions: $p \geq 0.05$ and $p \geq 0.01$ were converted to $p = 0.5$, $p < 0.05$ to $p = 0.025$, $p < 0.01$ to $p = 0.005$, $p < 0.001$ to $p = 0.0005$, and $p < 0.0001$ to $p = 0.00005$. In three instances, the p value in an article appeared as 0.0000; this was converted to 0.00005.

TABLE 2B. Overview of Published miRNA Studies in Blood Specimens of PD Patients and Controls Published Since 2017

Study	Country	N	Subspecimen	miRs	miRs MA	Featured miRs
Li, 2017[68]	China	60/60	Plasma	3	1	137, 124-3p
Cao,2017[69]	China	109/40	Serum	24	15	19b-3p, 195-5p, 24-3p
Fu, 2017[70]	China	15/15	PBMCs	1	1	21-5p
Schwienbacher, 2017[71]	Italy	50/50	Plasma	4	3	30a-5p
	Italy	49/49	Plasma	4	3	
	Italy	10/10	Plasma	4	n.a.[c]	
Zhang, 2017[72]	China	46[e]/49[e]	Plasma	4	2	433-3p, 133b
Bai, 2017[73]	China	80[e]/80[e]	Serum	4	3	29a-3p, 29b-3p, 29c-3p
Chen, 2017[74]	China	20/20	Plasma	8	2	4639-5p
	China	169/170	Plasma	1	n.a.[d]	
Yang, 2018[75]	China	30/30	Serum	3	n.a.[d]	—
Chen, 2018[76]	China	25/25	Plasma	15	4	27a-3p, let-7a-5p, let-7f-5p, 142-3p, 222-3p
Chi, 2018[77]	Portugal	19/13	PBMCs	19	1	126-5p, 29a-3p, 19b-3p
Yao, 2018[78]	China	52/48	Plasma	9	6	—
Jin, 2018[79]	China	46/46	Plasma	1	n.a.[d]	520d-5p
Caggiu, 2018[80]	Italy	37/43	PBMCs	2	1	155-5p, 146a-5p

Legend:
PBMCs = peripheral blood mononuclear cells. n.a. = not applicable (miRNA data not included because of population overlap or other reasons; see Materials and Methods);
[c]sample overlap (as reason for exclusion of data from meta-analysis);
[d]lack of 3 independent data sets for the miRNAs reported in this study (as reason for exclusion of data from meta-analysis).
[e]The effective sample size differs across individually tested miRNAs, numbers listed here represent the maximum effective sample size.

Statistical Analysis

Meta-Analyses. Whenever possible, we calculated fixed-effect and random-effects (see DerSimonian and Laird[15]) meta-analyses based on Hedges' g as a standardized mean difference between idiopathic PD and unaffected control individuals. Depending on data reporting in the individual studies,

TABLE 3. Overview of Published miRNA Studies in Cerebrospinal Fluid of PD Patients and Controls

Study	Country	N	miRs	miRs MA	Featured miRs
Burgos, 2014[22]	USA	57/65	16	4	—
Gui, 2015[81]	China	47/27	26	4	1-3p, 19b-3p, 153-3p, 409-3p, 10a-5p, let-7g-3p
	China	78/35	8	4	
Marques, 2016[82]	Netherlands	28/30	10	1	24-3p, 205-5p
Mo, 2016[83]	China	44/42	3	n.a.[d]	144-5p, 200a-3p, 542-3p

Legend: [d]lack of 3 independent data sets for the miRNAs reported in this study (as reason for exclusion of data from meta-analysis).

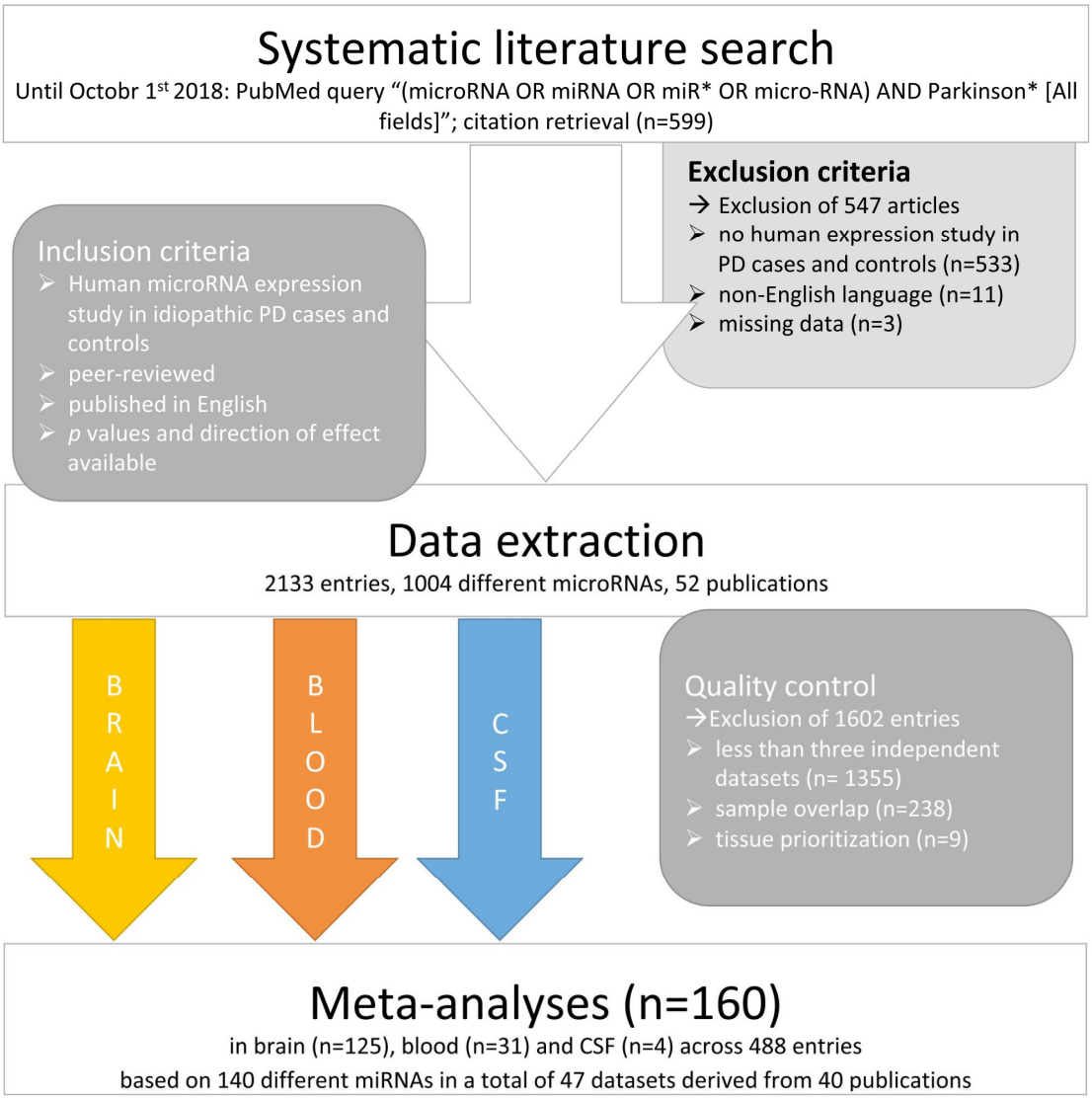

FIGURE 1: Flowchart of literature search, data extraction, and analysis of miRNA expression data. CSF = cerebrospinal fluid; miRNA = microRNA; PD = Parkinson's disease. [Color figure can be viewed at www.annalsofneurology.org]

Hedges' g was calculated using different approaches: It was either calculated based on means and standard deviations or based on mean differences and corresponding measures of variance. In cases where no direct effect sizes were provided, but the test statistic was described in sufficient detail, Hedges' g was approximated from the reported test statistics as described previously.[16] Whenever at least three independent data sets with Hedges' g estimates were available effect-size–based meta-analyses were calculated using the R package "meta" (https://cran.r-project.org/web/packages/meta/meta.pdf). Between-study heterogeneity of effect-size–based meta-analyses was quantified using the I^2 metric, which represents the estimate of percentage of heterogeneity that is beyond chance.

In case data of effect size estimates were not available, we performed meta-analyses on provided p values and directions of effects if these were available in ≥3 independent data sets using a customized R script transforming p values into signed z-scores using Stouffer's method[17] (https://www.r-project.org; available upon request), similar to an approach described previously for the meta-analysis of genetic association data.[18] This method allows to combine results even when effect size estimates and/or standard errors from individual studies are not available or are provided in different

units.[18] Briefly, the direction of effect and the *p* value observed in each data set were converted into a signed Z-score. Z-scores for each miRNA were then combined by calculating a weighted sum, with weights being proportional to the square root of the effective sample size for each data set. The primary meta-analysis for each miRNA was calculated based on the fixed-effect model and, if additional independent data were available, the *p*-value–based model. Random-effects models were calculated for comparison to the fixed-effect model. For diverging results between the fixed-effect and the random-effects models, forest plots, heterogeneity estimates, and effect directions across data sets were further investigated. Significance was defined using Bonferroni correction for multiple testing. This was based on the number of the primary meta-analyses performed across all three specimen strata (ie, $\alpha = 0.05/160 = 3.13 \times 10^{-4}$).

Classification of Study-Wide Significant Results According to the Presence of Heterogeneity. Study-wide significant meta-analysis results that showed no or little effect size heterogeneity (in the effect-size-based meta-analyses) and/or consistent direction of effects across data sets (in the *p*-value–based meta-analyses) were classified as showing "strong" support for a genuine involvement in PD. In the presence of heterogeneity, the random-effects model is considered more conservative than both the *p*-value–based and fixed-effect methods. However, because the random-effects model also tends to "penalize" consistent results that show heterogeneity on the same side of the null, we reinvestigated the forest plots of effect-size–based meta-analyses in such cases. If study-wide significant meta-analyses showed effect size heterogeneity attributed to variance of effect size estimates primarily at the same side of the null, we classified the respective miRNA as showing "strong" evidence and as "suggestive" if effect size estimates were on both sides of the null.

In cases where only *p*-value–based meta-analyses could be performed because of a lack of sufficient effect size data reported in individual studies, we also classified the consistency of effect direction using the above categories. Accordingly, miRNAs with meta-analysis results showing substantially differing directions of effect across independent data sets were labeled as providing "suggestive" and otherwise as providing "strong" evidence for an involvement in PD.

miRNA Target Gene Analysis. In order to assess indirectly whether any of the significantly differentially expressed miRNAs in brain may be involved in PD pathogenesis, we tested for a potential enrichment of their target genes in results of the latest genome-wide association study (GWAS) in PD.[2,3] To this end, summary statistics from 7,773,234 single-nucleotide polymorphisms (SNPs) were obtained from PDGene (http://www.pdgene.org),[3] and analyzed using two different approaches for miRNA target-site definition. First, we downloaded human miRNAs and corresponding experimentally validated miRNA targets from MiRTarBase (v. 6.1; http://mirtarbase.mbc.nctu.edu.tw/).[19] We used MiRTarBase because it lists miRNA-target interactions reported in the literature that have been experimentally validated, for example, by reporter assay, western blot, microarray, and/or next-generation sequencing (NGS) experiments. Second, we used brain-specific miRNA-target gene interactions predicted with AGO2 HITS-CLIP miRNA data published by Boudreau et al.[20] To this end, we mapped Ensembl gene identifiers from the data of Boudreau et al[20] to EntrezGene identifiers based on Ensembl (v. 87; http://www.ensembl.org). The corresponding gene sets from MiRTarBase and Bouddreau et al[20] were analyzed with Pascal[21] using 1000 Genomes samples (CEU) for assessment of linkage disequilibrium. Pascal combines SNP-based GWAS summary statistics to gene set scores and tests for enrichment of significant findings using a $\chi 2$ test and an empirical method.

In addition, we evaluated which top brain miRNAs bind to mRNAs from genes located in the established PD risk loci[2–4] (PD genes assigned for each locus according to Chang et al[4]) and to the established causal PD genes *LRRK2*, *SNCA*, *VPS35*, *PRKN*, *PINK1*, and *PARK7* (aka, *DJ1*).[6]

Furthermore, we evaluated whether any individual SNP (apart from the established, ie, genome-wide significant, risk SNPs) located in the miRNA target genes (±10kb) was significantly associated with PD in the GWAS data.[2,3] Adjustment for multiple testing was performed using Bonferroni correction for the number of tested target genes for all top miRNAs (ie, $\alpha = 0.05/532 = 9.40 \times 10^{-5}$). Finally, we investigated whether the respective PD-associated SNPs (or their proxies using a pair-wise linkage disequilibrium estimate of $r^2 > 0.6$ as threshold) may directly alter binding of the respective target miRNA(s). To this end, we mapped the SNPs and their proxies to the target sites of the top brain miRNAs as predicted by Targetscan (release 7.2; http://www.targetscan.org/vert_72/).

Results

Description of Eligible Studies

The PubMed search yielded 599 publications, which were screened for eligibility of inclusion. A total of 52 publications were eligible for initial data extraction. After QC, data from 47 independent data sets across 40 publications (Tables 1–3) were subsequently included in the meta-analyses. Reasons for the exclusion of eligible data sets from meta-analysis are summarized in Figure 1 and Tables 1–3.

miRNA expression data included in the meta-analyses were derived from brain tissue, CSF, and/or blood-derived samples (Tables 1–3). Eleven of the total of 47 data sets

included in the meta-analysis were based on brain (Table 1), 32 data sets on blood-derived samples (Tables 2A and 2B), and four data sets on CSF (Table 3). Only one of the included publications tested more than one of the three specimen types (blood and CSF[22]). Sampled brain regions of data sets included in the meta-analyses comprised substantia nigra/midbrain (n data sets = 6), neocortex (n = 4, comprising frontal, prefrontal, temporal, and anterior cingulate cortex), and amygdala (n = 1; Table 1). The median number of study participants per data set was 46 across all studies (interquartile range [IQR], 12–95; range, 4–250) irrespective of the specimen type analyzed. The median number of individuals was 11 (IQR, 8–16; range, 4–62) for brain tissue, 81 (IQR, 41–114; range, 13–250) for blood-derived specimens, and 93.5 (IQR, 70–115; range, 58–122) for analyses of CSF. Twenty-seven of the 47 independent data sets provided data (albeit sometimes only for a subset of miRNAs) that allowed for the calculation of Hedges' g as the standardized mean difference.

Across all 40 studies included in the analyses presented here, half of the eligible studies (20 of 40; 50%) stated explicitly that they had performed age matching in their study design. Furthermore, information on the age distribution in patients and controls was provided for 26 data sets, and this distribution was comparable in most instances (average difference in patients and controls across all 26 data sets: 2.5 years; Supplementary Table 1). Four studies indicated statistically significant differences in the age distribution between patients and controls. Similar observations were made for the reporting of sex matching (40% report sex matching; average difference, 3.1%; Supplementary Table 1).

Twenty-two of all 40 studies used a targeted ("candidate miRNA") approach to quantify miRNAs using RT-qPCR (n = 20 studies), northern blotting (n = 1), or a combination of methods (n = 1). The remaining 18 studies applied a hypothesis-free ("mirRnome-wide") screening approach using microarrays (n = 5), NGS (n = 6), or TaqMan array miRNA cards (n = 7). The five studies using microarrays as an initial hypothesis-free approach applied targeted quantification methods for the top miRNAs in the same samples for validation.

The median number of miRNAs analyzed per study and included in the meta-analyses presented here was 3 (IQR, 1–5), ranging from 1 to 123. Only four studies presented data on more than 100 miRNAs (Table 1). Overall, data for a total of 1,004 different miRNAs were reported across all studies, of which 140 had been assessed in at least three independent data sets in at least one specimen stratum and were thus eligible for meta-analysis (Supplementary Tables 2 and 3). Another 327 miRNAs had been assessed in two studies in at least one specimen type, and the remaining 537 had been assessed in only a single study in a single specimen type. Seventeen of the 140 miRNAs were meta-analyzed in both brain and blood strata, one miRNA was meta-analyzed in brain and CSF, and one miRNA in all three strata, overall resulting in 160 individual meta-analyses (Supplementary Tables 2 and 3).

Meta-Analysis Results

One hundred twenty-five meta-analyses were based on data collected in brain tissue, 31 in blood-derived samples, and four in CSF. Twenty-one of these meta-analyses were calculated based on effect sizes (Hedges' g) using fixed-effect and random-effects models (brain: n = 3, blood: n = 18; Supplementary Table 2; Figs 2 and 3). For more than half of these meta-analyses (13 of 21), additional data sets were available allowing extended meta-analyses based on *p* values (Supplementary Table 2).

The median number of data sets included per primary meta-analysis across all miRNAs in brain, blood, and CSF was 3 (maximum [max.] 4), 3 (max. 11), and 3 (max. 4), respectively. The median combined sample size across all miRNAs in brain, blood, and CSF was 88 (IQR, 87–98), 339 (IQR, 267–596), and 309 (IQR, 309.0–323.5), respectively. On average, approximately equal numbers of patients and controls were included in each meta-analysis (Supplementary Tables 2 and 3).

Three of the 125 miRNAs meta-analyzed in brain showed study-wide significant ($\alpha = 3.13 \times 10^{-4}$) differential expression in idiopathic PD versus controls subjects, with effect estimates pointing into the same direction of effect for each meta-analysis (classified as "strong" evidence; Table 4). One miRNA was upregulated (hsa-miR-497-5p, $p = 1.35 \times 10^{-4}$), while two (hsa-miR-132-3p, $p = 6.37 \times 10^{-5}$; hsa-miR-133b, $p = 1.90 \times 10^{-4}$) were downregulated in PD compared to control subjects (Table 4). Furthermore, the meta-analysis result for one brain miRNA (hsa-miR-628-5p) reached study-wide significance in the *p*-value–based model ($p = 1.67 \times 10^{-4}$; effect size estimates were not available), but effect directions were heterogeneous. Consequently, we classified this miRNA as showing "suggestive" evidence for differential expression (Table 4). In addition, 34 brain miRNAs showed nominally significant ($\alpha = 0.05$) differential expression (Supplementary Tables 2 and 3); however, these results did not survive multiple testing correction ($\alpha = 3.13 \times 10^{-4}$). Sensitivity analyses on the prioritization of multiple brain areas analyzed in the same samples showed that meta-analysis results were sufficiently robust regarding our prioritization procedure (Supplementary Table 4).

Ten of 31 meta-analyzed miRNAs from blood-derived samples showed study-wide significant ($\alpha = 3.13 \times 10^{-4}$) differential expression in idiopathic PD versus control subjects (*p* values ranging from 4.49×10^{-35} to 2.64×10^{-4}) with effect estimates nearly always pointing into the same direction in each meta-analysis ("strong" evidence, Table 5). All 10 miRNAs were downregulated in idiopathic PD compared to control subjects (Table 5). The miRNA with the most statistically

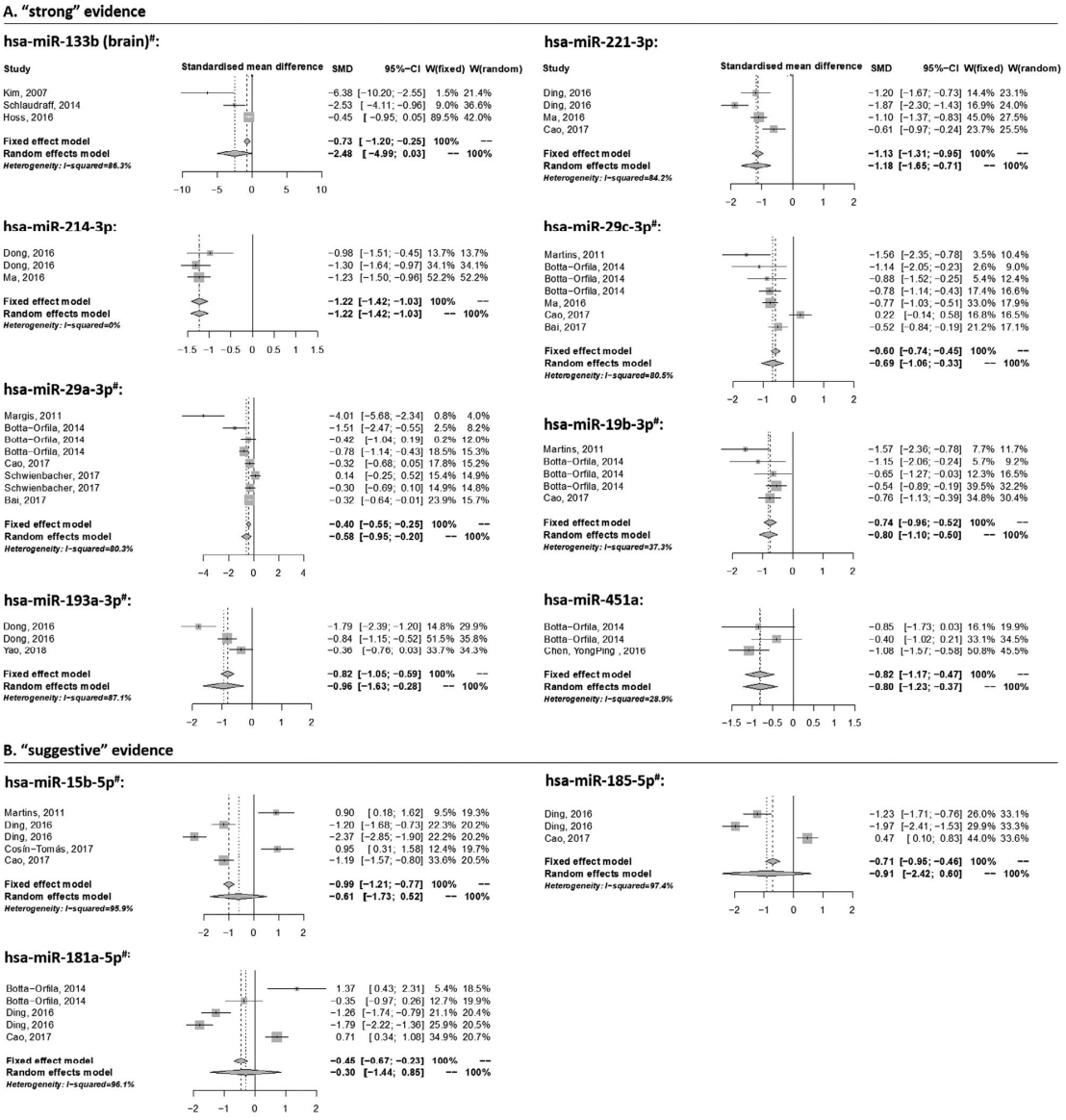

FIGURE 2: Forest plots of study-wide significant fixed-effect and random-effects meta-analyses on published miRNA expression data in idiopathic Parkinson's disease and unaffected control individuals. Study-wide significant meta-analysis results ($\alpha = 3.13 \times 10^{-4}$) were classified as showing "strong" and "suggestive" evidence for differential expression in Parkinson's disease according to heterogeneity assessments (see Materials and Methods). Note that for several miRNAs, extended data sets were available for *p*-value–based meta-analyses, which are therefore considered as primary meta-analyses (respective miRNAs are labeled with the pound symbol ("#"); also see Tables 4 and 5 for details). Also note that for a few significantly associated miRNAs, only *p*-value–based meta-analyses were performed (see Tables 4 and 5 for detail). CI = confidence interval; miRNA = microRNA; SMD = standardized mean difference.

significant differential expression in blood was hsa-miR-221-3p ($p = 4.49 \times 10^{-35}$). In addition, three miRNAs (ie, hsa-miR-15b-5p, hsa-miR-185-5p, and hsa-miR-181a-5p) showed study-wide significant differential expression in blood specimen in the fixed-effect meta-analyses in the presence of substantial in-between study heterogeneity, that is, with effect estimates on both sides of the null (Table 5). We therefore classified the results for these three miRNAs as "suggestive." Seven additional miRNAs showed nominally significant ($\alpha = 0.05$) differential expression in the primary meta-analyses (Supplementary Tables 2 and 3), but did not survive multiple testing ($\alpha = 3.13 \times 10^{-4}$).

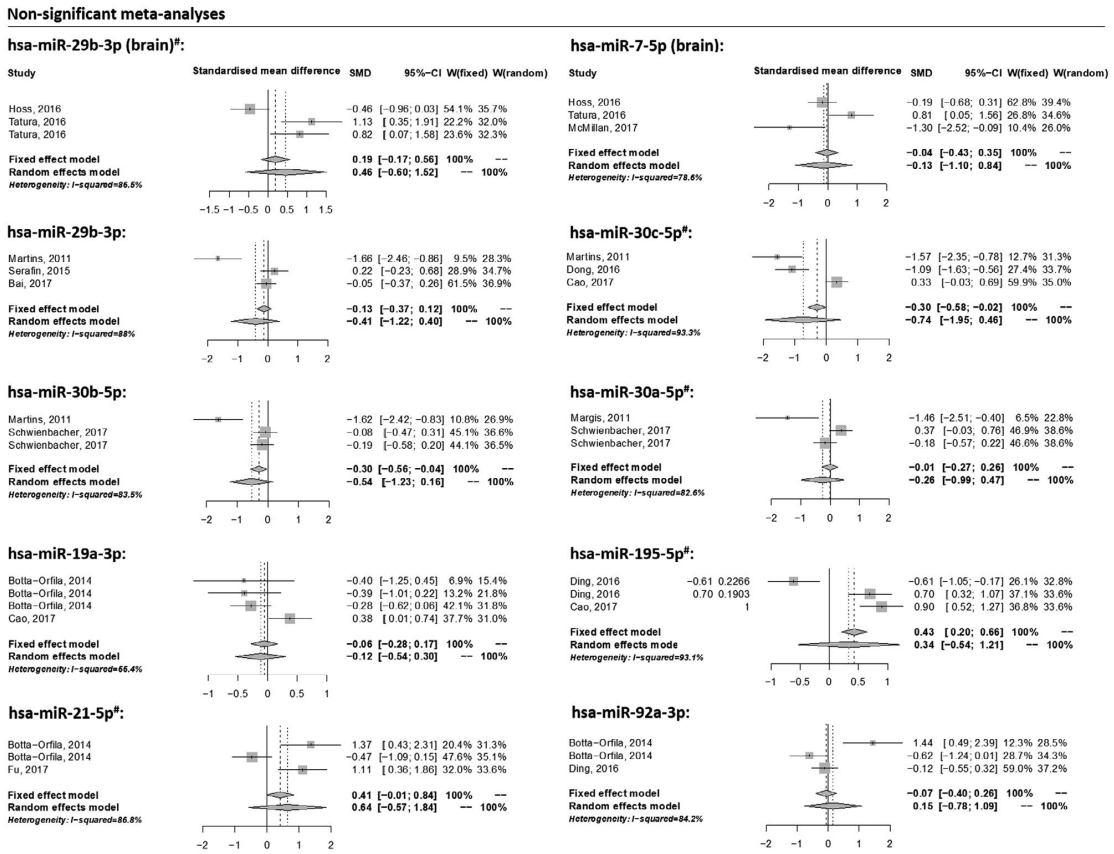

FIGURE 3: Forest plots of nonsignificant fixed-effect and random-effects meta-analyses on published miRNA expression data in idiopathic Parkinson's disease and unaffected control individuals. CI = confidence interval; miRNA = microRNA; SMD = standardized mean difference.

Of the four miRNAs meta-analyzed in CSF, none yielded significant results for differential expression in idiopathic PD versus control individuals (Supplementary Table 3).

Interestingly, hsa-miR-133b was study-wide significant in both brain ($p = 1.90 \times 10^{-4}$) and blood ($p = 2.64 \times 10^{-4}$) and was downregulated in both specimen groups. Furthermore, miRNAs hsa-miR-19b-3p, hsa-miR-185-5p, and hsa-miR-29a-3p showed at least nominally significant expression differences in both brain and blood. Hsa-miR-19b-3p and hsa-miR-185-5p were downregulated in both brain ($p = 7.29 \times 10^{-4}$ and $p = 0.0034$, respectively) and blood ($p = 2.68 \times 10^{-10}$ and $p = 4.84 \times 10^{-12}$, respectively) in PD versus controls. hsa-miR-29a-3p was upregulated in brain ($p = 0.0322$) and downregulated in blood ($p = 9.36 \times 10^{-12}$; Supplementary Tables 2 and 3).

Target Gene Analysis of Top Differentially Expressed Brain miRNAs

We assessed whether SNPs in or near genes that represent targets of the top candidate brain miRNAs (three classified as showing "strong" and one as showing "suggestive" evidence) may also contribute to PD risk (regardless of whether they alter protein function or gene expression by non-miRNA-mediated or by miRNA-mediated mechanisms). This may represent an independent line of evidence supporting a potential role of the respective brain miRNA(s) in PD pathophysiology. Based on published functional data available in miRTarBase[19] and on brain-specific HITS-CLIP data,[20] three of the four brain miRNAs (showing "strong" [n = 3] and "suggestive" [n = 1] evidence for differential expression) were found to target mRNAs from genes located in established PD risk loci or from causal PD genes. For instance, based on the available brain HITS-CLIP data, hsa-miR-132-3p binds to the mRNAs of *SNCA* and of *SCN3A* and hsa-miR-497-5p binds to the mRNA of *CCNT2* (Supplementary Table 5).

Considering all sets of genes targeted by any of the top four brain miRNAs, no set of targets showed significant enrichment ($\alpha = 0.05$) for genetic association with PD from GWAS data (Supplementary Table 6). However, the GWAS results of genetic variants mapping in target genes of the four brain miRNAs (after exclusion of the

TABLE 4. Significant Meta-Analysis Results of Differentially Expressed miRNAs in Brain Specimen of PD Patients and Controls

miRNA	N total (pts, ctrls)	Expr. per dataset	Overall expr.	*P*: FE | P	*P*: RE	I^2 (95% CI)	Evidence
132-3p[$]	84 (41,43)	-, -, -	Down	6.37E-05	n.a.	n.a.	strong
497-5p[$]	119 (65,54)	+, +, +, +	Up	1.35E-04	n.a.	n.a.	strong
133b	78 (41,37) | 90 (45,45)	-, -, -|-	Down	2.50E-04 | 1.90E-04	5.28E-02	86 (61;95)	strong[a]
628-5p[$]	88 (44,44)	-, +, +	Up	1.67E-04	n.a.	n.a.	sugg.[b]

Legend: This table displays the meta-analysis results of miRNAs for which *p*-value–based and, where applicable, fixed-effect (FE) and random-effects (RE) meta-analyses were performed. For several miRNAs (listed in Tables 4 and 5), the number of independent data sets available for *p*-value–based meta-analyses were larger than for the effect-size–based meta-analyses. In these instances, *p*-value–based meta-analyses were calculated and regarded as "primary" analyses and are also provided in this table. Details/results on these *p*-value–based meta-analysis comprising the extended number of data sets are provided second, separated by the symbol "|" in the relevant table cells. In some instances, as indicated, results were derived from *p*-value–based meta-analyses only.

[a]Heterogeneity attributed to variance of effect estimates only on one side of the null.

[b]Heterogeneity attributed to variance of effect estimates on both sides of the null/effect estimates pointing into both directions.

[$]Results derived from *p*-value–based meta-analyses only. N = number; CI = confidence interval; I^2 = amount of heterogeneity in percentage that is estimated to be beyond chance; data-set–specific expression = differential expression for the respective miRNA per data set included in the meta-analysis in PD patients compared to controls (for included studies, see Supplementary Material); overall expression = global direction of expression across all meta-analyzed data sets.

established risk loci already evaluated above) revealed nine additional loci that showed significant association with PD ($\alpha = 9.40 \times 10^{-5}$, Bonferroni-adjusted for the number of evaluated target genes [n = 532]; Supplementary Table 7).

Based on TargetScan predictions, a proxy (rs2977461) of one PD GWAS SNP (rs2944758; $r^2 = 0.62$) is located 19bp downstream of the seed site of miR-132-3p (chr8:141541307-141541314) in the 3'-untranslated region of *AGO2* and may thus possibly affect the binding of this miRNA to its target.

Comparison of miRNAs Featured in Original Publications Versus Meta-Analysis Results

Across all eligible studies, a total of 73 different miRNAs were "featured" in the original publications, that is, they were prominently highlighted as showing differential expression in PD patients versus controls in the abstract of the respective publication. Only eight (~11%; hsa-miR-1-3p, hsa-miR-7-5p, hsa-miR-30b-5p, hsa-miR-34b-3p, hsa-miR-146a-5p, hsa-miR-195-5p, hsa-miR-205-5p, and hsa-miR-214-3p) of these were featured in two studies and six (~8%; hsa-miR-19b-3p, hsa-miR-24-3p, hsa-miR-29a-3p, hsa-miR-29c-3p, hsa-miR-133b, and hsa-miR-221-3p) in more than two studies. More than half of these featured miRNAs (45 of 73; 62%) were meta-analyzed in our study. Of note, 13 of these 45 miRNAs (~29%) indeed showed study-wide significant association ($\alpha = 3.13 \times 10^{-4}$, with" strong" and "suggestive" evidence) in our meta-analyses, whereas an additional 10 (~22%) showed nominally significant association ($\alpha = 0.05$). In contrast, nearly half (ie, 22 of 45 miRNAs [49%]) that had been prominently highlighted in at least one publication did not show any significant results in our meta-analyses. In addition, and perhaps more importantly, miRNAs miR-497-5p and miR-628-5p, showing" strong" and "suggestive" evidence for association, respectively, in our brain-stratified meta-analyses, and hsa-miR-451a, showing study-wide significance with "strong" evidence in the blood-stratified meta-analyses, were not featured in any of the original studies.

Comparison of Original Versus Replication Evidence

To further assess the reproducibility of significant miRNA expression results, we compared all at least nominally significant *p* values from the original study with results from independent replication data only (replication data were combined by meta-analysis, where applicable; Fig 4). For 34 (21%) of all 160 meta-analyses, nominally significant (two-sided $\alpha = 0.05$) differential miRNA expression was recorded by us for the first study. Less than half of these results (n = 12; 35%) were replicated with at least nominal significance (one-sided $\alpha = 0.05$) when all available independent replication data were combined, and nine of these 12 results that replicated also yielded study-wide significance (two-sided $\alpha = 3.13 \times 10^{-4}$) upon meta-analysis of *all* data (ie, combining original and replication data). Interestingly, the failure of replication of original results

TABLE 5. Significant Meta-Analysis Results of Differentially Expressed miRNAs in Blood Specimen of PD Patients and Controls

miRNA	N total (pts, ctrls)	Expr. per dataset	Overall expr.	*P*: FE | P	*P*: RE	I^2 (95% CI)	Evidence
221-3p	596 (353, 243)	-, -, -, -	Down	4.49E-35	9.04E-07	84 (60;94)	Strong
214-3p	476 (260, 216)	-, -, -	Down	2.00E-34	2.00E-34	0 (0;80)	Strong
29c-3p	773 (436, 337) | 809 (444, 365)	-, -, -, -, -, +, -| -	Down	2.87E-15 | 3.00E-12	2.00E-04	81 (61;90)	Strong
29a-3p	711 (389, 322) | 1,029 (554, 475)	-, -, -, -, -, +, -, -| -,-,-	Down	2.80E-07 | 9.36E-12	2.70E-03	80 (62;90)	Strong[c]
19b-3p	371 (223, 148) | 657 (369, 288)	-, -, -, -, -| -,-	Down	2.71E-11 | 2.69E-10	2.13E-07	37 (0;77)	Strong
193a-3p	326 (174, 152) | 632 (343, 289)	-, -, -| -,-	Down	2.09E-12 | 3.04E-08	5.30E-03	87 (63;96)	Strong[a]
141-3p[$]	476 (260, 216)	-, -, -	Down	8.06E-07	n.a.	n.a.	Strong
451a	145 (54, 91)	-, -, -	Down	5.51E-06	3.00E-04	29 (0;93)	Strong
146a-5p[$]	411 (220, 191)	-, -, -	Down	9.88E-06	n.a.	n.a.	Strong
133b[$]	433 (227, 206)	-, -, -	Down	2.64E-04	n.a.	n.a.	Strong
15b-5p	419 (254, 165) | 669 (392, 277)	+, -, -, +, -| -	Down	3.18E-18 | 2.49E-12	2.90E-01	96 (93;98)	Sugg.[b]
185-5p	346 (215, 131) | 646 (378, 268)	-, -, +|-,-	Down	1.07E-08 | 4.84E-12	2.39E-01	97 (95;99)	Sugg.[b]
181a-5p	406 (245, 161) | 696 (403, 293)	+, -, -, -, +| -,-	Down	5.04E-05 | 2.21E-10	6.13E-01	96 (93;98)	Sugg.[b]

Legend: [a]Heterogeneity attributed to variance of effect estimates only on one side of the null.
[b]Heterogeneity attributed to variance of effect estimates on both sides of the null/effect estimates pointing into both directions.
[c]Heterogeneity attributed to variance of effect estimates predominately on one side of the null.
[$]Results derived from *p*-value–based meta-analyses only.

was predominately observed in CSF and brain whereas most blood-based findings showed good evidence for replication (Fig 4).

Discussion

Following a systematic literature search and data extraction, we analyzed data from all hitherto published eligible miRNA expression studies in PD patients versus controls. We identified 17 miRNAs that were significantly differentially expressed in brain or blood across at least three independent studies. Based on heterogeneity assessments, we classified 13 of these miRNA as showing "strong" evidence for differential expression and four miRNAs as showing "suggestive" evidence. Interestingly, some of the top brain miRNAs target mRNAs of genes that are central in PD pathophysiology. The most compelling finding relates to miRNA hsa-miR-132-3p binding to the mRNA of *SNCA*. To the best of our knowledge, our study represents the first quantitative assessment of published miRNA expression data in PD. Furthermore, we are not aware of any other neurodegenerative research field having applied a comparable approach to collate published miRNA expression results of individual miRNAs by meta-analysis. We are aware of one publication[23] that meta-analyzed sensitivity and specificity estimates of miRNA profiles in Alzheimer's disease in seven of >100 differential miRNA expression studies in that field. This approach, however, is substantially different from our approach, which aims to pinpoint individual miRNAs differentially expressed between affected and unaffected individuals. Therefore, our study not only provides unique insights into the current knowledge of individual miRNA expression differences in PD, but may also be taken as an example for performing equivalent analyses in other neurodegenerative diseases. In fact, our group is currently performing a similar field-wide analysis for differential miRNA expression in Alzheimer's disease (AD). Preliminary

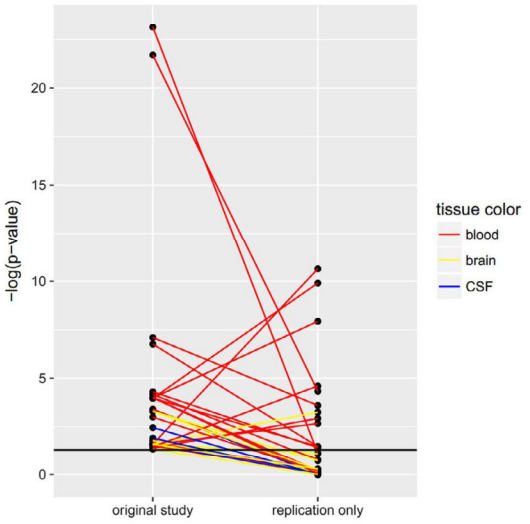

FIGURE 4: Comparisons of original and replication *p* values. This figure displays all at least nominally significant two-sided *p* values of the respective original studies (data from independent data sets derived from the original study were combined by meta-analysis where applicable) and the corresponding (one-sided) *p* value from all replication data only (combined by meta-analysis where applicable). Note that *p* values from all other meta-analyses in this article are two-sided; a one-sided *p* value was chosen here to take into account the directions of effect in the replication data. Corresponding *p* values of original and replication data are connected by a line (yellow line = brain-stratified results, red line = blood-stratified results, blue line = cerebrospinal fluid-stratified results). The y-axis shows the negative log of the *p* value (ie, larger values indicate more significant results). The horizontal black line corresponds to a *p* value of 0.05. CSF = cerebrospinal fluid.

results from that ongoing effort suggest that possibly up to three miRNAs (ie, hsa-miR-29c-3p, hsa-miR-146a-5p, and hsa-miR-451a) showing differential expression in blood in PD, also appear to be differentially expressed in blood from AD patients when compared to controls (Takousis et al, in preparation).

One of the strengths of this study is the increase in sample size (and thus power) by combining all eligible data into one statistical test. As outlined above, sample sizes of individual miRNA studies are often small, especially in studies of brain tissue. By meta-analysis, we were able to increase the sample size substantially. In addition, errors occurring only in a single data set will have a less pronounced impact on the resulting test statistic. Still, most of our brain-stratified meta-analyses (median, n = 88) are underpowered to detect only modest changes in miRNA expression. At the same time, significant results need to be considered with caution. Thus, a substantial increase in sample size should be one of the major objectives in future miRNA expression studies focusing on brain tissue.

Our study shows that the majority of miRNAs featured in the original publications or showing significant results in the first study cannot be replicated in independent investigations and do not have statistical support for differential expression in our meta-analyses. Along these lines, qualitative reviews on the role of miRNAs in PD are largely based on a (subjective) selection of the literature that does not hold up to systematic meta-analyses. For instance, in five recent articles reviewing the role of miRNAs in PD based on human expression or on experimental data[7,24–27] (including one systematic review[27]), 190 miRNAs were highlighted as being potentially relevant in PD (Supplementary Table 8). Of these, expression data were lacking or sparse for 113 (59%), that is, they could not be meta-analyzed here. Among the remaining 77 miRNAs highlighted by at least one review, only 13 (7% of the 190 miRNAs) showed evidence for differential expression in PD in our meta-analyses. Furthermore, three of our top miRNAs (hsa-miR-146a-5p, hsa-miR-497-5p, and hsa-miR-628-5p) were not mentioned in any of the five reviews. These observations highlight the need for independent replication and validation of proposed miRNAs as well as for regular quantitative—rather than merely qualitative—assessments of the available evidence in the literature.

Most of our significant results were based on blood expression data. Whereas these results will likely not reveal novel insights into PD's pathophysiology, these miRNAs may still have the potential to serve as "classification markers" for PD. It should also be noted that gene expression is not only tissue specific, but also variable over time. Thus, differential expression of miRNAs does not allow to draw conclusions on cause-effect relationships in PD. This is true for both blood and brain and for any investigation examining (prevalent) PD patients. In this context, it is noteworthy that all 11 miRNAs in the blood-based results appear to be "downregulated" in idiopathic PD as compared to control subjects. This may reflect changes in gene expression and/or cell compositions as a result of disease progression or maybe most likely treatment effects. Furthermore, in the brain-derived results, especially those from substantia nigra, it is also possible that expression differences might only reflect changes of cellular composition in the diseased tissue. Given that most studies normalize the results using general housekeeping genes, such effects will not necessarily be removed entirely. An alternative way to quantify miRNA expression would be to perform single-cell experiments in cells of interest (eg, dopaminergic neurons). However, although a meta-analysis has recently been published for mRNA-based transcriptomics studies applying laser capturing for single-cell analysis in the substantia nigra,[28] equivalent data on miRNAs are currently too sparse.

Furthermore, most publications do not provide any information on disease duration, severity, and treatment of patients and, for brain tissue, neuropathological progression markers. Thus, the impact of these factors on the respective miRNA results is impossible to assess adequately. In addition,

a study design that does not consider age and/or sex matching for patients and controls may produce biased gene expression results. As described in the Results section, the majority of data sets had comparable age and sex distributions in patients and controls. Notwithstanding, we cannot exclude that missing age and/or sex matching has had an impact on some of our meta-analysis results. Furthermore, we note that other variables, such as the use of different eligibility criteria and recruitment schemes and diverse specimen retrieval protocols, as well as different methods of RNA extraction, miRNA expression measurements, and different statistical methods, etc, may impact the results of any individual study and may thus be one of the causes of in-between study heterogeneity. However, the current number of independent individual data sets per miRNA is too small to investigate the impact of these variables systematically (eg, by performing sensitivity or metaregression analyses).

In the context of controlling the potential impact of external variables and in order to disentangle cause-effect relationships, the conduct of differential expression studies in animal models may be useful despite the potential limitations in translating these findings to the human system. To this end, we investigated (by screening titles and abstracts) how many of the 547 articles excluded from our systematic review would qualify for a similar meta-analytical approach based on animal models. This resulted in 11 differential miRNA expression studies in PD animal models. However, these studies were very heterogenous in that they investigated a range of different animals (mouse = 6 studies, rat = 2, drosophila = 2, *Caenorhabditis elegans* = 1) and focused mostly on nonoverlapping miRNAs. Therefore, although these results could be regarded as informative in the context of our study, the currently available published data are too sparse to yield meaningful meta-analyses or robust qualitative assessments.

In this study, whenever possible, we applied effect-size–based meta-analyses (n = 21) using the standardized mean difference (Hedges' g), which allowed us to also quantify effect size heterogeneity across the included data sets. Importantly, this list comprised 11 of the 17 miRNAs showing Bonferroni-corrected significance (with "strong" and "suggestive" evidence) in our primary meta-analyses. However, around 40% of all eligible studies did not provide precise effect estimates and/or variances and thus precluded the calculation of Hedges' g. In cases where effect-size–based meta-analyses were not possible (n = 135) or where data were available for additional data sets on the same miRNAs (n = 13 of 21 effect-size–based meta-analyses), we performed systematic *p*-value–based meta-analyses to collate the available published data. This is an established method often applied in the GWAS field,[18] and the *p* values of our fixed-effect meta-analyses corresponded well to those of the *p*-value–based meta-analyses on identical sets of data (Supplementary Table 9). However, using *p* values only does not allow to estimate the magnitude of gene expression differences, quantify the heterogeneity of estimated effect sizes, or perform additional analyses, such as testing for small-study effects, which can be indicative of publication or selective reporting bias.[29] However, except for the heterogeneity assessments, none of these additional analyses were possible for our effect-size–based data because of a lack of sufficient data. As proxy for in-between study heterogeneity for the *p*-value–based meta-analyses, we assessed the consistency of effect directions qualitatively across individual data sets for study-wide significant miRNAs. This revealed potential evidence for heterogeneity, that is, effect directions pointing in both directions of the null, for hsa-miR-628-5p expression in brain, and for to hsa-miR-15b-5p, hsa-miR-185-5p, and hsa-miR-181a-5p in blood, for which heterogeneity was already observed in the effect-size-based meta-analyses for a smaller subset of data. Because of this heterogeneity, we classified the overall evidence for differential expression for these four miRNAs as "suggestive" only (Tables 4 and 5).

Importantly, a proportion of publications did not report full *p* values (applicable to data from 19 of 40 publications and a total of 121 of 2,133 data entries), but reported them as "less than" or "greater than" a certain significance level. Here, we chose a conservative approach for including such data in our analyses (see Materials and Methods section). Furthermore, the quality of our analyses can, at best, only mirror the quality of the underlying publications from which data were extracted. We performed a range of QC checks to detect inconsistencies within studies, but cannot exclude that all errors were detected by this procedure. However, we do not expect any *systematic* error arising from errors and mistakes that may have remained undetected in the original publications. Nevertheless, these observations clearly highlight the need for a standardized and more transparent reporting of applied methodology, statistics, and results in miRNA expression studies.[30]

One additional limitation in combining data from the published domain is the potential presence of publication bias and/or selective reporting bias. Because of the lack of consistently reported effect size estimates in a part of eligible publications (see above), we were not able to assess potential hints for this bias quantitatively (eg, by regression analyses[31]). To address this concern, we evaluated each publication for evidence that only a subset of the generated expression results were reported in detail (Supplementary Table 10). For nearly two-thirds of all publications (ie, 25 of 40; 63%), we did not find evidence for selective reporting of expression results. Fifteen publications had generated more data than provided in the publication. Five of these studies provided the identifiers of the miRNAs for which detailed results were not provided. This list contained 10 of the 13 miRNAs differentially expressed in blood (with strong or suggestive evidence) according to our meta-analyses. This was attributed to few

large-scale studies that had only highlighted the most prominent miRNAs. Meta-analyses in other fields (eg, cancer) of miRNA and other regulatory RNA associations have pointed out the surprisingly high proportion of reported statistically significant results, which may be an indication of excess significance attributed to selective reporting.[32,33] This pattern was not as prominent in the studies that we analyzed, where 15 of the identified studies (Tables 1–3) did not feature any particular miRNAs eventually. In summary, we cannot exclude that selective reporting has inflated some of our meta-analysis results. Especially, the blood-based meta-analysis results need to be considered with caution and warrant independent replication.

In conclusion, by systematically combining data from all eligible miRNA expression studies published to date, we identified 13 miRNAs that were consistently differentially expressed in PD patients and controls in brain or blood. Future studies will need to increase the sample size for miRNA-based studies on brain tissue. Our study is the first to compile published miRNA expression data in the field of neurodegenerative diseases in a systematic and standardized way. Thus, it may serve as a model for combining these data in other related fields.

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Author Contributions

J.S., P.T., R.P., L.B., and C.M.L. contributed to the conception and design of the study. J.S., P.T., I.W., I.O.G.I., V.D., G.R., H.B., L.M., J.P.A.I., and C.M.L. contributed to the acquisition and analysis of data. J.S., P.T., L.B., and C.M.L. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

Nothing to report.

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Featured Article

Differential expression of microRNAs in Alzheimer's disease brain, blood, and cerebrospinal fluid

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Abstract

Introduction: Several microRNAs (miRNAs) have been implicated in Alzheimer's disease pathogenesis, but the evidence from individual case-control studies remains inconclusive.
Methods: A systematic literature review was performed, followed by standardized multistage data extraction, quality control, and meta-analyses on eligible data for brain, blood, and cerebrospinal fluid specimens. Results were compared with miRNAs reported in the abstracts of eligible studies or recent qualitative reviews to assess novelty.
Results: Data from 147 independent data sets across 107 publications were quantitatively assessed in 461 meta-analyses. Twenty-five, five, and 32 miRNAs showed studywide significant differential expression ($\alpha < 1{\cdot}08 \times 10^{-4}$) in brain, cerebrospinal fluid, and blood-derived specimens, respectively, with 5 miRNAs showing differential expression in both brain and blood. Of these 57 miRNAs, 13 had not been reported in the abstracts of previous original or review articles.
Discussion: Our systematic assessment of differential miRNA expression is the first of its kind in Alzheimer's disease and highlights several miRNAs of potential relevance.

Keywords: Alzheimer's disease; Dementia; Genetics; Epigenetics; Biomarker; Meta-analysis; Systematic review; Diagnosis; Prognosis; Pathophysiology

1. Background

In addition to genetic variants, epigenetic factors may play an important role in Alzheimer's disease (AD) pathogenesis, such as DNA methylation or the regulatory effects of small noncoding RNAs, in particular microRNAs (miRNAs). miRNAs are 18-25 nucleotides in length and primarily regulate gene expression at the post-transcriptional level, through recognition of specific binding sites located mainly in the 3′-untranslated region of their target messenger RNAs (mRNAs) [1]. Thus, changes in miRNA expression can lead to translational repression and, consequently, reductions in the levels of the respective proteins.

Owing to their crucial role in "fine-tuning" gene expression, miRNAs have been proposed as biomarkers and/or

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therapeutic targets for a range of human disorders, including AD [2–4]. A growing body of literature suggests that miRNAs are causally linked to AD by directly affecting the underlying pathogenic pathways, for example, by targeting *APP* [5] or *BACE1* expression [6,7], thereby altering the risk and/or progression of the disease [4,5]. However, despite their possible pathophysiological relevance in AD and their potential to serve as biomarkers, there is still no conclusive picture as to which miRNAs play the lead roles, for example, by showing consistent patterns of differential expression in AD versus healthy controls. Furthermore, interpretation of the existing data is severely hampered by methodological shortcomings of the individual studies, including small sample size and methodological heterogeneity, making it difficult to compare different studies.

The present work aimed at performing a systematic review and meta-analysis of all published studies assessing differential expression of miRNAs in AD cases versus controls in brain, blood, and cerebrospinal fluid (CSF) specimens. The main goal was to identify miRNAs that show significant and consistent differential expression when comparing results across publications. In line with similar efforts applied to results from genetic association studies developed by members of our group [8,9], we applied a standardized multistage data extraction and quality control protocol followed by meta-analysis on all resulting eligible data. Our work, which represents the first of its kind in AD, highlights 57 unique miRNAs showing consistent and highly significant differential expression across studies. This systematic review generates a knowledge base which could prove instrumental in future studies assessing the role of miRNAs in AD pathogenesis and their potential as AD biomarkers.

2. Methods

See Fig. 1 for a quantitative summary of the various steps underlying this systematic review; in addition, the Supplementary Material provides a full description of all methods applied in this study. In the following section, methods are only summarized to highlight the most essential features and check points of our study. Overall, the workflow and data collection procedures applied in this work are similar to those for genetic association studies developed previously by members of our group [8,9], and subsequently adapted to meta-analysis of miRNA expression studies [10]. In brief, this entailed a systematic literature search for miRNA expression studies in AD using the PubMed database, the systematic extraction of data from eligible studies into a project-specific database, and a range of data cleaning and reformatting steps followed by extensive plausibility and quality checking (e.g., alignment of miRNA identifiers to those listed in miRbase, v21 (http://www.mirbase.org), identification of duplicate publications and/or sample overlap across studies, and double checking of entries by independent team members). Statistical analyses of the remaining quality controlled data entailed meta-analyzing study-level results for miRNAs with at least three independent data points per specimen.

The meta-analyses themselves were performed using a customized R script (https://www.r-project.org; available on request) applying Stouffer's method [11,12] that is, converting reported *P* values into signed z-scores, which were then meta-analyzed using inverse variance weighting based on sample size, a method previously applied to the meta-analysis of mRNA/miRNA expression data by us and others [10,13]. Thresholds of statistical significance were defined after Bonferroni correction for multiple testing. This was based on the overall number of meta-analyses (n = 461) performed across all three strata resulting in a studywide α of $0{\cdot}05/461 = 1{\cdot}08 \times 10^{-4}$. Studywide significant results were further classified into "strong" and "suggestive" evidence based on consistency of effect direction across studies: to be classified as "strong" evidence, >80% of studies had to show the same direction of effect, else they are classified as "suggestive". This threshold proved to correlate well with formal effect size–based heterogeneity assessments using the I^2 metric in a similar study performed on differential miRNA expression in Parkinson's disease [10]. Target genes of miRNAs showing studywide significance were identified and evaluated based on next-generation sequencing data from human postmortem brain samples [14] and experimentally validated miRNA-target interactions from miRTarBase [15]. Finally, we performed genetic enrichment analyses using these target gene predictions based on GWAS summary statistics from the International Genomics of AD Project [16].

3. Results

3.1. Study characteristics

Our systematic literature search yielded a total of 895 publications, of which 107 were classified as eligible for inclusion after careful, multistage screening (Fig. 1). After quality control, data from 147 independent data sets across these 107 publications were subsequently extracted, entered into our database and included in the meta-analyses. Possible reasons for exclusion of publications/data sets from meta-analysis are summarized in Fig. 1.

miRNA expression data included in the meta-analyses were generated from specimens classified as derived from "brain", "CSF", and "blood". Of all 147 meta-analyzed data sets, 60 were generated from brain, 32 from CSF, and 53 from blood specimens. Two additional data sets utilized miRNA expression data from anterior nasal septum mucosa and olfactory bulb (Supplementary Table 1). Fifteen publications reported results from more than one

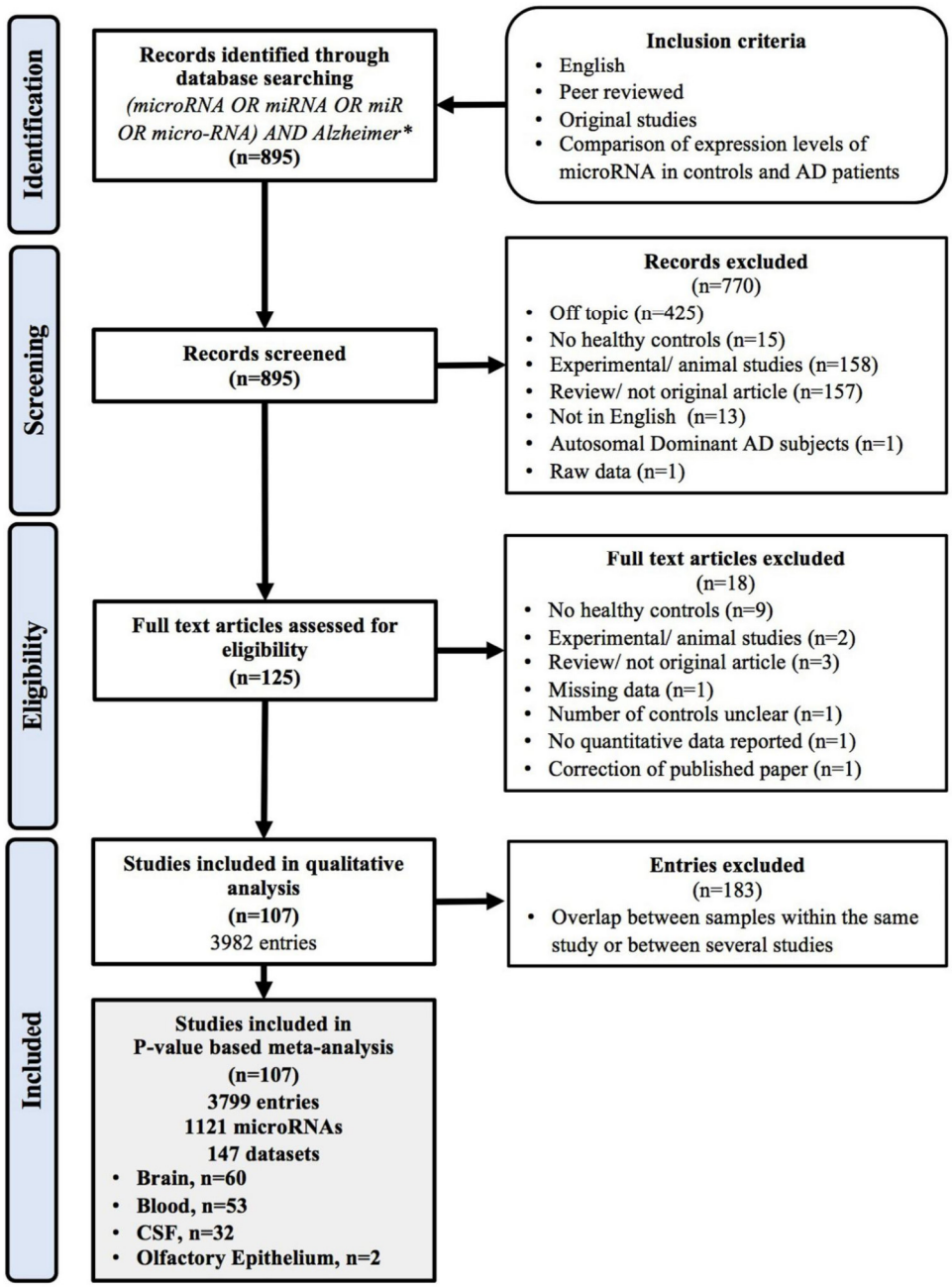

Fig. 1. Workflow implemented to identify eligible data sets.

specimen type: two from brain and CSF [5,17], four from brain and blood [18–21], and nine from CSF and blood [22–30]. Data sets included in the brain meta-analyses comprised specimen from different regions: basal forebrain/entorhinal (n = 3), hippocampus/medial temporal lobe (n = 31), frontal/prefrontal lobe (n = 14), and parietal lobe (n = 2). For four data sets, brain regions were not specified; six data sets consisted of two or more brain regions (Supplementary Table 1). The median sample size per data set was 34·5 across all studies (interquartile range [IQR] 17-68), 19 for brain (IQR 12-34), 38 for CSF (IQR 21-62), and 68 for blood (IQR 37-97).

Overall, data for a total of 1121 different miRNAs were reported across all studies. The median number of meta-analyzed miRNAs analyzed per study was four (IQR 2-13). Only nine studies used a large-scale approach, defined

here as reporting findings on more than 100 miRNAs, and data from all of these studies were included in our meta-analyses (Supplementary Table 1). Of all included miRNAs, 295 had been assessed in at least three independent data sets in at least one specimen type and were, thus, deemed eligible for meta-analysis. One hundred and twenty-three of the 295 miRNAs were meta-analyzed in more than one specimen type, that is, 58 in brain and blood, 20 in brain and CSF, two in CSF and blood, and 43 in all three specimen types. Overall, this resulted in sufficient data to conduct 461 individual meta-analyses for the present study (Supplementary Table 2).

3.2. Meta-analysis results

Two hundred and sixty meta-analyses were based on data collected using brain-derived, 66 using CSF-derived, and 135 using blood-derived specimens. The median number of data sets included per meta-analysis across all miRNAs was four in brain and blood, and three in CSF, with maximum values of 19, 10, and 11, respectively. The median combined sample size across all miRNAs in brain, CSF, and blood was 42·5 (IQR 23-85), 164 (IQR 98-205), and 259 (IQR 195-318), respectively.

Twenty-five of the 260 (10%) miRNAs meta-analyzed for brain showed studywide significant differential expression in AD cases versus controls with P values ranging from $4{\cdot}13 \times 10^{-13}$ to $9{\cdot}10 \times 10^{-5}$ (Table 1). Of these, 9 were classified as showing "strong" evidence based on the consistency of effect direction across studies (Methods). The three miRNAs with the most statistically significant and consistent differential expression were hsa-miR-125b-5p, hsa-miR-501-3p, and hsa-miR-138-5p. Twelve miRNAs were upregulated, whereas 13 were downregulated in AD compared with controls. In CSF, five of the 66 (8%) meta-analyzed miRNAs showed studywide significant differential expression in AD cases versus controls with P values ranging from $3{\cdot}48 \times 10^{-7}$ to $3{\cdot}76 \times 10^{-5}$ (Table 1; all showing consistent effect directions, classified here as "strong" evidence). All five were downregulated in AD compared with controls. The three miRNAs with the most statistically significant differential expression in CSF were hsa-miR-598-3p, hsa-miR-451a, and hsa-miR-9-5p. Finally, in blood, thirty-two of 135 (24%) meta-analyzed miRNAs showed studywide significant differential expression in AD cases versus controls with P values ranging from $9{\cdot}16 \times 10^{-24}$ to $4{\cdot}64 \times 10^{-5}$ (Table 1; 21 showing consistent effect directions, classified here as "strong" evidence). Thirteen miRNAs were upregulated, whereas 19 were downregulated in AD cases versus controls. The three miRNAs with the most statistically significant differential expression were hsa-miR-342-3p, hsa-miR-191-5p, and hsa-let-7d-5p. Thirty-eight additional miRNAs showed nominally significant differential expression across all three different specimen (see Supplementary Table 2 for a full list of meta-analysis results).

Interestingly, five miRNAs showed studywide significant differential expression in both brain and blood, that is, hsa-miR-181c-5p and hsa-miR-29c-3p were downregulated in both, whereas hsa-miR-125b-5p, hsa-miR-146a-5p, and hsa-miR-223-3p were upregulated in brain and downregulated in blood (Table 1). No overlaps in upregulated or downregulated miRNAs showing studywide significant differential expression were observed between CSF and either brain or blood.

3.3. miRNA target identification and genetic enrichment analyses

To map miRNA-target interactions of the studywide significant miRNAs, we used two sources of information. First, we interrogated Ago2 HITS-CLIP sequencing results derived from human brain samples [14], which highlighted nine genes previously implicated in AD (see Supplementary Material on how these were defined), that is, *APP, CCDC6, CD2AP, CLU, CNTNAP2, FERMT2, PICALM, PTK2B,* and *SORL1* (Fig. 2 and Supplementary Table 3a). Second, we used experimentally validated miRNA-target interactions posted on miRTarBase [15], which revealed 12 known AD genes, that is, *ADAMTS4, APP, CCDC6, CD2AP, CLU, FERMT2, HS3ST1, PICALM, PLCG2, SCIMP, SLC24A4,* and *SPI1* (Supplementary Table 3b; overlapping targets across both approaches are underlined). These observations provide additional support for a genuine involvement of the implicated miRNAs in AD pathogenesis.

In addition to these known AD genes, each miRNA was predicted to also interact with several targets not previously linked to AD. We were, thus, interested to assess whether the full sets of miRNA-specific targets showed an enrichment (using PASCAL) [31] of significant GWAS signals based on genomewide summary statistics published along with the International Genomics of AD Project study [16]. Although three nominally significant enrichments were observed, that is, with hsa-miR-17-3p, hsa-miR-7-1-3p, and hsa-miR-93-5p (Supplementary Table 3), none of these results reached studywide significance using false discovery rate controlling for multiple comparisons. Future work needs to assess the potential functional role, if any, of these non-AD genes targeted by the miRNAs highlighted in our meta-analyses.

3.4. Comparison of meta-analysis results to qualitative summaries of differential miRNA expression in AD

We generated two sets of qualitative reference data. First, we recorded miRNAs highlighted in the abstracts of the 107 publications forming the literature knowledge base of our study and compared these miRNAs with our meta-analysis

Table 1
Significant meta-analysis results of differentially expressed miRNAs in brain, CSF, and blood in Alzheimer's disease patients and controls (miRNAs not reported in the abstracts of previous original articles or in qualitative reviews, in italics)

miRNA	# Total (AD/HC)	# Data sets	Data set–specific expression	Overall expression	*P* value	Classification of evidence
Brain						
hsa-miR-125b-5p	122 (64,58)	11	+, +, +, +, +, +, +, +, +, +, +	Up	4.13E-13	Strong
hsa-miR-501-3p	68 (38,30)	4	+, +, +, +	Up	2.03E-11	Strong
hsa-miR-885-3p	23 (11,12)	3	-, -, +	Down	2.86E-11	Suggestive
hsa-miR-132-5p	57 (27,30)	5	-, -, +, -, -	Down	3.01E-10	Suggestive
hsa-miR-7-1-3p	23 (11,12)	3	-, +, +	Up	5.71E-09	Suggestive
hsa-miR-138-5p	161 (96,65)	6	-, -, -, -, -, -	Down	5.50E-08	Strong
hsa-miR-340-5p	40 (21,19)	4	-, +, -, +	Down	1.41E-07	Suggestive
hsa-miR-34a-5p	122 (65,57)	7	-, +, +, +, +, -, +	Up	1.89E-07	Suggestive
hsa-miR-195-5p	177 (104,73)	7	+, +, +, +, +, -, +	Up	3.74E-07	Strong
hsa-miR-129-5p	166 (100,66)	6	+, -, -, -, -, -	Down	3.84E-07	Strong
hsa-miR-146a-5p	235 (124,111)	12	+, -, +, +, +, +, -, +, -, +, +, +	Up	4.88E-07	Suggestive
hsa-miR-181c-5p	65 (31,34)	6	-, -, -, -, -, -	Down	9.49E-07	Strong
hsa-miR-129-2-3p	161 (95,66)	5	-, -, +, -, -	Down	1.16E-06	Suggestive
hsa-miR-223-3p	97 (57,40)	5	-, +, +, +, +	Up	1.65E-06	Suggestive
hsa-miR-454-3p	33 (16,17)	4	+, +, +, +	Up	4.86E-06	Strong
hsa-miR-363-3p	97 (57,40)	5	-, +, +, +, +	Up	6.57E-06	Suggestive
hsa-miR-487b-3p	97 (57,40)	5	+, -, -, -, +	Down	7.14E-06	Suggestive
hsa-miR-323a-3p	97 (55,42)	5	+, -, -, +, -	Down	1.05E-05	Suggestive
hsa-miR-769-5p	109 (62,47)	5	-, -, -, +, -	Down	1.33E-05	Suggestive
hsa-miR-152-3p	97 (55,42)	5	-, +, +, +, +	Up	1.54E-05	Suggestive
hsa-miR-455-5p	85 (51,34)	4	+, +, +, +	Up	1.58E-05	Strong
hsa-miR-488-3p	23 (11,12)	3	-, +, +	Up	2.53E-05	Suggestive
hsa-miR-29c-3p	89 (45,44)	5	-, -, -, -, -	Down	2.65E-05	Strong
hsa-miR-370-3p	97 (57,40)	5	+, -, -, -, +	Down	6.23E-05	Suggestive
hsa-miR-485-5p	113 (56,57)	6	+, -, -, -, -, +	Down	9.10E-05	Suggestive
CSF						
hsa-miR-598-3p	233 (112,121)	4	-, -, -, -	Down	3.48E-07	Strong
hsa-miR-451a	106 (49,57)	4	-, -, -, -	Down	4.86E-07	Strong
hsa-miR-9-5p	226 (111,115)	6	+, -, -, -, -, -	Down	1.22E-05	Strong
hsa-miR-127-3p	248 (119,129)	4	-, -, -, -	Down	2.79E-05	Strong
hsa-miR-139-5p	215 (103,112)	3	-, -, -	Down	3.76E-05	Strong
Blood						
hsa-miR-342-3p	703 (363,340)	6	-, -, -, -, -, -	Down	9.16E-24	Strong
hsa-miR-191-5p	674 (349,325)	8	-, +, -, -, -, -, -, -	Down	4.23E-16	Strong
hsa-let-7d-5p	703 (359,344)	8	-, -, -, -, -, +, -, -	Down	9.82E-15	Strong
hsa-miR-107	684 (394,290)	6	-, -, -, -, -, -	Down	4.26E-11	Strong
hsa-miR-425-5p	164 (97,67)	3	+, +, +	Up	1.73E-10	Strong
hsa-miR-361-5p	220 (120,100)	3	+, +, +	Up	3.01E-10	Strong
hsa-miR-98-5p	587 (305,282)	4	-, -, -, -	Down	4.19E-10	Strong
hsa-miR-671-3p	289 (150,139)	3	+, +, +	Up	3.10E-08	Strong
hsa-miR-31-5p	263 (134,129)	3	-, -, -	Down	3.78E-08	Strong
hsa-miR-5001-3p	244 (132,112)	3	+, +, +	Up	3.87E-08	Strong
hsa-let-7d-3p	182 (101,81)	3	+, +, +	Up	7.87E-08	Strong
hsa-miR-93-5p	568 (299,269)	7	-, -, +, -, -, -, -	Down	9.96E-08	Strong
hsa-miR-146a-5p	662 (349,313)	9	-, -, -, -, -, -, -, +, -	Down	1.21E-07	Strong
hsa-miR-125b-5p	986 (525,461)	9	+, -, -, -, -, -, +, -, -	Down	3.48E-07	Suggestive
hsa-miR-26a-5p	272 (151,121)	4	+, -, +, +	Up	5.17E-07	Suggestive
hsa-miR-181c-5p	443 (209,234)	4	-, +, -, -	Down	7.99E-07	Suggestive
hsa-miR-144-5p	212 (118,94)	3	-, -, -	Down	8.95E-07	Strong
hsa-miR-30d-5p	187 (104,83)	3	+, -, +	Up	1.04E-06	Suggestive
hsa-miR-128-3p	296 (159,137)	5	+, +, -, +, -	Up	1.53E-06	Suggestive
hsa-miR-210-3p	255 (130,125)	4	-, -, -, -	Down	2.22E-06	Strong
hsa-miR-17-3p	187 (104,83)	3	-, -, -	Down	2.40E-06	Strong
hsa-let-7a-5p	195 (108,87)	4	-, -, +, -	Down	5.00E-06	Suggestive
hsa-miR-29c-3p	327 (176,151)	5	-, -, +, -, -	Down	6.55E-06	Suggestive
hsa-miR-363-3p	212 (118,94)	3	+, +, -	Up	1.08E-05	Suggestive
hsa-let-7c-5p	187 (104,83)	3	-, -, -	Down	1.16E-05	Strong
hsa-miR-30a-5p	116 (73,43)	3	+, +, -	Up	1.39E-05	Suggestive
hsa-miR-885-5p	426 (215,211)	3	-, -, -	Down	1.84E-05	Strong
hsa-miR-550a-3p	182 (101,81)	3	+, +, +	Up	2.49E-05	Strong

(*Continued*)

Table 1
Significant meta-analysis results of differentially expressed miRNAs in brain, CSF, and blood in Alzheimer's disease patients and controls (miRNAs not reported in the abstracts of previous original articles or in qualitative reviews, in italics) (*Continued*)

miRNA	# Total (AD/HC)	# Data sets	Data set–specific expression	Overall expression	*P* value	Classification of evidence
hsa-miR-340-3p	182 (101,81)	3	+, -, +	Up	2.50E-05	Suggestive
hsa-miR-483-3p	517 (257,260)	3	-, -, -	Down	3.16E-05	Strong
hsa-miR-26b-3p	274 (147,127)	3	+, +, +	Up	3.90E-05	Strong
hsa-miR-143-3p	576 (305,271)	8	-, -, +, -, -, +, -, +	Down	4.64E-05	Suggestive

NOTE. classification of evidence is based on the heterogeneity assessments in the study by Schulz et al. [10]: "strong" evidence refers to meta-analyses with >80% of included studies showing the same direction of effect and "suggestive" otherwise.

results. In total, there were 124 different unique miRNAs reported in the abstracts of articles included in our study at varying levels of frequency (Supplementary Fig. 1). Thirty-one (25%) of these miRNAs were also found to be differentially expressed at studywide significance, and another 40 (i.e., overall 57%) at nominal significance in our meta-analyses (Supplementary Fig. 1). For the remaining 53 miRNAs, either no significant differential expression was observed here (i.e., for 30 [24%] miRNAs), or meta-analyses could not be performed because of a lack of sufficient data (i.e., for 23 [19%] miRNAs). Most importantly, 26 miRNAs that were found to be differentially expressed at studywide significance here had not been reported in the abstract of any of the individual research articles, 11 of these were classified as showing "strong" evidence for differential expression.

Second, we compared our quantitative findings with miRNAs highlighted in 17 recent qualitative reviews published since 2016 (see Supplementary Material for details on how publications were selected). Overall, these articles reported 201 miRNAs as being potentially relevant in AD pathogenesis but only 40 (20%) of these miRNAs showed studywide significant differential expression in our meta-analyses. Including nominally significant results extended this list to another 62 (i.e., overall 51%) overlapping miRNAs. Of the remaining 99 miRNAs, 49 (24%) showed no evidence of differential expression here, whereas 50 (25%) did not have sufficient data to be meta-analyzed. A total of 17 miRNAs showing studywide significant differential expression in our analyses were not highlighted in any of the 17 qualitative review articles. Four of these overlapped with miRNAs reported in abstracts of original articles (see the aforementioned), leaving a total of 13 miRNAs (of 57, i.e., 23%) which can be considered "novel" results of this study (italicized miRNA names in Table 1).

4. Discussion

This work represents a comprehensive and quantitative account of studies assessing differential miRNA expression in AD cases versus controls across three specimen types. We utilized a multipronged protocol combining systematic literature searches with rigorous data extraction, quality control, and double-checking procedures, followed by meta-analysis of eligible study data. The approach applied here was originally developed for and successfully applied to genetic association studies in AD and other fields [8,9], adapted to the specifics of studying miRNA expression. To the best of our knowledge, our study represents the first comprehensive systematic synthesis and quantitative assessment of miRNA differential expression data in the field of AD research.

Taken together, we identified 57 unique miRNAs showing studywide significant (i.e., $P < 1{\cdot}08 \times 10^{-4}$) differential expression in brain-derived, CSF-derived, and/or blood-derived specimens. Five of these miRNAs showed evidence for studywide differential expression in more than one tissue type and several of these miRNAs target established AD genes which may suggest their involvement in AD pathogenesis. Forty-four of the differentially expressed miRNAs were highlighted in the abstracts of publications eligible for data extraction in this study and/or qualitative review articles on the role of miRNA expression in AD. This means that nearly one quarter (i.e., 13) of the miRNAs identified here were not highlighted in previous publications and can, thus, be considered "novel" findings of our systematic analyses. Notably, nine of these 13 novel miRNAs were found to be differentially expressed in brain-derived specimen and according to published functional data, many of the novel miRNAs identified may play important roles in AD-related mechanisms (summarized in Supplementary Table 4). Alongside this publication we make the complete knowledge base collated for this study available to the community (Supplementary Table 5): this includes the database of all study-level miRNA expression data (with nearly 4000 entries across all 107 included studies) and details of all 461 meta-analysis results (in Supplementary Table 2).

More than half of the miRNAs showing studywide significant differential expression (i.e., 32 of 57) were identified by meta-analyses performed on blood-derived specimens. This finding could prove an important prerequisite for future investigations aimed at identifying miRNA signatures able to differentiate between healthy aging and AD, potentially in the prodromal stage. Further research into this topic is required to validate and extend our findings in prospective

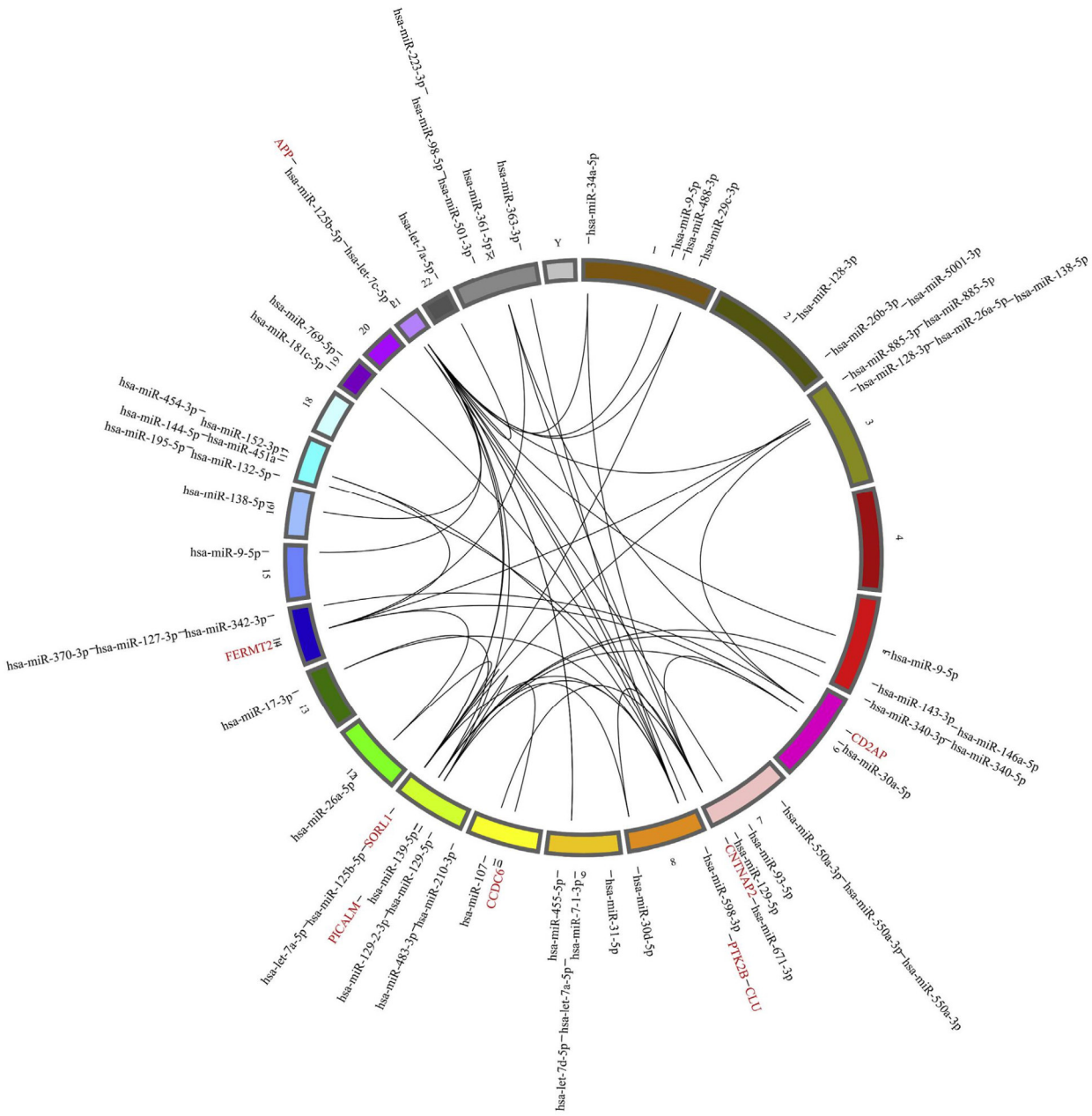

Fig. 2. Circos plot of chromosomal locations of all miRNAs showing studywide significant differential expression in the meta-analyses of AD cases versus controls. Connecting lines are between miRNAs and their target genes (as determined by Ago2 HITS-CLIP sequencing in postmortem human brain) [14] showing genomewide significant associations with AD risk from recent GWAS (see Supplementary Material). For more details on the AD-specific miRNA-target pairs displayed in this figure, see Supplementary Table 3a.

studies, for example, to assess the diagnostic and prognostic properties of the miRNAs highlighted by meta-analysis here. Such blood-based miRNA signature(s), perhaps in concert with other blood-based biomarkers and genetic predictors, would facilitate risk stratification for large-scale prevention programs and provide a window of opportunity for early therapeutic intervention. Such signature(s) could also serve as a tool to assess disease stage and monitor disease progression or therapeutic effects.

It is particularly noteworthy that five miRNAs showed studywide significant differential expression in both brain and blood: hsa-miR-181c-5p and hsa-miR-29c-3p were found to be downregulated in both, whereas hsa-miR-125b-5p, hsa-miR-146a-5p, and hsa-miR-223-3p were

upregulated in brain and downregulated in blood (Table 1). The fact that the same miRNAs show differential expression in different specimen types in AD versus controls emphasizes their potential role in AD and highlights their potential as future biomarkers. Different explanations have been proposed to explain the observation of opposing effect directions. For example, if the pathology is associated with a tissue-specific concentration change of a ubiquitously expressed miRNA, the effect on circulating miRNA concentration could be minimal because only a fraction of it derives from the affected tissue [32]. Alternatively, pathology-associated miRNA concentration changes may coincide with opposite changes in cellular miRNA secretion, thus counterbalancing the effect of altered miRNA expression [32]. Such a correlation is actually observed for one of the best established AD biomarkers; amyloid-β 42 ($A\beta_{42}$) levels are found to be increased in the brains of individuals with AD, but not in their CSF or blood, where $A\beta_{42}$ shows a marked decrease. Only future work will help to clarify the potential pathogenic and prognostic role of these and other miRNAs highlighted in our study.

Despite the systematic and comprehensive nature of our approach, our study and its results are subject to a range of limitations and potential sources of bias. First, our methods to identify relevant articles and the data reported therein may be erroneous, for example, owing to imperfect search strategies or mistakes made during the data entry and/or double-checking process. However, by using an adapted version of a carefully crafted data handling protocol already successfully applied to thousands of genetic association studies for different phenotypes [8,9], we do not expect data entry errors to have largely affected the main conclusions of our study. A related limitation relates to the "quality" of the primary articles which we did not specifically assess here. Poor quality studies (e.g., those applying inadequate data normalization or analysis strategies) may have affected the meta-analysis outcome to some degree.

The second and likely most important limitation of our study relates to the fact that meta-analyses in this report were based on synthesizing reported *P* values as test statistic and direction of differential expression ("up" or "down" in AD cases vs. controls). This method of quantitative data aggregation may appear relatively crude and simplistic as no direct effect estimates (e.g., fold change) or measures of precision (e.g., standard errors), which are typically utilized in quantitative research syntheses, were used. However, we note that these latter metrics were reported inconsistently and infrequently across the studies included in our analyses precluding effect size–based analyses (and thereby assessments of effect-size heterogeneity across study results) or potential biases, for example, publication bias or selective reporting bias. To gauge the overall impact of this issue, we performed a detailed comparison of effect size–based versus *P* value–based meta-analyses in the context our companion study on differential miRNA expression in Parkinson's disease [10]. These analyses clearly showed that *P* value–based results were essentially identical to effect size–based results in the absence of between-study heterogeneity. In the presence of heterogeneity, there tended to be greater differences, motivating us to classify studywide significant results into "strong" (i.e., very little heterogeneity) or "suggestive" (i.e., some evidence of heterogeneity [10]), and the same approach was taken here. Only future work adhering to standardized experimental and reporting standards will improve this situation in research aimed at synthesizing gene expression data across different datasets. Until then, the miRNAs highlighted in the meta-analyses presented here—especially those without evidence of between-study heterogeneity, that is, classified as "strong" evidence—likely represent the most promising candidates to show genuine differential expression in AD when compared with controls.

Third, we note that the sample sizes of individual studies aggregated in this work are comparatively small. This is likely due to the fact that the research probing for differential gene expression is still in an early phase with more powerful applications (e.g., mRNA or miRNA sequencing using next-generation technologies) only slowly becoming affordable to be performed on a larger scale. To a degree, the bias inherent in analyzing small sample sizes was attenuated here by combining the results of multiple independent small data sets by meta-analysis. However, even our largest analysis (i.e., that of hsa-miR-29a-3p in blood with a combined sample size of 1043 from ten independent studies [Supplementary Table 2]) is small compared with other fields, such as GWAS of complex traits where recent studies aggregated more than one million individuals by meta-analysis [33]. Until much larger miRNA expression studies are published (and meta-analyzed), the miRNAs highlighted here likely represent the most promising findings in the field.

Fourth, similar to gene expression data, evidence of differential miRNA expression cannot serve as indicator regarding cause or effect of the underlying pathophysiological process, disease progression, and/or treatment effects. Taking the AD brain as example, it is well established that the cell composition changes as the disease progresses; neuronal cells are depleted while the number of other cell types, for example, glial cells, can increase. Such changes can give rise to differential miRNA expression but do not necessarily reflect changes at the cellular level and/or imply the involvement of specific biochemical pathways, which is of primary interest to gain further insights into the pathophysiology of a given disease. Future studies could overcome this potential source of bias by measuring expression in specific cells rather than a heterogeneous tissue

specimen and by cell selection techniques such as laser capture microdissection. Furthermore, research on gene (including miRNA) expression should consistently provide detailed information about post-mortem interval, disease duration, severity and treatment before tissue donation, as this would enable assessment of how these factors may influence brain miRNA expression.

In conclusion, in our systematic and quantitative assessment of more than 100 individual studies, we identified 57 unique miRNAs showing studywide differential expression in AD versus controls, 35 of these with highly consistent effect directions across studies. Nearly a quarter of these miRNAs can be considered novel findings as they were not highlighted in either the abstracts of included studies or in recently published qualitative review articles, emphasizing the utility of the meta-analytic approach taken here. Taken together, the findings of this work have the potential to substantially advance research on miRNA (dys)function in AD and help pave the way, in concert with non-miRNA markers, for the development of improved predisease biomarker panels allowing an early detection and monitoring of the pathophysiologic changes underlying this devastating disease.

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Authors' contributions: Study concept and design was contributed by P.T., R.P., and L.B.. Analysis and interpretation of data was carried out by P.T., A.S., J.S., I.W., V.D., C.M.L., R.P., and L.B. Drafting of the manuscript was carried out by P.T., A.S., R.P., L.B. Critical revision of the manuscript for important intellectual content was performed by P.T., A.S., J.S., I.W., V.D., L.M., C.M.L., R.P., and L.B. Statistical analysis was performed by P.T., A.S., J.S., I.W., V.D., R.P., and L.B. J.S., I.W., V.D., and L.M. were responsible for administrative, technical, and material support. Study supervision was carried out by R.P. and L.B.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2019.06.4952.

RESEARCH IN CONTEXT

1. Systematic review: The authors performed a systematic literature search for microRNA (miRNA) expression studies in Alzheimer's disease (AD), limited to humans, based on the PubMed database (last search date: August 24, 2018) using the search term "(microRNA OR miRNA OR miR OR microRNA) AND Alzheimer*". The workflow and data collection procedures were similar to those for genetic association studies developed previously. The main aim was to identify original studies comparing the differential expression of miRNAs in AD cases versus controls.
2. Interpretation: Our systematic review led to the pooling of 107 eligible studies including data from 147 independent data sets reporting data for a total of 1121 different miRNAs. Across 461 meta-analyses, we found a total of 57 miRNAs to be differentially expressed at studywide significance. The distribution across tissues was 25, 5, and 32 in brain, CSF and blood-derived specimen, respectively. Five miRNAs showed differential expression in both brain and blood.
3. Future directions: We expect our results to make a substantial contribution to research on miRNA dysfunction in AD, and to potentially accelerate the search for novel pathophysiological biomarker candidates to improve the early diagnosis and monitoring of AD progression. The methodology developed by our group for this work can be readily applied to other diseases within neuroscience and beyond. Thus, in addition to representing an important step forward in deciphering microRNAs relevant in AD pathogenesis (differentially expressed in brain) and microRNAs possibly suitable as novel AD biomarkers (differentially expressed in blood), our approach may serve as a valuable example for the systematic elucidation of microRNAs in other fields.

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JAMA Neurology | Original Investigation

Association of Polygenic Risk Score With Cognitive Decline and Motor Progression in Parkinson Disease

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IMPORTANCE Genetic factors have a well-known influence on Parkinson disease (PD) susceptibility. The largest genome-wide association study (GWAS) identified 26 independent single-nucleotide polymorphisms (SNPs) associated with PD risk. Among patients, the course and severity of symptom progression is variable, and little is known about the potential association of genetic factors with phenotypic variance.

OBJECTIVE To assess whether GWAS-identified PD risk SNPs also have a cumulative association with the progression of cognitive and motor symptoms in patients with PD.

DESIGN, SETTING, AND PARTICIPANTS This longitudinal population-based cohort study of 285 patients of European ancestry with incident PD genotyped 23 GWAS SNPs. One hundred ninety-nine patients were followed up for a mean (SD) of 5.3 (2.1) years for progression (baseline: June 1, 2001, through November 31, 2007; follow-up: June 1, 2007, through August 31, 2013, with mortality surveillance through December 31, 2016); 57 patients had died or were too ill for follow-up, and 29 withdrew or could not be contacted. Movement disorder specialists repeatedly assessed PD symptom progression.

MAIN OUTCOMES MEASURES The combined association of PD risk loci, after creating a weighted polygenic risk score (PRS), with cognitive decline, motor progression, and survival, relying on Cox proportional hazards regression models and inverse probability weights to account for censoring.

RESULTS Of the 285 patients undergoing genotyping, 160 were men (56.1%) and 125 were women (43.9%); the mean (SD) age at diagnosis was 69.1 (10.4) years. The weighted PRS was associated with significantly faster cognitive decline, measured by change in the Mini-Mental State Examination (hazard ratio [HR] per 1 SD, 1.44; 95% CI, 1.00-2.07). The PRS was also associated with faster motor decline, measured by time to Hoehn & Yahr Scale stage 3 (HR, 1.34; 95% CI, 1.00-1.79) and change in Unified Parkinson's Disease Rating Scale part III score (HR, 1.42; 95% CI, 1.00-2.01).

CONCLUSIONS AND RELEVANCE Susceptibility SNPs for PD combined with a cumulative PRS were associated with faster motor and cognitive decline in patients. Thus, these genetic markers may be associated with not only PD susceptibility but also disease progression in multiple domains.

Supplemental content

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Parkinson disease (PD) is the second most common neurodegenerative disorder worldwide. Although PD is typically described in terms of motor dysfunction, nonmotor features are common.[1] Cognitive impairment in particular is frequent in PD and especially detrimental to patients' quality of life and increases in caregiver burden.[1,2] Furthermore, the rates of symptom development and symptom severity in PD are highly heterogeneous.[3] Identifying factors that influence progression could provide insight into etiologic pathways and ultimately prevention and therapies.

Idiopathic PD likely has a considerable genetic component.[4] Genome-wide association studies (GWASs) have successfully identified 26 independent single-nucleotide polymorphisms (SNPs) associated with PD at a genome-wide significance level ($\alpha = 5 \times 10^{-8}$).[5] The risk alleles of several of these PD loci may also lower the age of PD onset.[6-8] However, the cumulative influence of these risk loci on onset age, as assessed with a constructed polygenic risk score (PRS), is rather small.[6-8]

On the other hand, the collective association of these PD genetic risk loci with other PD phenotypes, including disease progression and survival, is largely unexplored. A Nordic study[9] generated a PRS based on 19 GWAS SNPs in 336 patients and found that the PRS estimated a faster time from diagnosis to Hoehn & Yahr Scale (H&Y)[10] stage 3 but was not associated with patient survival. To our knowledge, no study has yet assessed the association of a PD GWAS PRS with cognitive decline during disease progression.

Thus, the aim of the present study was to investigate for the first time, to our knowledge, the cumulative association of established PD genetic risk factors with cognitive impairment. Furthermore, we reexamined the association of the PRS with PD motor progression and survival.[9]

Key Points

Question Do genome-wide association study–identified susceptibility risk loci for Parkinson disease modify cognitive and motor symptom progression?

Findings In this population-based study of 285 patients with Parkinson disease, a higher polygenic risk score based on 23 Parkinson disease genome-wide association study–identified single-nucleotide polymorphisms was associated with faster cognitive decline and progression of motor symptoms. Results did not change after removing *GBA* loci from the risk score.

Meaning Susceptibility risk alleles had a cumulative association with cognitive and motor decline in Parkinson disease; these alleles may influence not only Parkinson disease susceptibility but also disease progression in multiple domains.

Methods

Participants

The Parkinson Environment and Gene (PEG) study is a population-based study in central California. More details on recruitment methods,[11,12] case definition criteria,[13] and the longitudinal patient cohort[14] have been published previously. Patients with idiopathic PD diagnosed less than 3 years previously were recruited from June 1, 2001, through November 31, 2007. Patients were confirmed as having clinically probable or possible PD by a team of movement disorder specialists, led by one of us (J.M.B.), and were generally cognitively unimpaired at baseline.[15] All procedures described were approved by the UCLA human subjects committee, and written informed consent was obtained from all participants.

Initially, 360 patients with PD were enrolled. We limited our study sample to patients of European ancestry (n = 285), all of whom are included in the survival analyses. We assessed symptom progression during 1 to 3 follow-up examinations by our movement disorder team (June 1, 2007, to August 31, 2013; mean [SD] follow-up, 5.3 [2.1] years; mean [SD] time from disease onset, 7.3 [2.8] years). We reexamined 199 patients (69.8%) during follow-up (57 had died or were too ill to participate in follow-up, and 29 withdrew or could not be contacted). A total of 192 participants provided the biosamples and data necessary for analyses of cognitive and motor symptom progression.

PD Symptom Assessment

Trained interviewers collected detailed information on demographic and risk factors. UCLA movement disorder specialists conducted in-person physical examinations to record progression for each participant. Motor symptoms were assessed with the Unified Parkinson's Disease Rating Scale part III (UPDRS-III; range, 0-108, with higher scores indicating worse motor symtoms).[16] If possible, patients were examined functionally while not receiving PD medications (off-score; 229 [80.4%] at the baseline examination and 146 [79.8%] at follow-up). For patients only examined while receiving medication (on-score), we estimated an off-score by adding the difference of the whole study population's mean off- and on-scores at the time of examination to the patient's on-score.[14] During the examination, the neurologists also assigned patients to one of the modified H&Y stages (1-5, with higher stages indicating worse motor symptoms) based on the clinical descriptions of each stage.[10] Cognitive function was assessed with the Mini-Mental State Examination (MMSE; range, 0-30, with lower scores indicating worse cognitive function)[17] at the same neurologic examination, although after the off-score UPDRS-III examination, patients were asked to take their PD medications as needed.

Outcomes

Survival

Continued mortality surveillance has been performed throughout the PEG follow-up, primarily through vital statistics data, review of public obituaries, and continued active follow-up with patients and their families. Although our patients were examined last by study neurologists in 2013, mortality surveillance extended to December 31, 2016, which was 16 years after our study began (2001), with as many as 19 years of passive follow-up for mortality for patients first diagnosed in 1998.

Table 1. Demographic and Outcome Characteristics of the Follow-up Cohort

Characteristic	Data
Age at diagnosis, mean (SD), y	69.1 (10.4)
Age at baseline examination, mean (SD), y	71.1 (10.3)
Male, No./total No. (%)	160/285 (56.1)
PD duration at baseline examination, mean (SD), y	2.0 (1.4)
PD duration at last follow-up, mean (SD), y	7.3 (2.8)
Follow-up, mean (SD), y[a]	5.3 (2.1)
Survival	
Registered deaths, No./total No. (%)	166/285 (58.2)
Observation time from baseline examination, mean (SD), y[b]	8.1 (3.7)
Age at death, mean (SD), y	81.4 (7.3)
MMSE cognition score (n = 191)[c]	
Mean change per 5 y, mean (SD)	−0.9 (2.5)
Fast cognitive decline (decrease in score ≥4 points), No./total No. (%)	32/191 (16.8)
Observation time from baseline examination, mean (SD), y[b]	5.2 (2.1)
Mean change per 5 y among 32 patients with fast cognitive decline	−5.1 (3.7)
H&Y stage (n = 192)[a,d]	
Baseline ≥3, No./total No. (%)	23/192 (12.0)
Observed conversion from <3 to ≥3, No./total No. (%)	57/192 (29.7)
Observation time from baseline examination, mean (SD), y[b]	4.9 (2.0)
UPDRS-III motor score (n = 183)[e,f]	
Change per 5 y, mean (SD)	8.2 (12.5)
Fast motor progression (increase in score ≥20 points), No./total No. (%)	41/183 (22.4)
Observation time from baseline examination, mean (SD), y[b]	5.6 (1.8)
Change per 5 y among 183 patients with fast motor progression, mean (SD)	24.4 (18.7)

Abbreviations: H&Y, Hoehn & Yahr Scale; MMSE, Mini-Mental State Examination; PD, Parkinson disease; PRS, polygenic risk score; UPDRS-III, Unified Parkinson's Disease Rating Scale part III.

[a] Includes 192 patients with available data.

[b] Indicates time to event or censoring.

[c] Scores range from 0 to 30, with lower scores indicating worse cognitive function.

[d] Stages range from 1 to 5, with higher stages indicating worse motor symptoms.

[e] Includes 183 patients.

[f] Scores range from 0 to 108, with higher scores indicating worse motor symptoms.

Cognitive Decline

Cognitive decline was determined with the MMSE. Multiple studies investigating reliable change indices, which provide estimates of the probability that an individual's change in test scores is not due to chance, have suggested that a reliable change in MMSE score during long intervals is 3 to 4 points.[18,19] Because our mean (SD) follow-up was 5.3 (2.1) years (**Table 1**), we defined cognitive decline as a 4-point decrease from baseline MMSE score and time to event as the time from the baseline to follow-up examinations in which a 4-point decrease was first measured.

Motor Decline

Changes of 2.5 to 5.2 points on the UPDRS-III motor score represent clinically meaningful differences.[20] We thus defined fast motor progression, based on the mean 5.3 years of follow-up, as a 20-point increase in UPDRS-III score (mean of 4 points per year) and time to event as the time from the baseline to follow-up examinations in which a 20-point increase was first measured.

H&Y Stage

Previous research using a PRS investigated conversion to H&Y stage 3.[9] Time to conversion to H&Y stage 3 was defined as the time from the baseline to first follow-up examinations in which the patient scored at least stage 3. The 23 patients with this score at baseline (Table 1) were excluded from these analyses.

Genotyping and Calculation of the Weighted PRS

Participants provided biospecimens (blood or saliva) from which we extracted DNA. The SNPs were selected based on published GWASs (eTable 1 in the Supplement). Genotyping was performed using a high-throughput system (BioMark HD; Fluidigm Corporation). Of all 26 SNPs showing genome-wide significant evidence for association with PD risk in the latest GWAS meta-analysis,[5] 23 SNPs were successfully genotyped. The genotyping assays of SNPs rs8118008 and rs7681154 did not work properly, and rs13201101 failed our genotyping efficiency criterion (ie, <97%). The genotyping efficiency for the remaining 23 SNPs was greater than 99.6%. Furthermore, all SNPs were in Hardy-Weinberg equilibrium in PEG controls (significance cutoff of $\alpha = .002$ based on Bonferroni correction for 23 SNPs) as determined based on Pearson χ^2 test implemented in the PLINK tool set (version 1.07; http://zzz.bwh.harvard.edu/plink/).

The PRS was calculated using R software (version 3.3.1; https://www.r-project.org). This score is defined as the sum of the number of risk alleles per individual weighted by their β coefficient (ie, their effect size estimate corresponding to the logarithm of the odds ratio, as described by Nalls et al[5] and Purcell et al[21]). We extracted the β coefficients from the combined discovery and replication stage analysis of Nalls et al[5] so that the weights were ascertained independently from the data set used for the present study. We standardized the PRS by z transformation.

Statistical Analysis

To assess associations between the PRS and the survival and progression outcomes, we used Cox proportional hazards regression models to estimate hazard ratios (HRs) with 95% CI and a Wald χ^2 test for significance ($\alpha = .05$). Each outcome was modeled individually. In each model, we controlled for sex and age at diagnosis. For the MMSE and UPDRS-III, we chose not to analyze the rate of change as a continuous variable. Symptom progression is likely to be nonlinear; thus, we selected analyzing time to meaningful differences based on changes in UPDRS-III motor and MMSE scores (as described in the Outcomes subsection of the Methods section).

When estimating the associations for progression outcomes, we were forced to restrict our analysis to an uncensored subset of the cohort for whom we had ascertained out-

Table 2. Estimated HRs for Clinical Outcomes in PD and PRS[a]

	Full PRS		PRS excluding *GBA* loci		Pihlstrøm et al,[9] 2016	
Outcome	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Survival	1.08 (0.79-1.47)	.62	1.09 (0.78-1.52)	.63	1.13 (0.88-1.45)	.32
Time to MMSE 4-point decrease[b]	1.44 (1.00-2.07)	.049	1.42 (0.98-2.07)	.07	NA	NA
Time to UPDRS III 20-point increase[c]	1.42 (1.00-2.01)	.051	1.50 (1.05-2.14)	.03	NA	NA
Time to conversion to H&Y stage ≥3[d]	1.34 (1.00-1.79)	.054	1.36 (1.01-1.83)	.045	1.29 (1.06-1.56)	.01

Abbreviations: HR, hazard ratio; H&Y, Hoehn & Yahr Scale; MMSE, Mini-Mental State Examination; NA, not applicable (outcome not investigated); PD, Parkinson disease; PRS, polygenic risk score; UPDRS-III, Unified Parkinson's Disease Rating Scale part III.

[a] Cox proportional hazards regression models were used to estimate HRs, including sex and age at diagnosis as covariates. Estimates for time to MMSE, UPDRS-III, and H&Y events were obtained using inverse probability of censoring weighting. *P* values were calculated using the Wald χ^2 test.

[b] Scores range from 0 to 30, with lower scores indicating worse cognitive function.

[c] Scores range from 0 to 108, with higher scores indicating worse motor symptoms.

[d] Stages range from 1 to 5, with higher stages indicating worse motor symptoms.

come information (n = 192). To account for the possibility of selection bias from right censoring, we used inverse probability of censoring weights (IPCW) to create a pseudopopulation mimicking the total population before censoring in distribution of measured covariates.[22] We then estimated the associations between the PRS and progression outcomes in the pseudopopulation. The outcomes of participants lost to or unavailable for follow-up are represented in the pseudopopulation by increasing the weight given to the outcomes of participants with similar measured covariates who were included (ie, had outcome information). We used logistic regression to calculate the probability of being censored, including covariates in the model that may be related to loss to follow-up and PD progression (age at PD diagnosis, sex, years of schooling, baseline MMSE score, baseline UPDRS-III score, and H&Y stage 3) and the PRS.

We conducted sensitivity analyses excluding the 2 *GBA* (OMIM 606463) SNPs (rs35749011 and rs114138760) from the PRS to assess whether results were driven by *GBA*. We used SAS software (version 9.4; SAS Institute) for analyses. Unless otherwise indicated, data are expressed as mean (SD).

Results

Of the 285 patients undergoing genotyping, 160 were men (56.1%) and 125 were women (43.9%); the mean age at diagnosis was 69.1 (10.4) years. Demographic and outcome frequencies and characteristics of the European ancestry–only cohort can be found in Table 1. The mean duration of PD at the baseline examination was 2.0 (1.4) years. The cohort was followed up for a mean of 5.3 (2.1) years for symptom progression (ie, 7.3 [2.8] years after onset of disease).

Of the 285 European ancestry–only participants enrolled at baseline, 166 (58.2%) died, with a mean time to death or censoring from baseline of 8.1 (3.7) years. Among the 192 participants with follow-up data, the mean change in the MMSE was −0.9 (2.5) points per 5 years. However, among the 32 of 191 patients with a decrease of at least 4 points (16.8%), the mean change was −5.1 (3.7) points per 5 years. Among the 183 patients with data available, the mean change in the UPDRS-III score was 8.2 (12.5) points per 5 years and 24.4 (18.7) points per 5 years among the 41 patients with an increase in score of 20 points or more (22.4%) (Table 1). Correlations among our outcome results in the follow-up cohort can be found in eTable 2 in the Supplement. Although motor outcomes (change in the UPDRS-III score and H&Y stage 3) were correlated (ρ = 0.43; *P* < .001), the cognitive outcome was only weakly correlated with other outcomes (range, ρ = 0.17 to ρ = 0.26).

Table 2 provides the results for the PRS and each outcome analyzed. Estimates for progression events were obtained using IPCW; eTable 3 in the Supplement gives the estimates from analyses that did not apply IPCW. We did not observe an association between the PRS and survival. However, we found that a higher PRS was associated with faster time to cognitive and motor events. We found that the PRS was associated with faster (4-point) decrease on the MMSE, such that a 1-SD increase in PRS corresponds to an HR of 1.44 (95% CI, 1.00-2.07). The effect size remained similar when we removed *GBA* from the PRS (HR, 1.42; 95% CI, 0.98-2.07). A higher PRS also tended to be associated with motor symptom progression, including a 20-point UPDRS-III increase (HR, 1.42; 95% CI, 1.00-2.01) and progression to H&Y stage 3 (HR, 1.34; 95% CI, 1.00-1.79). Again, results were similar and formally statistically significant when we removed *GBA* SNPs from the score for a UPDRS-III 20-point decrease (HR, 1.50; 95% CI, 1.05-2.14) and progression to H&Y stage 3 (HR, 1.36; 95% CI, 1.01-1.83; Table 2).

Discussion

We assessed the association of a PRS with disease progression in a longitudinal population-based cohort of patients with PD. We newly suggest that the PRS, based on the established PD GWAS risk loci, is associated with cognitive decline and validates previous findings of a collective association of these risk variants with motor symptom progression. Furthermore, we support the previous observation[9] that PD risk variants are not cumulatively associated with survival among patients.

Cognitive impairment and dementia are well established disorders in PD.[23] As many as 80% of patients with PD who are alive 10 years after diagnosis are expected to develop dementia.[23] Despite the high prevalence, our understanding of pathogenic mechanisms is limited, and treatments are largely unsuccessful.

To our knowledge, all patient cohorts investigating cognitive impairment in PD have used candidate gene approaches. For instance, *GBA*, one of the most important genetic risk factors for PD, has also been implicated in cognitive impairment in PD.[24-26] Other studies have assessed the role of *LRRK2*,[27-29] *MAPT*,[30,31] and *SNCA*[32,33] for dementia in PD, but results have often been inconclusive or not been replicated independently. For the first time to our knowledge, we linked the cumulative burden of PD genetic risk factors with patients' cognitive decline.

Although the MMSE is among the most widely used screening instruments for cognitive impairment, not all changes in score reflect true clinical change.[18] Furthermore, multiple cut points have been suggested as thresholds for cognitive impairment and dementia, mostly 26 or less to 24 or less.[34,35] Multiple independent studies have shown that changes of 3 to 4 points in MMSE score during longer intervals are necessary to conclude with 90% confidence that an individual has experienced a reliable functional change.[18,19,36] Thus, we selected a 4-point decrease in MMSE score to define a meaningful cognitive decline (by definition, all patients with a 4-point decrease will have scores ≤26).

The only study to explore progression of motor symptoms with a PRS (based on 19 PD SNPs)[9] followed up 336 Nordic patients in tertiary care and reported faster progression to H&Y stage 3 with increasing score (HR per 1 SD, 1.29; 95% CI, 1.06-1.56). Because PD is a gradually progressive disorder, moving from one H&Y stage to another generally takes several years, and H&Y stage 3 signifies the point when disability advances from mild to moderate and the appearance of postural instability.[10] We have replicated this finding in our own community-based study (HR per 1 SD, 1.34; 95% CI, 1.00-1.79), providing additional evidence for a multiloci genetic component in PD motor progression.

We also examined changes in UPDRS-III motor scores. Shulman et al[20] reported that changes of 2.5 to 5.2 points on the UPDRS-III represent clinically meaningful differences, with 10.8 points corresponding to a 1-stage change on the H&Y. We selected a 20-point increase in UPDRS-III score, corresponding to a clinically meaningful 4-point annual change in score during follow-up. We found a higher PRS to be associated with a faster time to a 20-point increase. As expected, an increase of 20 points on the UPDRS-III and conversion to H&Y stage 3 were correlated in our population (Spearman coefficient, $r = 0.43$; $P < .001$) (eTable 2 in the Supplement), indicating that both measures likely capture some of the same features of motor progression. The UPDRS-III and H&Y were assessed throughout the study by the same team of movement disorder specialists, led by one of us (J.M.B.), trained to evaluate patients in a standardized fashion. Thus, we did not expect much misclassification owing to differences in how clinicians conducted examinations. However, 89 of our participants (31.2%) could not perform at least 1 neurologic examination while in a functional off-medication state. We estimated UPDRS-III off-scores based on the study population means. However, this procedure is still likely to cause some misclassification of motor severity for patients with on-scores. We expect this misclassification to be nondifferential with respect to the PRS because the PRS does not predict medication status at any of the examinations. In addition, not enough patients had only on-score examinations to determine whether our off-score findings are consistent across medication status. This determination would be interesting when considering whether attributed genetic effects may also be related to levodopa responsiveness. Furthermore, although the MMSE was done after the UPDRS-III motor examinations, patients were allowed to take PD medications as needed after motor examinations.

The influence of *GBA* mutations on PD risk is well established. The weight of evidence supports that carriers of *GBA* mutations are not only at a higher risk of PD[37-40] but also have an earlier age at onset and are more likely to develop cognitive dysfunction.[26,41,42] Thus, we conducted sensitivity analyses after excluding variants in the *GBA* locus to determine whether our results for the PRS were driven by *GBA*. Estimated effect sizes did not change meaningfully (Table 2), suggesting that the remaining multiple genetic variants together contribute to faster progression cumulatively.

Strengths and Limitations

Our study is one of less than a handful of population-based prospective PD cohorts worldwide and the only study, to our knowledge, to investigate PD risk loci with a PRS and cognitive decline. Although we were unable to follow up all patients with PD enrolled at baseline because loss to follow-up occurred primarily owing to death and illness, we conducted IPCW statistical analyses to account for selective survival. This form of internal adjustment corrects for censoring, which is modeled as a function of measured baseline risk factors and PD symptom levels that affect censoring and the PD progression end points under study. However, this method can only account for factors included in the censoring model. Other unmeasured factors likely influence loss to follow-up, and thus some remaining selection bias is possible.

A notable strength in our study is the well characterized PD population. All our patients were personally examined by the same UCLA movement disorder specialists throughout the study, which minimizes outcome misclassification. Furthermore, follow-up began early in the disease course (within 3 years of diagnosis), allowing us to track the natural history of progression. Because of our community-based design, our results are more generalizable to average PD populations than patient cohorts assembled at tertiary care centers.

Conclusions

Ultimately, identifying genetic markers associated with faster progression may help elucidate pathogenesis and inform further research. Although replication is needed, our results support the collective involvement of PD susceptibility risk alleles in cognitive and motor decline, suggesting that these alleles may be associated with not only PD susceptibility but also disease progression in multiple domains. Our findings support a polygenic architecture contributing to PD progression, as has been suggested for PD susceptibility, and suggest that progression in PD may, at least in part, be driven by an accumulation of many common genetic variants, each individually having a relatively small effect size.

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7 Eidesstattliche Erklärung

Ich erkläre hiermit, dass ich diese Dissertation selbstständig ohne Hilfe Dritter und ohne Benutzung anderer als der angegebenen Quellen und Hilfsmittel verfasst habe. Alle den benutzten Quellen wörtlich oder sinngemäß entnommenen Stellen sind als solche einzeln kenntlich gemacht.

Diese Arbeit ist bislang keiner anderen Prüfungsbehörde vorgelegt worden und auch nicht veröffentlicht worden.

Ich bin mir bewusst, dass eine falsche Erklärung rechtliche Folgen haben wird.

_____

Ort, Datum, Unterschrift

8 Lebenslauf

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Publikationen

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2017 Lill. Schulz, Takousis, Wohlers, Duchrow, Itua, Binder, Ioannidis, Perneczky, Bertram, Systematic meta-analyses identify differentially expressed miRNAs in Parkinson's disease. European Human Genetics Conference 2017, Kopenhagen.

Auszeichnungen

2017	Promotionsstipendium “Lübecker Exzellenzmedizin”
2013	Auszeichnung mit dem Abiturpreis der Deutschen Mathematiker-Vereinigung
2013	und dem Abiturpreis der Gesellschaft Deutscher Chemiker 2013
2007-2010	mehrfache erfolgreiche Teilnahme in der Landesstufe der Mathematikolympiade