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Longitudinale Veränderungen der Kortikalen Dicke von Adoleszent*innen mit Autismus-Spektrum-Störung und deren Assoziation mit Restriktiven und Repetitiven Verhaltensweisen

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1 Zusammenfassung

Der Artikel „Longitudinal changes in cortical thickness in adolescents with autism spectrum disorder and their association with restricted and repetitive behaviors“ von

Bieneck et al. (2021), veröffentlicht in „Genes“, beschäftigt sich mit der Frage nach einem Zusammenhang zwischen der intra-individuellen Entwicklung der Kortexdicke von Adoleszent*innen mit Autismus-Spektrum-Störung (ASS) und der Schwere restriktiver und repetitiver Verhaltensweisen (restricted and repetitive behaviors = RRB) im Verlauf dieser Lebensphase. Darüber hinaus untersuchen wir potentielle Anreicherungen von Genen, bei denen eine Assoziation mit ASS bekannt ist, in entsprechenden Hirnregionen mit abweichender Entwicklung der Kortikalen Dicke (Cortical Thickness = CT). Ziel der Studie ist es mikroskopische und makroskopische Ätiologien der ASS miteinander zu verknüpfen und diese entsprechenden klinischen Phänotypen zuzuordnen.

Die Basis dieser Forschungsarbeit bilden Daten, die im Rahmen einer longitudinalen Studie zur Gehirnentwicklung bei ASS während der Adoleszenz an der Kinder- und Jugendpsychiatrie des Universitätsklinikums in Frankfurt erhoben worden sind. Die Stichprobe setzt sich zusammen aus insgesamt 70 Proband*innen im Alter zwischen 11 und 18 Jahren zum ersten Testzeitpunkt (T1), davon N=33 mit diagnostizierter ASS und N=37 neurotypische Kontrollen. Alle Proband*innen erhalten im ersten Jahr (T1) einen strukturellen Magnetresonanztomographie-Scan (sMRT), der im Abstand von zwei Jahren (T2) wiederholt wird. Die Quantifizierung der Symptomschwere erfolgt in beiden Gruppen mittels eines klinischen Fragebogens zu restriktivem und repetitivem Verhalten an beiden Messzeitpunkten (T1, T2).

Die sMRT-Scans durchlaufen für die Auswertung ein Softwarepaket, um eine virtuelle Rekonstruktion der kortikalen Oberfläche von jedem T1-gewichteten Bild abzuleiten. Die Software gibt die intraindividuelle Veränderung der CT an jedem Vertex an. Mittels statistischer Analysen unter Verwendung eines generalisierten linearen Modells (GLM) werden Vertex-weise Unterschiede in der Veränderung der CT zwischen beiden Gruppen herausgearbeitet und mit der Änderung der Symptomschwere von RRBs korreliert.

Der dritte Schritt der Analyse umfasst die Auswertung potentieller genetischer Korrelate atypischer CT-Entwicklung der Adoleszent*innen mit ASS. Hierfür wird ein Hirnatlas

(Allen Human Brain Atlas) hinzugezogen, der Informationen zur räumlichen Verteilung der Expression von Genen im menschlichen Cortex enthält. Dieser wird mit den Hirnregionen, die eine abweichende Veränderung der CT in Autist*innen zeigen, abgeglichen, und eine entsprechende Liste an Genen wird abgeleitet. Es folgt eine Analyse dieser Genliste im Hinblick auf Anreicherungen von Genen, die in Verbindung mit ASS stehen. Dieser Vorgang wird mit den Hirnregionen wiederholt, die eine Korrelation der abweichenden CT-Entwicklung mit der Veränderung der Symptomschwere von RRBs in den autistischen Teilnehmenden zeigen. Die daraus gewonnene Genliste wird auf eine vermehrte Anreicherung (i.e. Enrichment) von Genen untersucht, die in Verbindung mit restriktivem und repetitivem Verhalten stehen.

Die Ergebnisse dieser Studie zeigen eine signifikant verminderte Abnahme der CT in verschiedenen Hirnregionen von Personen mit ASS, die funktionell mit autistischen Symptomen und Verhaltensweisen gekoppelt sind. Eben diese Regionen zeigen zudem eine vermehrte Anreicherung von Genen, die ebenfalls eine Assoziation mit ASS aufweisen. Eine Verbindung von strukturellen und klinischen Parametern zeigt sich durch die Korrelation abnehmender CT in bestimmten Hirnregionen mit einer verminderten Schwere restriktiver und repetitiver Verhaltensweisen im Verlauf.
Diese Untersuchungen weisen auf Verbindungen des neuroanatomischen Parameters CT mit genetischen Grundlagen der ASS hin und zeigen einen Zusammenhang dieser Korrelationen mit unterschiedlichen Ausprägungen des klinischen autistischen Phänotyps.

2 Abstract

The study “Longitudinal changes in cortical thickness in adolescents with autism spectrum disorder and their association with restricted and repetitive behaviors” by Bieneck et al. (2021) published in “Genes” examines intra- and inter-individual differences in the developmental trajectory of cortical thickness (CT) in childhood and adolescence, as well as their genomic underpinnings, in 33 individuals with autism spectrum disorder (ASD) and 37 typically developing controls (TD; aged 11–18 years). Moreover, Bieneck et al. link regional atypical CT development to intra-individual variations in restricted and repetitive behavior (RRB) over a two-year time period. Last, gene enrichment for genes known to be associated with ASD at a genetic and transcriptomic level within the spatial patterns of the neuroanatomical differences in CT is tested. The aim of this study is to establish the link between microscopic and macroscopic pathology in ASD, as well as their relationship with different clinical ASD phenotypes.

This study used data provided by an ongoing longitudinal study examining brain development during adolescence in individuals with ASD and in TD controls at two timepoints (T1, T2) separated by ~2 years. Structural magnetic resonance imaging scans (sMRI) are acquired at T1 and T2 for each participant (N=70). Differences in variations in RRB between ASD and TD are measured by a clinical questionnaire at both timepoints. For virtual reconstruction of the cortical surface of each sMRI scan, a software package is used to measure intraindividual differences in CT at each vertex. Using a general linear model (GLM), vertex-wise between-group differences in CT and their correlations with changes in symptom severity of RRB are examined.

The third step of the analysis links observed differences in the CT trajectory of participants with ASD to potential genetic underpinnings. Therefore, the spatial gene expression data, provided by the Allan human brain atlas (AHBA), is compared to the brain regions with altering CT trajectories in ASD. The resulting gene list is tested for an enrichment of genes that have previously been linked to ASD on the genetic and transcriptomic level. This step is re-run for brain regions showing an association between altering CT development and change in RRB severity in the ASD group.

Subsequently, a gene enrichment analysis is performed to test for an enrichment of genes that have previously been associated with RRB.

The results of this study show significantly reduced cortical thinning in individuals with ASD in several of the brain regions functionally related to wider autism symptoms and traits (e.g., fronto-temporal and cingulate cortices) compared to TD. Intra-individual differences in CT development also correlate with within-subject variability in changes of RRB severity. Furthermore, we report that the spatial patterns of the neuroanatomical differences in CT development are enriched for genes known to be associated with ASD at a genetic and transcriptomic level. However, there was no enrichment for RRB-linked-genes in brain regions with a correlation between RRB symptom severity and change in CT.

These findings represent an important step towards characterizing the neuroanatomical underpinnings of ASD across development based upon measures of CT. Moreover, they provide important novel insights into the link between microscopic and macroscopic pathology in ASD, as well as their relationship with different clinical ASD phenotypes.

3 Übergreifende Zusammenfassung

3.1. Einleitung

Die Autismus-Spektrum-Störung (ASS) zählt nach ICD-10 zu den „tiefgreifenden Entwicklungsstörungen“ (F84) und betrifft nahezu 1% der gesamten Bevölkerung[1]. Personen mit ASS haben Defizite in verbaler und nonverbaler sozialer Interaktion und Kommunikation, weisen repetitive und stereotypische Verhaltensweisen auf und zeigen spezielle Sonderinteressen[2]. Erste Symptome zeigen sich in der Regel im Alter von 2 Jahren und können über die gesamte menschliche Lebensspanne persistieren[3]. Die Diagnostik der ASS setzt zwar die oben genannte Symptomtriade voraus, allerdings zeigt sich eine große klinische Heterogenität des Krankheitsbildes. Die Begründung hierfür liegt vor allem in ihrer komplexen genetischen Ätiologie, in Variationen der Symptomschwere und dem häufigen Vorliegen komorbider Störungen[4]. Zudem hat man bis heute geringe Kenntnis über die neurobiologischen Mechanismen der Pathogenese, weshalb eine spezifische und effektive pharmakologische Therapie der ASS bislang fehlt. Bei Personen mit ASS finden sich allerdings Abnormalitäten in der Gehirnanatomie sowie in deren Konnektivität[5]. Bisherige Forschungsergebnisse legen eine Störung im Prozess der Hirnentwicklung nahe, die sich in spezifischen Autismus-assoziierten Regionen abbildet. Dies betrifft vor allem die neuroanatomische Ebene isolierter Hirnregionen sowie die Entwicklung neuronaler Systeme und Schaltkreise des zentralen Nervensystems. Um die neurobiologischen Grundlagen der Pathogenese zu entschlüsseln, ist es demnach erforderlich, Personen mit ASS in unterschiedlichen Entwicklungsstadien zu untersuchen. Zudem müssen Unterschiede im Phänotyp des Gehirns mit den Veränderungen in Beziehung gesetzt werden, die bei der typischen Hirnentwicklung bekannt sind. Bis heute stammt das meiste Wissen über die Neuropathologie der ASS hauptsächlich von Bildgebungsstudien, die sich auf die frühkindliche Hirnentwicklung konzentrieren. Veränderungen während der Adoleszenz bleiben hingegen weitgehend unbekannt[6-11]. Allerdings herrscht Einigkeit darüber, dass sich der Prozess der Hirnreifung während der Adoleszenz bis zum frühen Erwachsenenalter fortsetzt. Da dieser Lebensabschnitt von rapider Hirnentwicklung geprägt ist, gibt er bereits die Richtung für die Reife und damit einhergehende klinische Verhaltensweisen im Erwachsenenalter vor. Daher ist es wichtig, die anatomische Entwicklungskurve auch während der Adoleszenz zu untersuchen.

Eine alternierende Hirnentwicklung bei Menschen mit ASS zeigt sich am konsequentesten im morphologischen Parameter der Kortikalen Dicke (Cortical Thickness = CT). Der die CT beschreibt hier die engste Distanz von der äußeren (pia mater) zur inneren (weiße Substanz) Begrenzung des Kortex an jedem Vertex der rekonstruierten Oberfläche. Das Prinzip eines atypischen Entwicklungsverlaufes der CT bei Personen mit ASS ist gut etabliert, auch wenn die Berichte hinsichtlich der Richtung des Unterschiedes zur neurotypischen Entwicklung variieren. Beispielsweise beschreibt eine longitudinale Studie von Zielinski et al. (2014) ein übermäßiges Wachstum des kortikalen Mantels während der frühen Kindheit, gefolgt von einem akzelerierten Rückgang im mittleren Kindesalter, resultierend in einer Phase der „Normalisierung“ im Erwachsenenalter von Autist*innen. Hingegen berichtet Wallace et al. (2010) in einer Querschnittsuntersuchung von einer akzelerierten statt einer verlangsamten Abnahme der CT im Erwachsenenalter von Personen mit ASS. Diese unterschiedlichen Ergebnisse zeigen exemplarisch Inkonsistenzen neuroanatomischer Forschungsergebnisse zwischen Querschnittsstudien und Studien mit einem longitudinalen Design auf, die altersabhängige Veränderungen der Neuroanatomie der ASS untersuchen. Daher sind weitere longitudinale Ansätze nötig, um die vorherigen Ergebnisse zu replizieren und validieren.

Trotz dieser unterschiedlichen Ergebnisse herrscht Einigkeit darüber, dass insbesondere frontotemporale und frontoparietale Hirnregionen bei der ASS betroffen sind und sich hier die stärksten atypischen Verläufe der neurologischen Entwicklungskurve zeigen[9,12,13]. Die meisten dieser Hirnregionen sind wesentliche Bestandteile des sogenannten „sozialen Gehirns“, das höhere sozio-kognitive Fähigkeiten steuert[14]. Zahlreiche Studien, die sich mit dem Zusammenhang neuroanatomischer und symptomatischer Charakteristika der ASS beschäftigen, konzentrieren sich hauptsächlich auf Einschränkungen in sozialer Interaktion und Kommunikation von Autist*innen. Über neuroanatomische Grundlagen der restriktiven und repetitiven Verhaltensweisen (restricted and repetitive behaviors = RRB), die mit der aktuellen Edition der DSM-V Kriterien kürzlich in den Fokus gerückt sind, ist hingegen wenig bekannt[15]. Auf Verhaltensebene können RRBs einerseits unterteilt werden in „repetitiv sensomotorische“ (niederes Level) Verhaltensweisen, die stereotype Bewegungen und die wiederholte Verwendung spezifischer Objekte beinhalten. Die

andere Ebene (höheres Level) ist definiert durch „Insistenz auf Gleichheit“ und meint ritualisierte Gewohnheiten und das Bestehen auf bewährte Routinen[16].
Forschungsergebnisse weisen darauf hin, dass die Schwere repetitiver Verhaltenswiesen bei der ASS mit Abweichungen in subkortikalen Strukturen korrelieren könnte, speziell in Teilen des frontostriatalen Netzwerks (bspw. dem Volumen des Nucleus Caudatus in den Basalganglien[17,18]). Wenige Studien haben allerdings die Assoziation zwischen repetitiven Verhaltensweisen und der kortikalen Neuroanatomie über einen längeren Zeitraum untersucht. Aufgrund der wesentlichen Rolle des Kortex im frontostriatalen Bahnsystem ist es wichtig, Hirn-Verhaltenskorrelationen nicht nur auf subkortikaler Ebene zu untersuchen, sondern vielmehr Veränderungen in kortikalen Maßen und deren Auswirkung auf die frontostriatale Konnektivität zu analysieren.
Zudem sind RRBs bekanntermaßen mit sensorischen Symptomen der Autismus-Spektrum-Störung assoziiert (z. B. hyperreaktiven Verhaltensweisen[19]), welche eine wichtige Rolle bei der Klassifizierung von ASS-Subtypen spielen können [20].
Die interindividuelle Heterogenität der Neurobiologie der ASS findet sich jedoch nicht nur in der Neuroanatomie, sondern auch in der Genetik dieser Entwicklungsstörung. Hunderte genetische Varianten sind bisher mit der ASS in Verbindung gebracht worden. Somit liegt es nahe, nicht von einem einzelnen „Autismus-Gendefekt“ auszugehen, sondern vielmehr eine hochkomplexe, polygenetische zugrundeliegende Genstruktur der ASS anzunehmen[21]. Im letzten Schritt dieser Analyse testen wir deshalb die Hypothese, dass Gene, die kürzlich mit ASS in Verbindung gebracht worden sind, in Hirnregionen mit atypischer CT-Entwicklung während der Adoleszenz in ASS überrepräsentiert sind.
Zusammenfassend muss für ein vollumfängliches Verständnis der Ätiologie von ASS die Untersuchung der 3 Ebenen Gehirn, Verhalten und Genetik erfolgen. Bisher sind eher soziale Verhaltensweisen und deren Zusammenhang zur Gehirnanatomie untersucht worden, selten repetitive. Eine zusätzliche Betrachtung der genetischen Hintergründe fehlt bislang fast vollständig. Daher beschäftigt sich der dieser Dissertation zugrundeliegende Artikel „Longitudinal changes in cortical thickness in adolescents with autism spectrum disorder and their association with restricted and repetitive behaviors“ von Bieneck et al. (2021), veröffentlicht im Fachjournal „Genes“, mit der Frage nach einem Zusammenhang zwischen einer intra-individuellen Entwicklungsrate der

Kortexdicke von Adoleszent*innen mit Autismus-Spektrum-Störung und der Veränderung in der Schwere restriktiver und repetitiver Verhaltensweisen im Verlauf dieser Lebensphase. Die kortikalen Veränderungen werden in einem letzten Schritt mit Genen korreliert, die bekannterweise eine Verbindung zur ASS aufweisen. Das Ziel dieser Studie ist somit, neue Einsichten in die Verknüpfung von mikroskopischer und makroskopischer Pathologie der ASS sowie deren Beziehung zu unterschiedlichen klinischen Phänotypen zu erhalten. Alles in allem sind die drei Datenmodalitäten (CT- und RRB-Veränderungen sowie Genanreicherungen bei ASS) bisher isoliert untersucht worden. Diese Studie ist daher die erste, die mit Hilfe eines longitudinalen Designs CT-Wachstumskurven von Adoleszent*innen mit ASS mit deren Entwicklungsverläufen von RRBs assoziiert und spezifische genetische und genauere transkriptomische Grundlagen aufzeigt.

Als übergeordnetes Ziel sollen in weiterführenden Studien aus diesen Erkenntnissen MRT-basierte Biomarker (z.B. die CT_{spc}) entwickelt werden, mithilfe derer das klinische Outcome von ASS Patient*innen vorhergesagt werden soll. Bestenfalls werden somit Vorhersagen über Charakteristika der Symptomatik eines/einer Patient*in, sowie deren Intensität im jeweiligen Lebensabschnitt getroffen. Dies ist ein wichtiger Schritt, um eine bessere Einteilung in Untergruppen dieses komplexen Krankheitsbildes vorzunehmen, und um eine zielgerichtete Therapie basierend auf unterschiedlichen Pathophysiologien zu entwickeln.

3.2. Darstellung der Publikation

Die Analysen erfolgen im Rahmen der longitudinalen Studie „BrainMap“, die die Gehirnentwicklung bei Personen mit und ohne ASS während der Adoleszenz an der Kinder- und Jugendpsychiatrie in Frankfurt untersucht (DFG-finanziertes Projekt, Projekt Nr. 271513085). Eingeschlossen sind Daten von 70 Kindern und Jugendlichen zwischen 11 und 18 Jahren, die zu zwei Messzeitpunkten untersucht werden. Es sind sowohl Kinder und Jugendliche mit einer klinischen ASS-Diagnose als auch neurotypische Mädchen und Jungen untersucht worden, die hinsichtlich ihres Alters, ihrer Händigkeit und ihres durchschnittlichen Intelligenzquotienten (IQ) gematcht sind.

Die klinische ASS-Diagnose ist im Rahmen der Studie mithilfe der Goldstandard Diagnostikinstrumente, der Diagnostischen Beobachtungsskala für Autistische

Störungen – 2 (ADOS-2)[22] und dem Diagnostischen Interview für Autismus – Revidiert (ADI-R)[23] bestätigt. Bei allen Studienteilnehmer*innen liefert die Auswertung der Repetitiven Verhaltensskala – Revidiert (RBS-R)[24] zu beiden Zeitpunkten (T1, T2) Informationen über die Veränderung der Symptomschwere der RRBs über die Zeit. Hierbei wird ein Vier-Faktoren-Modell verwendet, welches exzessives Festhalten an Routinen und ritualisierten Verhaltensmustern (F1), stereotypes (F2), selbstverletzendes (F3) und zwanghaftes Verhalten (F4) einschließt.

Die neuroanatomische Analyse erfolgt mit Hilfe von struktureller Magnetresonanztomographie (sMRT) zu T1 und T2 bei allen Teilnehmenden, um die individuelle Hirnstruktur und Konnektivität über die Zeit zu erfassen. Die Vorverarbeitung der sMRT Scans erfolgt mit Hilfe der Software FreeSurfer v6.0.0 software (https://surfer.nmr.mgh.harvard.edu/, accessed on 11 November 2020)[25]. Hierbei wird die kortikale Oberfläche von jedem T1-gewichteten Bild der Teilnehmenden rekonstruiert. Im Anschluss daran kann die longitudinale Veränderung aus den rekonstruierten Oberflächenabbildungen der CT abgeleitet werden. Die Vertex-weisen Kalkulationen der CT-Veränderungen zwischen den beiden Messzeitpunkten werden als „symmetrierte prozentuale Veränderung“ (= symmetrized percent change, CT_{spc}) angegeben. Der „Longitudinal Stream“ von FreeSurfer[26] berechnet somit die CT_{spc} nicht nur aus der Differenz der Kortexdicke zwischen T1 und T2, sondern auch unter Berücksichtigung der durchschnittlichen Kortexdicke jedes einzelnen Studienteilnehmenden.

Die oberflächenbasierten statistischen Analysen erfolgen mit Hilfe der SurfStat Toolbox[27] (http://www.math.mcgill.ca/keith/surfstat, accessed on 11 November 2020) für Matlab (R2021a; MathWorks) sowie mit Hilfe von R (Version 4.0.5) für die statischen Berechnungen (www.r-project.org, accessed on 16 December 2020). Im ersten Schritt wird ein Verallgemeinertes Lineares Modell (= generalized linear model, GLM) gefittet, welches sich am besten für die Berechnung der Gruppenunterschiede in der CT_{spc} zwischen Autist*innen und neurotypischen Teilnehmenden eignet. Die Auswahl der einzelnen Faktoren des Regressionsmodells erfolgte hierbei über die Anwendung eines stufenweisen „Step-Up“-Modells. Im zweiten Schritt wird ein zweites GLM dazu verwendet, um eine potentielle Assoziation zwischen der CT_{spc} und der intraindividuellen Veränderung der RRBs zu berechnen. Letztere wird quantifiziert als Veränderung der

Gesamtpunktzahl des individuellen RBSR zwischen T1 und T2 (ΔRBS-$R_{(T2-T1)}$). Da die Varianz in ΔRBS-$R_{(T2-T1)}$ in unserer Untersuchung im Wesentlichen durch die ASS-Gruppe verursacht wurde, werden die Hirn-Verhaltenskorrelationen nicht nur in der Gesamtstichprobe, sondern vor allem auch innerhalb der reinen ASS-Gruppe berechnet.

Für die Analyse der potentiell zugrundeliegenden genetischen Mechanismen der beobachteten neuroanatomischen Gruppenunterschiede wird ein Atlas für räumliche Genexpression verwendet. Der Allen Human Brain Atlas (AHBA[28]) fungiert als molekulare Karte des Gehirns und wird in dieser Studie für eine Genexpressionsdekodierungsanalyse (= Gene Expression Decoding Analysis, GEDA[29]) verwendet. Hierfür werden 20.787 Protein kodierende Gene statistisch auf ein räumliches Expressionsmuster getestet, das mit dem räumlichen Verteilungsmuster der neuroanatomischen Unterschiede – gemessen durch die Vertex-weisen CT Analysen – abgeglichen wird. Nach der Applikation einer statistischen Schwelle von $p < 0.05$ wird eine Liste an potentiellen Kandidatengenen abgeleitet. In einem weiteren Schritt folgt die Testung dieser Genliste auf eine Anreicherung von Genen, denen eine Beteiligung an der ASS durch genetische und transkriptomische Untersuchungen zugesprochen worden ist[30-34]. In diesem Schritt erfolgt zudem die Untersuchung dieser Liste auf spezifische Zelltypanreicherungen[34]. Dieser Analyseprozess wird wiederholt für die Hirnregionen, die eine Assoziation zwischen CT_{spc} und $\Delta RBSR_{(T2-T1)}$ zeigen. Hier erfolgt der Abgleich mit dem räumlichen Verteilungsmuster von Genen, die mit RRBs in genetischen Studien in Verbindung gebracht worden sind[33].

In der klinischen Auswertung zeigt sich bei der Mehrheit der Personen mit ASS (60,1%) eine Abnahme der RBS-R-Gesamtwerte über den gemessenen Zeitraum von 24 Monaten 33,3% haben höhere RBS-R-Werte zu T2 und bei 6% gibt es keine Veränderung zwischen beiden Messzeitpunkten. In der Kontrollgruppe hingegen bleiben die RBS-R Gesamtwerte bei den meisten Proband*innen (57%) gleich, 21,6% zeigen verminderte und 21,2% erhöhte Messwerte zu T2.

Die neuroanatomische Analyse ergibt in beiden Gruppen insgesamt eine Abnahme der kortikalen Dicke über den gesamten Kortex hinweg mit zunehmendem Alter (bzw. zwischen T1 und T2). Allerdings zeigen Proband*innen mit ASS verglichen mit der neurotypischen Kontrollgruppe eine verminderte Abnahme der CT. Nur im Bereich der

Insula kommt es zu einer stärkeren Reduktion der Kortexdicke innerhalb der ASS Gruppe.

Ein Zusammenhang zwischen CT_{spc} und $\Delta RBS\text{-}R_{(T2-T1)}$ lässt sich in der Gesamtstichprobe in 6 Regionen nachweisen (u.a. in frontalen und temporalen Arealen, sowie Teilen des Cingulums). In diesen Hirnregionen ist eine Abnahme der kortikalen Dicke zwischen T1 und T2 mit einer abnehmenden RBS-R-Symptomatik assoziiert. In der Gruppe der Personen mit ASS zeigt sich ebenfalls eine Assoziation zwischen sich abmildernden RRBs und der Ausdünnung des Kortex. Dieser Zusammenhang wird in drei Regionen beobachtet, die insbesondere temporale Hirnabschnitte, sowie das Cingulum und die Insula enthalten.

Der letzte Schritt beleuchtet eine vermehrte Expression bestimmter Genmodule, die bekannterweise in ASS herunterreguliert sind, in den Hirnarealen, die eine abweichende CT_{spc} bei den Autist*innen zeigen. Die ermittelten Module repräsentieren vor allem Gene, die in der rezeptorvermittelten Signalübertragung und an synaptischer Transmission beteiligt sind[30,31]. Anreicherungen an in ASS hochregulierten Genen sind nicht gefunden worden. Die Analyse für bestimmte Zelltypanreicherungen in den Hirnbereichen mit atypischer CT_{spc}-Entwicklung der ASS-Gruppe zeigt Anreicherungen von Genen, die in exzitatorischen Neuronen der Kortexschichten L2/L3 sowie in der synaptischen Funktion und in Transkriptionsfaktoren in L4 dysreguliert sind[34]. Die ermittelten Regionen, in denen ein signifikanter Zusammenhang zwischen $\Delta RBS\text{-}R_{(T2-T1)}$ und CT_{spc} besteht, weisen keine Anreicherung für Gene, die durch Tao et al. (2016) mit RRBs in Verbindung gebracht worden sind, auf.

Ein weiterer Schritt der genetischen Analyse beinhaltet die Untersuchung auf die Anreicherung von Genen, die mit RRBs in Verbindung stehen, in Hirnarealen, die einen Zusammenhang zwischen der Veränderung der Kortexdicke und der Zu- oder Abnahme in RRBs vorweisen. Die signifikanten Gruppenunterschiede der CT_{spc} in dieser Studie finden sich vor allem in frontotemporalen Regionen und dem Cingulären Cortex. Gerade diese Regionen weisen Genanreicherungen von ASS-Risikogenen und Genexpressionsmodulen, die bekanntermaßen im autistischen Kortex runterreguliert sind, auf. Zudem beobachten wir in dieser Untersuchung eine Anreicherung von Genen, die Zelltransmissionsprozessen spezifischer Zellen zugrunde liegen. Insbesondere von exzitatorischen Neuronen der Kortexschichten L2, L3 und L4[34].

Zusammenfassend weisen unsere Ergebnisse auf eine Assoziation zwischen Abweichungen in der Entwicklungskurve der kortikalen Dicke bei Personen mit ASS und Variationen von involvierten Genen hin. Außerdem legt diese Studie eine Verbindung von CT-Veränderungen (in frontotemporalen, frontoparietalen und cingulären Regionen) und intraindividuellen Veränderungen in der Symptomschwere von restriktiven und repetitiven Verhaltensweisen in der Adoleszenz bei Personen mit ASS dar. Damit erweitert diese Untersuchung vorherige Berichte von atypischen Hirnentwicklungen bei ASS und verbindet diese Unterschiede mit spezifischen ASS-Symptomen.

3.3. Diskussion

Im Gegensatz zu vorherigen Studien zur CT-Entwicklung bei ASS[35,9,11,13] beobachten wir eine verminderte statt einer verstärkten Abnahme der CT in der ASS-Gruppe im Vergleich zur Kontrollgruppe. Diese abweichenden Ergebnisse bestätigen die Inkonsistenzen vorliegender Bildgebungsstudien in den regionalen Entwicklungsunterschieden der CT zwischen Querschnitts- und longitudinalen Stichproben, abhängig von der untersuchten Altersgruppe. Während manche Studien von zunehmender Abnahme berichten, finden andere eine verlangsamte Abnahme des Kortex bei ASS über die Zeit[36]. Insbesondere Querschnittstudien, die einen Zeitraum untersuchen, der Kindheit und Adoleszenz zusammenfasst, berichten von verstärkter Abnahme der Kortexdicke bei ASS[10,13,10,12,37]. Diese Studien merken allerdings an, dass eine Untersuchung der neurologischen Entwicklungskurve in den einzelnen Altersphasen separat erfolgen sollte. Hier ist die longitudinale Studie von Zielinski et al. (2014) anzuführen, die eine Unterteilung der Proband*innen in unterschiedliche Altersgruppen vornimmt[38]. Personen mit ASS zeigen hier zum Zeitpunkt der frühen Kindheit kortikale Verdickung, gefolgt von verstärkter Ausdünnung des Kortex während der Adoleszenz, resultierend in einer verminderten Ausdünnung im frühen Erwachsenenalter. Hierbei überschneiden sich die CT-Wachstumskurven der ASS- und der Kontrollgruppe zwischen der Kindheit und Adoleszenz (10-20 Jahre). An diesem Punkt zeigen sich keine signifikanten Unterschiede zwischen beiden Gruppen. Basierend auf diesen Ergebnissen, könnten sich die CT-Entwicklungsunterschiede der ASS-Gruppe in unserem untersuchten Altersspektrum (11-18 Jahre) „pseudonormalisieren“. Genauer gesagt sollten kaum oder keine Gruppenunterschiede

zu T1 und/oder T2 festgestellt werden. Dennoch berichten wir von signifikanten Unterschieden in der CT Entwicklung zwischen beiden Zeitpunkten. Genauer findet sich in einer Region (linke Insula) eine verstärkte Abnahme der CT, und mehrere Hirnareale zeigen eine verminderte Ausdünnung des Kortex in der ASS-Gruppe. Solche Diskrepanzen zu anderen Studien können teilweise durch Unterschiede in Stichprobencharakteristika und analytischen Ansätzen erklärt werden. Beispielsweise ist das Altersspektrum dieser Stichprobe enger gewählt als bei Zielinski et al. 2014 (11-18 Jahre vs. 3-36 Jahre), zudem schließt unser Studiendesign auch weibliche Proband*innen ein[38].

Bisherige Studien zur Untersuchung der Hirnentwicklung während der Kindheit und Adoleszenz stellen den Prozess der Hirnreife als „invertierte U-Form“ dar, die ihr Maximum während der Kindheit bzw. früher Adoleszenz erreicht (beispielsweise basierend auf Messungen des kortikalen Volumens und des Kontrastes zwischen grauer und weißer Substanz)[8,39]. Die gleichen Kurvenmerkmale werden für die Entwicklungskurve der CT berichtet, wenn auch mit früheren Peaks (Männer/Jungen = 8,6 Jahre, Frauen/Mädchen = 8,4 Jahre), gefolgt von kortikaler Ausdünnung[40]. Demzufolge steht die Abnahme der Kortexdicke während der späten Kindheit bzw. frühen Adoleszenz in Einklang mit neurotypischen Reifeprozessen während dieser Entwicklungsphase, sodass die analysierte verminderte Abnahme der CT in unserer Stichprobe eine weniger rapide oder stagnierende Reifung suggeriert. Auf zellulärer Ebene ist die kortikale Ausdünnung bereits ebenfalls mit dem Prozess des „Synaptic Pruning“ (Eliminierung von bestehenden Synapsen) in Verbindung gebracht worden. Bei diesem Mechanismus der Hirnreifung werden neue Zellverbindungen aufgebaut und redundante nicht genutzte Verbindungen aktiv eliminiert. Histologische Studien berichten, dass die Synapsendichte im mittleren frontalen Gyrus, in welchem wir eine verminderte kortikale Ausdünnung gefunden haben, bis zum Alter von 7 Jahren postnatal zunimmt. Dieser Phase folgt ein Abschnitt des „Synaptic Pruning“ bis zum frühen Erwachsenenalter[41]. Die Eliminierung von Synapsen ist wichtig für den Aufbau eines komplexen Nervensystems und findet seine Parallele in der Abnahme der kortikalen Dicke während der Hirnreifung[42,43]. Unsere Erkenntnisse weisen darauf hin, dass der Kortex bei Personen mit ASS in spezifischen Hirnregionen langsamer reift. Von einer verzögerten kortikalen Ausdünnung bei ASS während der Adoleszenz berichtet

ebenfalls eine Studie von Razhnahan et al. (2010), insbesondere den linken mittleren temporalen und den rechten superioren frontalen Gyrus betreffend[44]. Zusammengefasst zeigen die makroskopischen Unterschiede, die wir im autistischen Gehirn beobachten, in die Richtung spezifischer zugrundeliegender Mechanismen, die bei Personen mit ASS dysreguliert sein könnten – zum Beispiel „Synaptic Pruning“ sowie dendritische und axonale Verzweigung.

Die atypische Hirnentwicklung bei ASS ist bereits in Verbindung mit der Symptomschwere autistischer Verhaltensweisen gebracht worden[2]. Hier liegt der Fokus allerdings auf dem Zusammenhang von atypischer Kortexentwicklung und sozialer Beeinträchtigung bei Personen mit ASS. Gleichzeitig bleiben Untersuchungen der Beziehung zwischen Neuroanatomie und RRBs bei Personen mit ASS in der Literatur unterrepräsentiert. Diese Studie nimmt sich diesem Problem an und untersucht die Auswirkungen der Veränderungen in der CT auf die Symptomschwere von RRBs. Letztere stehen außerdem in Verbindung mit sensorischen Symptomen, die sich als vielversprechende Kandidaten für die Zergliederung der Heterogenität in ASS herausstellen[20]. Verhaltensweisen niederer Level (einschließlich selbstverletzenden Verhaltens) treten häufiger bei jüngeren Kindern mit ASS auf und bei jenen mit geringer Intelligenz[45,46]. Diese Tendenz können wir mit den Beobachtungen in unserer Stichprobe bestätigen, da die adoleszente ASS-Gruppe ein nur sehr geringes Maß an selbstverletzendem Verhalten zeigt. Zudem berichten wir in Übereinstimmung mit anderen Studien von einer Abnahme der Gesamtpunktzahl des RBS-R und somit der Symptomschwere der RRBs in der ASS-Gruppe über den untersuchten Zeitraum, was in dieser Altersgruppe üblich ist[47,24].

Vereinzelte Studien berichten signifikante Korrelationen zwischen der Symptomschwere repetitiver Verhaltensweisen und Messwerten der Hirnanatomie bei ASS. Bis heute wurden diese Hirn-Verhaltenskorrelationen hauptsächlich in subkortikalen Strukturen (Nucleus Caudatus, Basalganglien) beobachtet, die an Frontalkortexregionen anschließen und einen Frontostriatalen Schaltkreis bilden[17,48]. Genauer gesagt wird hier der Zusammenhang zwischen einem zunehmendem Volumen des linken Nucleus Caudatus mit selbstverletzendem Verhalten in Jungen mit ASS dargestellt, während zwanghaftes und ritualisiertes Verhalten signifikante positive Korrelationen mit den Volumina beider Nuclei Caudati aufweist[48]. Weiterhin steht eine Zunahme der

funktionellen Konnektivität zwischen dem linken Nucleus Accumbens und einer Region des rechten prämotorischen Kortex/mittleren frontalen Gyrus in Verbindung mit erhöhter Symptomschwere von repetitiven Verhaltensweisen bei Kindern und Adoleszenten mit ASS[49]. Mit unserer Untersuchung zeigen wir zudem eine Assoziation zwischen intraindividuellen Variationen in RRBs und Veränderungen der CT bei Personen mit ASS in kortikalen Abschnitten, die postzentrale, parietale, frontale und temporale Regionen umfasst. Unsere Ergebnisse stimmen nicht nur mit vorherigen Untersuchungen überein, die eine entscheidende Rolle der frontostriatalen Schaltkreise hinsichtlich der Mediation von RRBs während der Entwicklung suggerieren. Vielmehr erweitern wir diese Erkenntnisse, indem wir von einer Korrelation mit den kortikalen Aspekten dieser Bahnsysteme berichten und weisen hiermit auf potentielle Zusammenhänge dysfunktionaler kortikostriataler Konnektivität aufgrund von atypischen Veränderungen der kortikalen Dicke in Adoleszent*innen mit ASS hin.
Ziel des letzten Analyseschrittes stellt die Verknüpfung der von uns auf neuroanatomischer Ebene beobachteten makroskopischen Unterschiede mit potentiellen genetischen Grundlagen der ASS dar. Bisher haben wenige Studien die zugrundeliegenden genetischen und molekularen Mechanismen der CT-Abweichungen bei ASS untersucht. Romero-Garcia et al. (2019) demonstriert in seiner Studie, dass CT-Unterschiede während der Kindheit stark assoziiert sind mit Genen, die in synaptischen Übertragungswegen involviert sind[50]. Diese Gene sind bekannterweise im postmortalen ASS-Kortex runterreguliert[31,50]. Von ähnlichen Ergebnissen ist kürzlich von unserer Arbeitsgemeinschaft bei der Untersuchung von CT-Unterschieden in einer großen klinisch heterogenen Stichprobe berichtet worden[51]. Die aktuellen Ergebnisse konvergieren mit den vorherigen Erkenntnissen, indem wir Anreicherungen für Gene und Co-Expressionsmodule nachweisen, die bekannterweise in ASS runterreguliert sind. Die heruntergefahrenen Module M16 und M10 sind bekanntermaßen in neuronale Aktivitäten und Synaptischer Funktion involviert[31], was zu Defiziten im „Synaptic Pruning“ und zu verlangsamter Kortikaler Ausdünnung führen könnte[52]. Zudem sind viele ASS-Risikogene daran beteiligt, die Reifung exzitatorischer und inhibitorischer neuronaler Bahnen zu beeinträchtigen[32]. Die Ergebnisse entsprechen folglich genetischen Studien, die von dysregulierten Genen berichten, die in synaptischer Transmission exzitatorischer Neurone über kortikale Schichten hinweg (L2, L3, L4[34])

involviert sind. Somit stützen wir vorherige Beobachtungen, die eine den ASS-Phänotypen zugrundeliegende exzitatorisch-inhibitorische Imbalance beschreiben[53]. Wir haben keine signifikanten Anreicherungen von Genen gefunden, die kürzlich mit repetitiven Verhaltensweisen in ASS in Verbindung gebracht worden sind[33]. Allerdings ist die von Tao et al. (2016) bereitgestellte Genliste vergleichsweise klein und unspezifisch im Hinblick auf ihre funktionelle Beteiligung[33]. Ihre funktionale Rolle in der Hirnentwicklung bleibt daher zu ermitteln. Insgesamt ergänzen die durch uns nachgewiesenen Genanreicherungen von ASS-zugehörigen Gensets die biologische Plausibilität der Ergebnisse bildgebender Verfahren und verbinden die atypische CT-Entwicklung mit spezifischen ätiologischen Mechanismen der ASS.

4 Publikationsübersicht

Die vorliegende publikationsbasierte Dissertation basiert auf der folgenden Originalarbeit, die im Verlauf angehängt ist.

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5 Publikation

Longitudinal Changes in Cortical Thickness in Adolescents with Autism Spectrum Disorder and Their Association with Restricted and Repetitive Behaviors

genes

MDPI

Article

Longitudinal Changes in Cortical Thickness in Adolescents with Autism Spectrum Disorder and Their Association with Restricted and Repetitive Behaviors

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Abstract: The neuroanatomy of autism spectrum disorder (ASD) shows highly heterogeneous developmental trajectories across individuals. Mapping atypical brain development onto clinical phenotypes, and establishing their molecular underpinnings, is therefore crucial for patient stratification and subtyping. In this longitudinal study we examined intra- and inter-individual differences in the developmental trajectory of cortical thickness (CT) in childhood and adolescence, and their genomic underpinnings, in 33 individuals with ASD and 37 typically developing controls (aged 11–18 years). Moreover, we aimed to link regional atypical CT development to intra-individual variations in restricted and repetitive behavior (RRB) over a two-year time period. Individuals with ASD showed significantly reduced cortical thinning in several of the brain regions functionally related to wider autism symptoms and traits (e.g., fronto-temporal and cingulate cortices). The spatial patterns of the neuroanatomical differences in CT were enriched for genes known to be associated with ASD at a genetic and transcriptomic level. Further, intra-individual differences in CT correlated with within-subject variability in the severity of RRBs. Our findings represent an important step towards characterizing the neuroanatomical underpinnings of ASD across development based upon measures of CT. Moreover, our findings provide important novel insights into the link between microscopic and macroscopic pathology in ASD, as well as their relationship with different clinical ASD phenotypes.

Keywords: autism spectrum disorder; cortical thickness; restricted and repetitive behaviors; genetics

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by impairments in (1) social communication and interaction, (2) repetitive and restricted behaviors and interests, and atypical sensory responses (DSM-5, 2013; [1]). However, the clinical phenotype of ASD is highly heterogeneous both within (e.g., across age) and between (e.g., in terms of the severity and profile of core and associated symptoms [2]) individuals. Similarly, there exists large inter-individual heterogeneity in the neurobiology of ASD, including in the neuroanatomy and genetics [2,3]. For example, while neuroimaging studies in ASD agree on an atypical developmental trajectory of brain maturation in affected individuals ([2,4,5]), the reported spatial distribution of these differences reveal a high level of diversity across development [6]. Moreover, ASD has been linked to hundreds

of genetic variants. This suggests that ASD is not a single gene disorder, but has a highly complex (poly)genetic architecture [7]. Combined, these findings highlight inherent difficulties in linking differences in the brain structure to etiological factors, and determining their impact on clinical outcomes in ASD.

While a wealth of studies have explored the neural networks underpinning ASD [8], the reported differences vary in terms of the specific patterns and sign (i.e., direction) [2]. Moreover, the patterns of differences vary for different morphometric features, including volumetric and geometric measures of the neuroanatomy [9–11]. One of the morphological parameters in which atypicalities in ASD have been reported most consistently, is in the cortical thickness (CT); i.e., the closest distance between the outer (i.e., pial) and inner (i.e., white matter) boundary at each vertex on the tessellated surface [12]. It is well established that the developmental trajectory of CT in ASD deviates from the neurotypical trajectory, even though reports vary with regard to the direction of the difference. For example, in a longitudinal study by Zielinski et al. (2014), ASD was characterized by an overgrowth of the cortical mantle during early childhood, followed by an accelerated decline in mid-childhood, and a phase of 'normalization' during adulthood [5]. Contrarily, Wallace et al. (2010) reported an accelerated—rather than decelerated—cortical thinning during adulthood in a cross-sectional sample of ASD individuals [11]. These studies highlight some of the inconsistencies of neuroanatomical findings derived from longitudinal and cross-sectional studies designed to investigate age-related changes in the neuroanatomy of ASD. Hence, further longitudinal studies are required to replicate and validate these earlier findings.

Despite these differing results, studies agree that fronto-temporal and fronto-parietal brain regions [13–15] are particularly affected in ASD, and display the most atypical neurodevelopmental trajectory relative to other brain regions. Overall, neuroanatomical differences in ASD have been reported in (1) fronto-temporal and fronto-parietal brain networks including the medial, orbitofrontal (OFC) and inferior frontal (IFG) cortices, the posterior parietal cortex, the superior temporal sulcus (STS) and the fusiform gyrus; (2) limbic brain regions such as the amygdala–hippocampal complex, the thalamus, and cingulate regions; (3) the fronto-striatal circuitry including parts of the basal ganglia, the anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC); and (4) the cerebellum [2,8]. Most of these brain regions are integral parts of the so-called 'social brain' that underpins higher socio-cognitive functioning [16]. However, less is known about the neuroanatomical underpinnings of restricted and repetitive behaviors (RRBs), which, with the most recent edition of the DSM-V, have only recently become the focus of attention. [1]. Emerging research suggests that the severity of repetitive behaviors in ASD may be correlated with differences in subcortical structures, particularly in parts of the fronto-striatal circuitry (e.g., the volume of the caudate nucleus in the basal ganglia [17,18]). However, few studies have investigated the association between repetitive behaviors and cortical neuroanatomy (e.g., CT) across time. Due to the crucial role of the cortex in fronto-striatal pathways [19], it is important to not only assess brain–behavior correlations at a subcortical level, but to furthermore analyze changes in cortical measures and their impact on fronto-striatal connectivity.

Therefore, in this study we compared changes in the CT of children and adolescents with ASD to those of typically developing (TD) controls, and examined the relationship of CT differences with variations in repetitive behavior (as measured by the Restricted and Repetitive Behavior Scale-Revised (RBS-R) [20,21]) over time. We focused on RRB because most neuroanatomical studies to date have examined brain–behavioral correlations with social impairments, leaving RRB largely unexplored. Furthermore, RRBs are known to be associated with sensory symptoms (e.g., hyperresponsive behaviors; [22]), which have been highlighted as promising new candidates for subtyping ASD individuals [23]. In contrast to most previous studies that have examined neurodevelopmental trajectories in CT during early to mid-childhood [4,13,24–27], we focused on the developmental changes in CT during late childhood and adolescence based on a narrower age range (11–18 years).

This allowed us to investigate a crucial stage in development during which CT growth curves in ASD and TD controls typically overlap [5], even though ASD symptoms persist across the human life span. Last, using the spatial gene expression data provided by the Allen Human Brain Atlas (AHBA; [28]), we tested the hypothesis that brain regions with atypical CT development during adolescence in ASD are enriched for genes that have previously been linked to ASD on the genetic and transcriptomic level.

2. Materials and Methods

2.1. Participants

This study used data provided by an ongoing longitudinal study examining brain development during adolescence in individuals with ASD and in TD controls at two timepoints separated by ~2 years (see Figure 1). The total sample consisted of n = 70 individuals of between 11 and 18 years at timepoint 1, out of which n = 33 participants had a diagnosis of ASD, and n = 37 were TD controls. Groups were matched for age, sex, and full-scale IQ (FSIQ; see Table 1). ASD was assessed using gold-standard diagnostic tools, i.e., the German version of the Autism Diagnostic Interview-Revised (ADI-R; [29,30]) and the second edition of the Autism Diagnostic Observation Schedule (ADOS-2; [31,32]). Repetitive behaviors were examined in all participants using the German version of the Repetitive Behavior Scale-Revised (RBS-R) at both assessment timepoints [20,21]. In accordance with Kästel et al. (2014), we used a four-factor model, containing persistent (F1), stereotyped (F2), self-injurious (F3), and compulsive (F4) behaviors to compute the RBS-R subscales. A full list of inclusion and exclusion criteria and the clinical characteristics of the sample is provided in the Supplementary Materials (see Supplementary Methods 1 and Supplementary Table S1). All participants (guardians of participants below 18 years of age), gave informed written consent. The study was approved by the Ethics Committee of the Faculty of Medicine of Goethe University, Frankfurt.

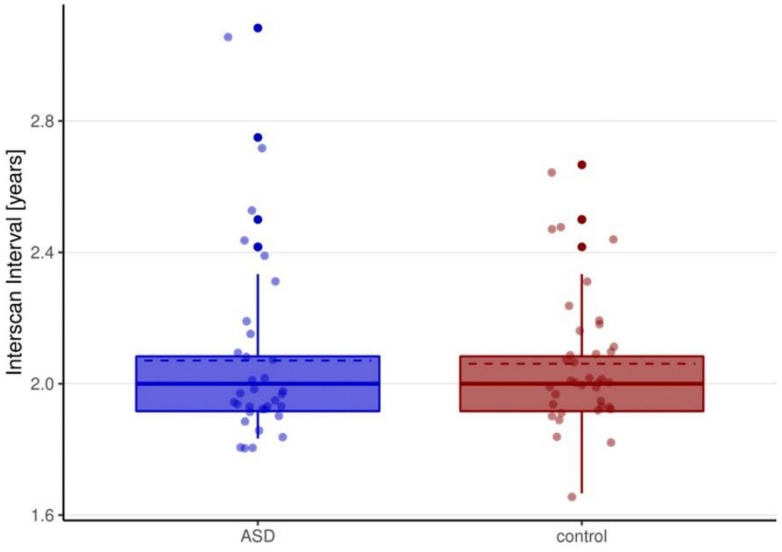


Figure 1. Interscan Interval (ISI) of the ASD and control group. Time between the two scanning timepoints in the ASD group (blue) and the control group (red). Dashed lines represent mean scores of ISI, solid lines represent the median scores of ISI.

Table 1. Sample Characteristics.

	ASD (n = 33)	TD Controls (n = 37)	Group Comparison		
			t	x^2	p
Age [years]					
T1	14.48 ± 2.51	13.76 ± 2.36	1.25		0.22
T2	16.61 ± 2.47	15.89 ± 2.45	1.21		0.23
Interscan-Interval (ISI) [years]	2.07 ± 0.28	2.06 ± 0.20	0.17		0.87
Sex (male/female)	27/6	30/7		<0.001	1.00
Full-scale IQ (FSIQ)	101.38 ± 13.19	107.07 ± 11.52	−1.91		0.06
Handedness (right/left)	29/4	34/3		0.025	0.87
ADI-R *Social Interaction*	17.06 ± 4.96	-			
ADI-R *Communication*	13.06 ± 4.12	-			
ADI-R *Repetitive Behaviors*	4.76 ± 2.50	-			
ADOS *CSS*					
T1	5.79 ± 2.71	-			
T2 [1]	5.95 ± 2.22	-			
RBS-R total					
T1	25.18 ± 18.80	2.11 ± 3.67	6.9		<0.001 ***
T2	22.48 ± 17.21	2.30 ± 4.07	6.58		<0.001 ***
Δ(T2-T1)	−2.70 ± 16.00	+0.19 ± 3.01	−1.02		0.3148
RBS-R *persistent*					
T1	16.88 ± 11.26	1.59 ± 2.85	7.59		<0.001 ***
T2	14.97 ± 10.39	1.81 ± 03.23	6.98		<0.001 ***
Δ(T2-T1)	1.91 ± 9.43	−0.22 ± 2.27	1.26		0.22
RBS-R *stereotyped*					
T1	3.06 ± 3.18	0.30 ± 0.62	4.91		<0.001 ***
T2	2.70 ± 3.66	0.08 ± 0.28	4.09		<0.001 ***
Δ(T2-T1)	0.36 ± 3.63	0.22 ± 0.67	0.23		0.82
RBS-R *self-injurious*					
T1	0.88 ± 2.16	0.05 ± 0.23	2.18		<0.001 ***
T2	1.06 ± 1.84	0.03 ± 0.16	3.22		<0.001 ***
Δ(T2-T1)	−0.18 ± 2.51	0.03 ± 0.29	−0.48		0.64
RBS-R *compulsive*					
T1	4.36 ± 5.17	0.16 ± 0.60	4.64		<0.001 ***
T2	3.76 ± 4.57	0.38 ± 1.14	4.14		<0.001 ***
Δ(T2-T1)	0.61 ± 3.17	−0.22 ± 0.71	1.46		0.15
Total Brain Volume [l]					
T1	1.18 ± 1.00	1.24 ± 0.94	−2.60		<0.05 *
T2	1.17 ± 0.10	1.23 ± 0.10	−2.49		<0.05 *
Δ(T2-T1)	−0.01 ± 0.02	−0.01 ± 0.02	−0.20		0.8445
Total Surface Area [m^2]					
T1	0.18 ± 0.015	0.19 ± 0.02	−2.33		<0.05 *
T2	0.18 ± 0.16	0.19 ± 0.02	−2.28		<0.05 *
Δ(T2-T1)	−0.02 ± 0.002	−0.002 ± 0.002	−0.16		0.877
Mean Cortical Thickness [mm]					
T1	2.75 ± 0.10	2.78 ± 0.08	−1.15		0.2555
T2	2.68 ± 0.08	2.70 ± 0.08	−0.81		0.4221
Δ(T2-T1)	−0.07 ± 0.05	−0.08 ± 0.04	0.89		0.3759

Note: Data expressed as mean ± standard deviation; Abbreviations: ASD: Autism Spectrum Disorder, TD: Typical Developing, T1: timepoint 1, T2: timepoint 2, ADI-R: Autism Diagnostic Interview-Revised [29], RBS-R: Repetitive Behavior Scale-Revised [20,21], ADOS: Autism Observation Schedule, *CSS*: Calibrated Severity Score [33], mm: millimeter, l: liter, m^2: square meter, t: t-value, x^2: Pearson's chi-squared test, p: p value, ***: $p < 0.001$, *: $p < 0.05$; [1] data based on n = 21 individuals.

2.2. MRI Data Acquisition

All MRI data were acquired at the Brain Imaging Centre (BIC), Frankfurt using a contemporary MRI scanner operating at 3 Tesla (Magnetom Trio, Siemens Medical Systems, Erlangen, Germany). High-resolution structural ADNI MPRAGE sequences were acquired with full head coverage using an 8-channel head coil (slice thickness = 1.0 mm, in-plane

resolution = 1.0 × 1.0 mm^2, repetition time (TR) = 2300 ms, echo time (TE) = 2.2 ms, flip angle = 9°, field of view = 26.5 cm, slice number = 176).

2.3. Cortical Surface Reconstruction Using FreeSurfer

Image processing and cortical reconstruction for all MRI scans was performed using FreeSurfer v6.0.0 software (https://surfer.nmr.mgh.harvard.edu/, accessed on 11 November 2020). Here, models of the cortical surface are created for each T1-weighted image, i.e., one image per subject and timepoint, by implementing well validated and fully automated procedures. These have been extensively described in previous studies [34–36]. The reconstructed scans were further processed using FreeSurfer's longitudinal stream to derive estimates of the vertex-wise change in CT [34]. Here, an unbiased within-subject template (i.e., 'base' image) is initially created using cubic spline interpolation reflecting the average anatomy of each subject across time to reduce the confounding effect of inter-individual morphological variability [37,38]. Subsequent processing steps of the single timepoints, including skull stripping, Talairach transforms, and atlas registration, are then based upon this common information from the within-subject template. This significantly improves the reliability and statistical power [34]. More information on the quality assurance procedures is provided in the Supplementary Materials (see Supplementary Methods 2). CT was calculated based on the longitudinal scans as the closest distance from the outer (i.e., pial) to the inner (i.e., white matter) boundary at each vertex on the tessellated surface [12]. Vertex-wise estimates of the longitudinal CT change were expressed as the Symmetrized Percent Change (CT_{spc}) within the framework of FreeSurfer's longitudinal stream (https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalTwoStageModel, accessed on 11 November 2020), which is calculated as

$$CT_{spc} = 100 * \frac{\text{rate}}{\text{average}}$$

With the rage $= 0.5 * (CT_{T1} + CT_{T2})$, and rate $= CT_{T2} - (\frac{CT_{T1}}{ISI})$. Hence, this parameter represents change the in CT of individuals from T1 to T2 at each vertex, while accounting for the average thickness across the cortex and intra-individual noise. Vertex-wise estimates of CT_{spc} were registered to a common space surface template (i.e., fsaverage in FreeSurfer) and smoothed using a 10-mm surface-based smoothing kernel, to increase the ability to detect population changes.

2.4. Statistical Analyses

Surface-based statistical analyses were performed using the SurfStat toolbox (http://www.math.mcgill.ca/keith/surfstat, accessed on 11 November 2020) for Matlab (R2021a; MathWorks), and R (version 4.0.5) for the Statistical Computing (www.r-project.org, accessed on 16 December 2020). Missing data in RBS-R (maximum 3 out of 43 items per person) was input by predictive mean matching using the 'mice' package in R [39]. Between-group differences in age at both timepoints, ISI, sex, FSIQ, handedness, ASD symptom severity, total brain measures at both timepoints and their change, were assessed via *t*-test or χ^2-test (see Table 1). We initially applied a step-up model selection procedure using a nested model comparison to identify the general linear model (GLM) that best fitted our CT data (for more information see Supplementary Methods 3). Based on the best-fitting model, vertex-wise between-group differences in CT_{spc} (Y) were subsequently examined by applying a GLM with the diagnostic group and sex as the fixed-effects factors, and the linear and quadratic age at T1, FSIQ, and ISI as the continuous covariates, i.e.,

$$\gamma_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Sex} + \beta_3 \text{Age}_{T1} + \beta_4 \text{Age}_{T1}^2 + \beta_5 \text{FSIQ} + \beta_6 \text{ISI} + \varepsilon_i \quad (1)$$

where ε_i is the residual error at vertex i. Between-group differences were estimated from the coefficient β_1, and normalized by the corresponding standard error.

In a second analysis step, we examined the association between the developmental change in CT (CT_{spc}) and developmental changes in the severity of general autism symptoms and repetitive behaviors across adolescence, which was quantified as the intra-individual change in the RBS-R total score from T1 to T2 (ΔRBS-$R_{(T2\text{-}T1)}$). The employed GLM was derived using a step-up model selection procedure that assessed the goodness-of-fit upon inclusion of the ΔRBS-$R_{(T2\text{-}T1)}$ and the ΔRBS-$R_{(T2\text{-}T1)}$-by-group interaction (see Supplementary Methods 3). Based on the model comparison, the following model was fitted

$$\gamma_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Sex} + \beta_3 \text{Age}_{T1} + \beta_4 \text{Age}_{T1}^2 + \beta_5 \text{FSIQ} + \beta_6 \text{ISA} + \beta_7 \Delta\text{RBSR}_{(T2-T1)} + \varepsilon_i \quad (2)$$

We did not covary for the mean CT across the cortex, as this is already accounted for in the computation of CT_{spc}. Since variance in ΔRBS-$R_{(T2\text{-}T1)}$ was mainly driven by individuals within the ASD group (see Supplementary Figure S1), brain-behavioral correlations were examined both in the total sample and within the ASD individuals. The main effect of ΔRBS-$R_{(T2\text{-}T1)}$ on CT_{spc} was then calculated from the respective coefficient β_7. In the ASD group, associations between regional deviations from the neurotypical developmental trajectory of CT and developmental changes in autism symptoms and repetitive behaviors were further examined via Pearson correlation.

In all GLMs, the continuous covariates were mean centered across groups to improve interpretability of the coefficients. In the subanalysis of individuals with ASD, mean centering was performed across all included participants with ASD. Corrections for multiple comparisons across the whole brain were performed using 'random field theory' (RFT)-based cluster analysis for nonisotropic images with a cluster-forming and cluster-based significance threshold of $p < 0.05$ (2-tailed; [40]).

2.5. Gene Expression Decoding Analysis

To identify the potential genetic underpinnings of the observed neuroanatomical findings, we used the spatial gene expression data provided by the Allen Human Brain Atlas (AHBA; [28]) to perform a gene expression decoding analysis (GEDA; [41]). Here, a total of 20,787 protein coding genes were statistically tested for a spatial pattern of expression that was similar to the spatial pattern of neuroanatomical differences highlighted by the vertex-wise analyses of CT, e.g., the *t*-map for the between-group difference in CT_{spc}. The resulting gene list was thresholded at $p < 0.05$ (see Figure 2a; for more information on the methodological approach further see Supplementary Methods 4). This liberal threshold was selected as this analysis did not constitute a hypothesis test per se, but rather a selection step to provide a list of potential candidate genes. Subsequently, the resultant gene list was tested for enrichment using lists of genes that have previously been implicated in ASD by genetic and transcriptomic studies [42–46]. At the genetic level, this included ASD risk genes with de novo and rare variants [44], and GWAS-significant ASD risk genes with common variants [47]. At the transcriptomic level, this included genes that are (i) differentially expressed (i.e., upregulated or downregulated) in postmortem cortical tissue in ASD [43], and in specific neuronal cell types in ASD [45], and (ii) genes of differentially expressed coregulated modules in ASD [42,48]. We also included the ASD-gene list compiled by the SFARI gene database (categories S,1,2,3 downloaded 11 November 2020 from https://gene.sfari.org/).

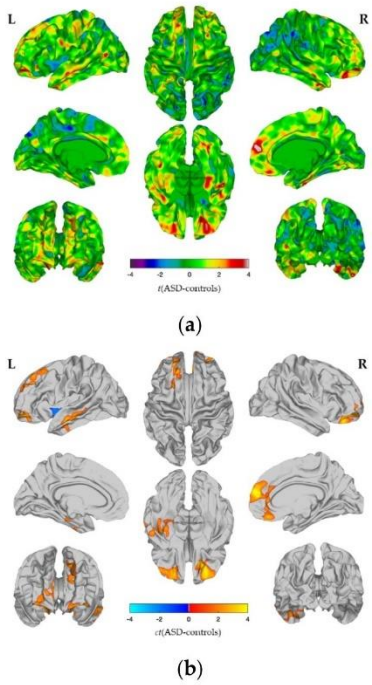


Figure 2. Vertex-wise between-group differences in cortical thickness (CT). Vertex-wise between-group differences in Scheme 1 to T2, i.e., the rate of CT change with respect to the average CT, in individuals with autism spectrum disorder (ASD) compared to neurotypical controls. (**a**) *t*-test statistic for the contrast ASD minus controls (unthresholded); (**b**) Positive clusters (orange to yellow) indicate significantly less cortical thinning, negative clusters (blue to cyan) indicate significantly stronger cortical thinning in ASD (RFT-based cluster corrected, $p < 0.05$, two-tailed). Abbreviations: L: left hemisphere, R: right hemisphere.

Furthermore, we examined the *t*-map associated with the main effect of RBS-R variation over time (see Figure 3a) for enrichment of genes previously linked to repetitive behavior in ASD [46]. All enrichment tests were performed using the GeneOverlap package in R (10.18129/B9.bioc.GeneOverlap, accessed on 20 January 2021), which generated enrichment odds ratios (OR), hypergeometric *p* values, and FDR-corrected *p* values (p_{adj}). Only comparisons with $p_{adj} < 0.05$ were interpreted further (more details on the methodological approach of the enrichment analysis are provided in the Supplementary Methods 5).

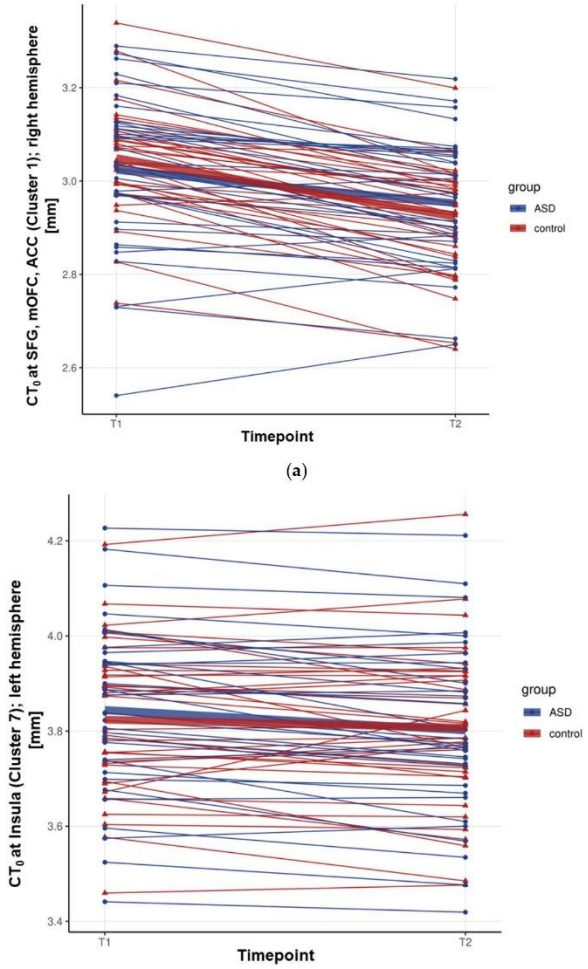


Figure 3. Cluster-wise mean Cortical Thickness (CT) at timepoint 1 (T1) and timepoint 2 (T2). Cluster-wise estimates of the mean CT of individuals at T1 and T2 for the clusters in which a significant between-group difference was observed between individuals with autism spectrum disorder (ASD) (marked in blue) and typically developing (TD) controls (marked in red) in the main analysis, i.e., the main effect of the group for a developmental change in CT. Displayed are two clusters, (**a**) cluster 1 is an example of the first six clusters out of seven, in which, overall, developmental cortical thinning was more pronounced in TD controls as compared to individuals with ASD; and (**b**) cluster 7, which was the only cluster, where individuals with ASD, overall, showed more pronounced developmental cortical thinning relative to TD controls. Abbreviations: SFG: superior frontal gyrus; mOFC: medial orbital frontal cortex; ACC: anterior cingulate cortex.

3. Results

3.1. Subject Demographics

There were no significant differences between individuals with ASD and TD controls in their age ($t(66) = 1.25$, $p = 0.22$), ISI ($t(58) = 0.17$, $p = 0.87$), FSIQ ($t(64) = -1.91$, $p = 0.06$), or in the distribution of sex ($\chi 2(1) < 0.001$, $p = 1.00$) and handedness ($\chi 2(1) = 0.025$,

$p = 0.87$). Furthermore, there was no significant difference in the mean CT at T1 or T2 (T1 ($t(61) = -1.15$, $p = 0.26$); T2 ($t(68)_{meanCTT2} = -0.81$, $p = 0.42$)), or in the overall CT change between T1 and T2 (Δ(T2-T1): $t(62) = 0.89$, $p = 0.38$). However, we observed a significant between-group difference in the total brain volume (T1: $t(66)_{CVT1} = -2.6$, $p < 0.05$; T2: $t(67)_{CVT2} = -2.49$, $p < 0.05$; Δ(T2-T1): $t(68)_{\Delta CV} = -0.2$, $p = 0.85$) and total surface area (SA) at both timepoints (T1: $t(66)_{SAT1}$= -2.33, $p < 0.05$; T2: $t(66)_{SAT2} = -2.28$, $p < 0.05$; Δ(T2-T1): $t(68)_{\Delta SA} = -0.16$, $p = 0.88$). There was no significant difference in the total ΔCV or total ΔSA over time. For further detailed statistical details, see Table 1.

Intra-Individual Differences in RBS-R Total Severity Scores over Time

The majority of ASD individuals (60.1%) showed a decrease in total RBS-R scores between T1 and T2 (maximum decrease [maxD] = −47), 33.3% had higher RBS-R scores at T2 (maximum increase [maxI] = +35), and 6% did not change between T1 and T2 (ΔRBS-$R_{total\ severity}$(T2-T1) = 0). In contrast, most TD controls (57%) showed no change in RBS-R over time. Here, 21.6% had a decrease (maxD = −8), while an increase in the RBS-R total score was observed in 21.2% (maxI= +8; see Supplementary Table S2).

3.2. Between-Group Differences in CT_{spc}

In both groups, we observed widespread cortical thinning across the cortex with increasing age (i.e., between T1 and T2). However, individuals with ASD showed reduced (i.e., decelerated) cortical thinning relative to TD controls, particularly in the fronto-cingulate and temporal regions. More specifically, individuals with ASD showed significant reductions in cortical thinning in the bilateral superior frontal gyrus (approximate Brodmann areas [BA] 4/6/8/10), the rostral middle frontal gyrus (BA 9/46), the right medial orbital frontal cortex (BA 11/32), the rostral anterior cingulate cortex (BA 24/33), the left pars orbitalis (BA 47), the left inferior temporal (BA 20), the middle temporal gyrus (BA 21), and the fusiform and parahippocampal gyrus (BA 17–19/37). An increased cortical thinning in individuals with ASD as compared to TD controls was observed in the left insula exclusively (BA 13) (see Figure 2a,b, Figure 3a,b and Table 2).

Table 2. Clusters with significant between-group differences in the estimated developmental change in cortical thickness (CT_{spc}) from T1 to T2.

Contrast	Cluster	Region Labels	Hemisphere	BA	Vertices	Talairach			t_{max}	$p_{cluster}$
						x	y	z		
ASD > Control										
	1	Superior frontal gyrus, medial orbital frontal cortex, rostral anterior cingulate cortex	R	4, 6, 8, 10, 11, 24, 32, 33	2358	10	50	11	5.07	9.59×10^{-5}
	2	Lateral orbital frontal cortex, rostral middle frontal gyrus	R	9–11, 45–47	1716	25	44	−10	3.99	8.27×10^{-4}
	3	Superior frontal gyrus	L	4, 6, 8, 10	1662	−16	37	41	3.13	9.83×10^{-4}
	4	Fusiform gyrus, parahippocampal gyrus, inferior temporal gyrus	L	20, 28, 34–37	1374	−23	−24	16	2.94	4.83×10^{-3}
	5	Lateral orbital frontal cortex, rostral middle frontal gyrus, pars orbitalis	L	6, 8–11, 45–47	966	−22	43	−12	3.32	1.87×10^{-2}
	6	Middle temporal gyrus	L	21	1318	−48	−13	−14	3.01	2.18×10^{-2}
ASD < Control										
	7	Insula	L	13	580	−35	−4	−6	−1.67	3.84×10^{-2}

Note: Hemisphere: L: Left, R: Right; BA: approximate Brodmann area(s); ASD: Autism Spectrum Disorder; Vertices: number of vertices within the cluster; t_{max}: maximum t-statistic within the cluster; p-cluster: cluster-corrected p value.

3.3. Brain–Behavioural Correlations

To examine the association between cortical thinning and variability and measures of symptom severity over time, we correlated the mean CT_{spc} of individuals from clusters with significant between-group differences, with the severity of autism symptoms at T1 (i.e., total and subdomain scores of the ADOS, ADI-R, and RBS-R; see Supplementary Figure S2). We observed a significant negative correlation between CT_{spc} in the right

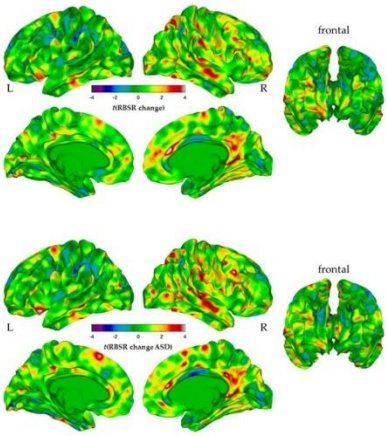
lateral orbital frontal cortex and right rostral middle frontal gyrus (cluster 2 in Table 2) and a change in self-injurious behavior as measured by the RBS-R. We also observed a significant negative correlation between CT_{spc} and a change in stereotypic behavior in the left middle temporal gyrus (cluster 6 in Table 2). Hence, a reduction in cortical thinning was associated with stable or worsening symptom severity from T1 to T2. However, none of these correlations remained significant following FDR correction for multiple comparisons; hence, our findings should be interpreted as preliminary.

3.4. Association between a Longitudinal Change in CT and a Change in Repetitive Behaviors

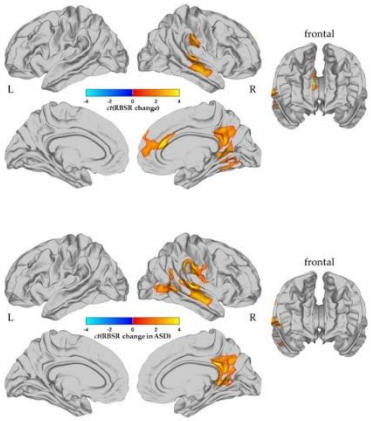
When examining the main effect of RBS-R_{Δ} in the entire sample (i.e., individuals with ASD and TD controls), we identified six clusters where a change in RBS-R was significantly associated with a change in CT (RFT-based cluster correction, $p < 0.05$, 2-tailed). These clusters included the right superior frontal gyrus (BA 4/6/8/10), the caudal anterior cingulate cortex (BA 24/33), the supramarginal gyrus (BA 40), the precuneus cortex (BA 7/31), the isthmus cingulate cortex (BA 29/30), the superior temporal gyrus (BA 22/42), the middle temporal gyrus (BA 21), as well as the lingual and fusiform gyri (BA 17/18/19/37) (see Supplementary Table S3). In these brain regions, cortical thinning between T1 and T2 was associated with less severe RBS-R symptoms over time (Supplementary Figure S4). These associations did not differ significantly by group, i.e., including an RBS-R change-by-group interaction term did not significantly improve the model fit (see Supplementary Figure S3). Because the main effect of RBS-R change on CT_{spc} was mainly driven by variability within ASD individuals, we also repeated the analysis within the ASD group. Here, a reduction in RBS-R was related to a decrease in CT in three significant clusters, including the right superior temporal gyrus (BA 22/42), the middle temporal gyrus (BA 21), the inferior parietal cortex (BA 39), the banks superior temporal sulcus (BA 22/42), the lateral occipital cortex (BA 17–19), the transverse temporal cortex (BA 41), the precuneus cortex (BA 7/31), the isthmus cingulate cortex (BA 29/30), the supramarginal gyrus (BA 40), the postcentral gyrus (BA 1/2/3), and the right insula (BA 13) (see Figure 4a,b).

3.5. Gene Set Enrichment Analyses

To link the observed differences in the CT trajectory in ASD to the potential genetic underpinnings, we performed a gene expression decoding analysis of our main output maps. The t-map of between-group differences in CT_{spc} (see Figure 1a) was significantly correlated with the pattern of expression of N = 2589 genes (nominal $p < 0.05$). Within this gene set, we found an enrichment for ASD candidate genes, and for genes that are differentially expressed during childhood and adolescence in ASD (see Figure 5a). More specifically, the t-map showed an enrichment for gene coexpression modules that are known to be downregulated in ASD, namely ASD.DEG.down [43], CTX.down.M4 [42], CTX.down.M10 [42], and CTX.down.M16 [42]. According to the international data base of the bioinformatics initiative, 'Gene Ontology' (GO), these coexpression modules represent genes involved in receptor signaling (ASD.DEG.down; [43]) and synaptic transmission (CTX.M4, CTX.M10, CTX.M16; [42]) [49,50]. We found no enrichment of the expression modules known to be upregulated in ASD. Furthermore, we observed an enrichment for ASD risk genes known to affect synaptic signaling, both in excitatory and inhibitory neurons [44] (see Figure 5a). We also performed a cell-type enrichment analysis for the t-map of between-group differences in CT_{spc}. We observed a significant enrichment for genes that are dysregulated in excitatory neurons in cortical layers L2/L3 and in L4 synaptic function and transcription factors [45] (see Figure 5b). The odds ratios for all modules and adjusted p values are displayed in Figure 5.



(**a**)



(**b**)

Figure 4. Main effect of a change in restricted and repetitive behaviors (RRBs) on vertex-wise differences in the change in Cortical Thickness (CT_{spc}). A significant main effect of developmental changes in the total severity in RRBs was quantified as the difference in the total severity on the RBS-R between timepoint 1 and timepoint 2 $\Delta RBSR_{total\ severity}$(T2-T1)], on vertex-wise differences in the developmental change in CT (CT_{spc}) across the total sample [upper figures in (**a**) and (**b**)], and only within individuals with ASD [bottom figures in (**a**) and (**b**)]. The unthresholded t-maps are displayed in (**a**) and the random field theory (RFT)-based, cluster corrected ($p < 0.05$, 2-tailed) difference maps, determined following multiple comparisons are in (**b**). Associations between increases in the RBS-R severity from T1 to T2 with vertex-wise changes in CT were marked in yellow to red (left panel), respectively, and in red to yellow (right panel), and associations between decreases in RBS-R severity from T1 to T2 with vertex-wise changes in CT were marked in cyan to purple (left panel), respectively, and in blue to cyan (right panel). Abbreviations: L: left hemisphere, R: right hemisphere. The green to red color scale indicates regions with an increased CT in the ASD group relative to the control group, while the blue to violet color scale indicates vertices with a decreased CT in the ASD group relative to the control group.

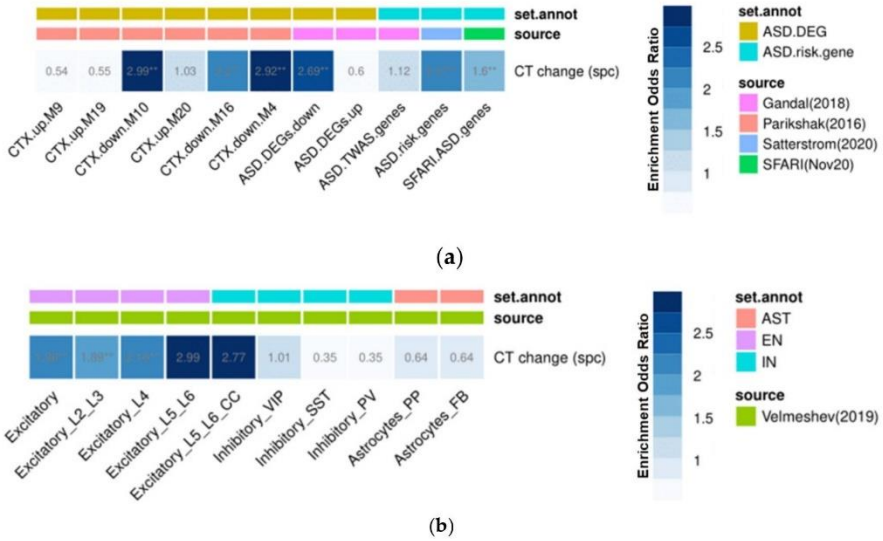


Figure 5. Genomic and cellular underpinnings of neurodevelopmental deviations in the change in Cortical Thickness (CT_{spc}) in ASD. The enrichment analysis of genomic underpinnings of the symmetrized percent change in CT (CT_{spc}) was based on the *t*-map of statistical between-group differences in the developmental change in CT during adolescence in individuals with autism spectrum disorder (ASD), relative to neurotypical controls (Figure 2a) and various gene sets. (**a**) The significant odds-ratios (OR) at an FDR-rate *p* value of < 0.05 resulting for genes expressed in the *t*-map. Gene sets were subdivided into sets with differential gene expression in ASD (ASD.DEG) and into sets containing ASD risk genes (ASD.risk.gene). Gene set annotation (set.annot) and labeling were determined by their original publication [42–45]. Abbreviations: up: upregulated expression in ASD, down: downregulated expression in ASD, CTX: cortex, DEG: differential gene expression, **: significant odds-ratios at an FDR-rate *p* value of < 0.05. (**b**) The set with an enrichment of genes particularly expressed in the upper layer cortex neurons and crucial for brain development, as reported by Velmeshev et al. (2019). Annotations were defined by the three cell types: astrocytes (AST), excitatory neurons (EN), and inhibitory neurons (IN). Abbreviations: L2–6: cortical layers, CC: cortico-cortical, SST: somatostatin, PV: parvalbumin, PP: protoplastic, FB: fibrous.

We also tested for an enrichment of gene sets by examining the main effect of RBS-R change in ASD (see Figure 3a), which revealed a set of N = 711 significant genes. However, this gene set was not significantly enriched for genes that have previously been associated with RBS by Tao et al. (2016; [46]; see Supplementary Figure S6).

4. Discussion

The aim of this study was to compare the longitudinal neurodevelopmental trajectories of CT in adolescents with ASD to those in TD controls, and to examine their association with intra-individual variation in repetitive behaviors. Moreover, to move towards bridging the gap between macroscopic and microscopic pathology, we examined the spatial patterns of between-group differences in CT for enrichment in (i) genes known to be associated with ASD, and (ii) genes that have previously been linked to repetitive and stereotyped behavior. We identified significant between-group differences in CT_{spc}, particularly in frontotemporal regions and the cingulate cortex. These brain regions were also enriched for ASD risk genes, and gene expression modules that are known to be downregulated in the cortex of ASD individuals. Additionally, we observed a significant enrichment for genes underpinning cell transmission processes in specific cell types, particularly in the excitatory neurons of the L2, L3 and L4 cortical layers [45]. Taken together, our findings suggest that differences in the development of CT in ASD are associated with genetic variations in the genes known to be implicated in this condition. Moreover, our study revealed an

association between developmental changes in CT (in fronto-temporal, fronto-parietal, and cingulate regions) and intra-individual changes in the severity of repetitive behaviors across adolescence in ASD individuals. Hence, our study extends previous reports of atypical brain development in ASD and links these developmental differences to specific autism symptoms.

In contrast to previous studies examining CT development in ASD [13,14,27,51], we observed a reduced cortical thinning in the ASD group compared to the TD controls. This is line with the fact that, depending on the examined age group, in ASD the existing structural neuroimaging studies of regional developmental differences in CT in cross-sectional and longitudinal samples remain highly inconsistent. Further longitudinal studies are hence required to replicate, validate, and add onto these earlier findings. While some studies report an increased (i.e., accelerated) thinning, others report decreased (i.e., slowed down) thinning of the cortex in ASD over time [2]. More specifically, during childhood and adolescence, cross-sectional studies mostly reported enhanced thinning in ASD relative to the neurotypical trajectory [11,15,26], although the importance of subdividing the neurodevelopmental trajectory into different developmental stages has also been noted. For example, one prior longitudinal study classified individuals into distinct age groups [5]. Here, the period of early childhood was marked by cortical thickening, followed by accelerated thinning in adolescence, and decelerated thinning in early adulthood. Hence, the CT growth curves of ASD and TD controls intersected between childhood and adolescence (10–20 years), during which time no significant differences were observed. Based on these findings, CT differences in the age range we examined in our sample (i.e., 11–18 years) could be expected to 'pseudonormalize' in ASD, i.e., few or no differences between groups might be observed when examining between-group differences at T1 and/or T2 [5]. Nonetheless, we observed significant differences in CT development between these times points; i.e., we observed one cluster with an accelerated decrease in CT (the left insula), and several clusters with decelerated cortical thinning in ASD (e.g., in the right and left fronto-temporal regions and the right cingulate cortex). Such discrepancies with other studies might be partially explained by differences in the sample characteristics and analytical approaches. For example, in comparison to Zielinski et al. (2014), the age range examined in our study was narrower (11–18 years vs. 3–36 years, respectively), and we also included female participants [5].

Previous neuroimaging studies examining brain development during childhood and adolescence have characterized the developmental trajectory of brain maturation as an 'inverted U-shape' that reaches its maximum during childhood/early adolescence (e.g., based on measures of cortical volume and the grey-white matter tissue contrast) [25,52]. The same curve characteristics have been reported for the developmental trajectory of CT, albeit with earlier peaks (males = 8.6 years, females = 8.4 years), followed by cortical thinning [53]. Consequently, cortical thinning during late childhood/early adolescence is commensurate with maturational processes during this stage of development, so the reduced cortical thinning we observed in our sample might suggest a less rapid or stagnant maturation in ASD. On the cellular level, cortical thinning has also been related to experience-dependent (i.e., learning dependent) synaptic pruning. For example, it is known from histological studies, that the synaptic density in the middle frontal gyrus, where we found less cortical thinning in ASD, shows a postnatal increase in density until the age of around 7 years, followed by a period of synaptic pruning until early adulthood [54]. Synapse elimination is important for the development of complex neural systems, and is paralleled by cortical thinning during brain development [55,56]. Our findings indicate that the cortex in ASD might mature more slowly in specific regions of the brain. Decelerated cortical thinning in ASD during adolescence has also been reported in a study by Raznahan et al. (2010), particularly in the left middle temporal and right superior frontal gyrus [57]. In sum, the macroscopic differences we observe in the brain in ASD might point towards specific underlying mechanisms, such as selective synaptic elimination and the arborization of dendrites and axons, which may be dysregulated in ASD.

Atypical brain development in ASD has previously been related to the severity of ASD-related symptoms and behaviors [8]. However, most studies to date have focused on the relationship between atypical cortex development and social impairments in ASD, commonly measured by ADOS and SRS [58,59]. Meanwhile, investigations of the relationship between neuroanatomy and repetitive/restricted behaviors (RRBs) in ASD remain under-represented in the literature. In our study, we addressed this issue by adding a second analysis stream, where we examined the impact of developmental changes in CT on the severity of RRBs, which are also linked to sensory symptoms that are emerging as promising candidates for parsing heterogeneity in autism [23]. On the behavioral level, RRBs can be divided into 'repetitive sensory motor' (lower-level) and 'insistence on sameness' (higher-level) behaviors. Stereotyped movements and the repetitive use of specific objects define the former, and ritualistic habits and insistence on well-established routines the latter [60]. Lower-level RRBs (including self-injurious behavior) occur more frequently in younger children with ASD, and in those with lower levels of intelligence [61,62]. This was also observed in our sample, where ASD individuals showed minimal self-injurious behavior (see Supplementary Figure S1) [21,63]. Moreover, in agreement with other studies, we found that in the severity of RRB symptoms, there was a decrease in the total severity score between the two examined timepoints in ASD, which is common within this age range [21,63]. Several studies have reported significant correlations between the severity of repetitive behaviors and measures of brain anatomy in ASD. To date, such brain–behavior correlations have mainly been observed in subcortical structures (caudate nucleus and basal ganglia), connecting to the frontal cortical regions and forming a "fronto-striatal circuit" [17,64]. More specifically, an increased volume of the left caudate nucleus was associated with self-injurious behavior in boys with ASD, while compulsive and ritualistic behaviors showed significant positive correlations with bilateral caudate nuclei volumes [64]. Additionally, an increased functional connectivity between the left nucleus accumbens and a cluster in the right premotor cortex/middle frontal gyrus was related to more severe symptoms of repetitive behavior in children and adolescents with ASD [65]. Here, we report there is also an association between intra-individual variations in RRBs and changes in CT also in cortical regions that include the postcentral, parietal, frontal, and temporal regions in ASD. Our findings are thus in agreement with previous results in suggesting a crucial role of the fronto-striatal neurocircuitry in mediating RRB symptoms across development. We further extend these findings by reporting a correlation between the cortical aspect of this circuitry, indicating the importance of dysfunctional cortico-striatal connectivity due to atypical changes in cortical thickness in adolescents with ASD.

Last, we aimed to link the macroscopic differences we observed at the neuroanatomical level to potential genomic mechanisms that have previously been linked to ASD. So far, few studies have examined the genetic and molecular mechanisms underpinning CT differences in ASD. A recent study by Romero-Garcia et al. (2019) demonstrated that differences in cortical thickness (CT) during childhood were robustly associated with genes involved in synaptic transmission pathways, which are known to be downregulated in the postmortem ASD cortex [42,66]. Similar results were also recently reported by our group when examining CT differences in ASD in a large and clinically heterogeneous sample [67]. The present results converge with these previous findings, as we observed an enrichment for genes and coexpressed modules that are known to be downregulated in ASD. ASD downregulated modules M16 and M10 are known to be involved in neuronal activity and synaptic function [42], which may contribute to deficits in synaptic-pruning and reduced cortical thinning [68]. Moreover, many ASD risk genes are likely to affect the maturation of excitatory and inhibitory neuronal pathways [44]. Our neuroimaging findings are also in line with genetic studies reporting a dysregulation of genes involved in the synaptic transmission of excitatory neurons across cortical layers (L2, L3, L4; [45]), and substantiate prior reports of an impaired excitatory–inhibitory balance underlying autism phenotypes [69]. We found no significant enrichment for genes previously linked to

repetitive behaviors in ASD [46]. However, the RRB gene lists provided by Tao et al. (2016) are relatively small, and unspecific with regard to their functional involvement [46], and their functional role in brain development remains to be established. Overall, however, our finding of an enrichment for ASD-related gene sets adds to the biological plausibility of our neuroimaging findings, and links atypical CT development to specific etiological mechanisms in ASD.

Taken together, these three data modalities (CT change in ASD, RRB change in ASD, gene enrichment in ASD) have predominantly been studied in isolation. Our study is therefore among the first to use a longitudinal design to associate CT trajectories in adolescents with ASD to their development of RRBs, and to add specific genetic, and more precisely transcriptomic underpinning.

The present study needs to be interpreted in the light of several limitations. First, our strict exclusion criteria and MRI quality assessment resulted in a comparatively small sample size. Larger studies of longitudinal samples are therefore needed to replicate our findings. Moreover, although RRBs are a common clinical feature of ASD, they are not unique to this condition [70]. Future studies linking developmental changes in repetitive behaviors to intra-individual variability in brain structure, using a transdiagnostic approach, are therefore needed to establish whether the observed brain–behavioral correlations are specific to ASD or generalized across mental health conditions. Moreover, here we focused on cortical regions. However, previous studies have also linked RRBs to subcortical regions, including the striatum [71,72]. Therefore, additional neuroimaging studies should extend the set of brain regions when characterizing the neuroanatomy underpinning RRBs. Last, the AHBA currently provides the most extensive source of anatomic and genomic information. However, it is based on adult donors. While we examined some adult participants (up to 18 years at T1 and up to 21 at T2), the AHBA does not fully cover our age range (11–21 years). We thus acknowledge the importance of repeating our analyses using gene expression data from young adults, once these become available.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/genes12122024/s1, Figure S1: Distribution of longitudinal changes in the RBS-R subdomain and total severity across groups, Figure S2: Correlation between symptom severity domains and group-differences in the symmetrized percent change in cortical thickness (CT_{spc}), Figure S3: Interaction effect of longitudinal RBS-R changes-by-group on longitudinal changes in cortical thickness, Figure S4: Correlation between the symmetrized percent change in CT (CTspc) in ASD and the change in restricted and repetitive behavior (RRB), Figure S5: Significant between-group differences in cortical thickness estimates in scans that did not vs. did undergo the longitudinal stream in FreeSurfer, Figure S6: Genomic underpinnings of the main effect of RBS-R, Table S1: Overview of exclusion reasons, Table S2: Intra-individual differences in RBS-R total severity scores over time, Table S3: Main effect of a longitudinal change in RBS-R total severity on a longitudinal change in measures of cortical thickness. Supplementary Method 1: Sample description, including in- and exclusion criteria, Supplementary Method 2: Image processing and Quality Assessment, Supplementary Method 3: Model fitting using nested model comparison, Supplementary Method 4: Gene decoding analysis (GEDA), Supplementary Method 5: Enrichment analysis.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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6 Darstellung des eigenen Anteils an der Publikation

Die Analysen der Publikation „Longitudinal changes in cortical thickness in adolescents with autism spectrum disorder and their association with restricted and repetitive behaviors“ sind im Rahmen der longitudinalen Studie „BrainMap“ erfolgt, die die Gehirnentwicklung bei Personen mit und ohne ASS während der Adoleszenz an der Kinder- und Jugendpsychiatrie in Frankfurt untersucht (DFG-finanziertes Projekt, Projekt Nr. 271513085).
Die Publikation ist in gemeinschaftlicher Arbeit unter der Leitung von Frau Prof. Dr. Christine Ecker ausgearbeitet worden. Mein Eigenanteil setzt sich wie folgt zusammen:

In Absprache mit meinen Kolleg*innen habe ich folgende Tätigkeit in kompletter Eigenleistung durchgeführt: Editierung und Nachbessern der rekonstruierten kortikalen Oberfläche aller T1 gewichteten MRT Aufnahmen, sowie das Matching der Stichproben. Zudem habe ich die statistischen und genetischen Analysen in Matlab und R durchgeführt, Visualisierungen erstellt und den Originalentwurf der Publikation verfasst.

In überwiegender Eigenarbeit mit Unterstützung durch Frau Dr. Anke Bletsch und Frau Dr. Caroline Mann ist die Ausarbeitung der statistischen Modelle erfolgt. Bei der technischen Durchführung, sowie bei der Erstellung der Rechenskripte für R und Matlab bin ich von Herrn Dr. Tim Schäfer unterstützt worden. Die Vorbereitung der genetischen Analysen erfolgten mit Unterstützung durch Frau Prof. Dr. Christine Ecker und Frau Dr. Anke Bletsch.

Die Korrekturen und die Revisionen des Manuskriptes für die Publikation sind in enger Zusammenarbeit mit Frau Prof. Christine Ecker und Frau Dr. Caroline Mann erfolgt.

Eine genaue Auflistung der Beiträge der einzelnen Autoren lässt sich zudem in der Publikation nachlesen.

7 Literaturverzeichnis

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8 Anhang

8.1. Supplement der Publikation

Supplementary Methods

Sample description, including in- and exclusion criteria

We included children and adolescents aged between 11 and 18 years at timepoint one (T1) with a current clinical diagnosis of autism spectrum disorder (ASD) based on DSM-5 or ICD10 criteria [1, 2]. All individuals with ASD met diagnostic cutoffs in the reciprocal social interaction (cutoff = 10), communication (cutoff = 8), and repetitive behaviors domain (cutoff = 3) of the Autism Diagnostic Interview-Revised (ADI-R) [3, 4]. The Autism Diagnostic Observation Schedule (ADOS-2) [5, 6] was conducted in all participants with ASD at their first assessment (T1), but only in 21 subjects at their second appointment (T2) and was not used as inclusion criteria. Depending on the subjects' age, different modules of the ADOS (i.e. module
3 and module 4) were applied between individuals and within individuals across timepoints.
To allow comparability of ADOS total severity and subdomain scores, ADOS Calibrated Severity Scores (CSS) were calculated [7]. To estimate overall intellectual ability, the Wechsler Abbreviated Scale of Intelligence (WASI-I) [8] was conducted at T1 as a short version of the Wechsler Intelligence Scales for Children (WISC-IV) [9] or Wechsler Adult Intelligence Scale (WAIS-IV) [10], providing indices of verbal, non-verbal, and full-scale IQ (FSIQ). We excluded participants with FSIQ < 70. As an exception, one female in the ASD group with FSIQ = 67 was included, to ensure groups were as gender-balanced as possible.

Further, this study contained the following exclusion criteria for all participants: contraindications to MRI, a history of major psychiatric or developmental disorder (e.g. psychosis), head injury, genetic disorders associated with ASD (e.g. fragile-X syndrome, tuberous sclerosis), as well as any medical condition affecting brain morphometry and function. In this study we included one participant with epilepsy. However, abnormalities in brain anatomy that may cause this disease or vice versa can be differentiated from those who can be related to behavioral patterns of ASD, due to the longitudinal study design. Furthermore, we excluded participants with a history of

any drug abuse (including alcohol). Given the high number of individuals with ASD taking regular medication [11–13], participants on stable medication were, however, included.

Image processing and Quality Assessment

Image processing and cortical reconstruction were initially performed for N= 210 MRI scans (i.e. scans from n=105 subjects at timepoint 1 (T1) and timepoint 2 (T2), respectively) from an ongoing longitudinal study following the longitudinal stream in FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalTwoStageModel). Here, models of the cortical surface are created for each T1-weighted image, i.e. one at T1 and one at T2 for each subject. These images are then used to create an unbiased within-subject template (i.e. base) that represents the average anatomy of each participant across time. The longitudinally processed cross-sectional images are subsequently derived by aligning the subjects' crosssectional scans at each timepoint with the base. Stringent and standardized quality assessments were performed for all scans, i.e. five scans per subject (cross_T1, cross_T2, base, long_T1, long_T2) to ensure maximum data quality. Initially, all scans were visually examined by three independent raters, who could either (0) reject 'as is' (n= 12 or 2.29%), mostly due to severe (motion) artefacts and/or the existence of extra-brain tissue that precluded a successful FreeSurfer reconstruction, (1) accept a reconstruction 'as is' (n= 457 or 87.05%), or (2) prescribe manual editing (n= 56 or 10.67%) in case of smaller (i.e. 'local') reconstruction errors. We performed edits to the pial surface in case of interference of parts of the dura mater, skull or blood vessels with surface formation, which were erroneously identified as grey matter (gm). Segmentation errors of the white matter (wm) required edits to the white surface. Gm edits where performed in the longitudinally processed cross-sectional data (i.e. long_T1 and long_T2 scans), while wm edits were performed in the base template. After manual edits were performed, the images were re-preprocessed and visually re-assessed, to assure that editing improved the data quality. All 45 manually edited scans benefited from the process and were therefore included in the statistical analysis. However, other individuals were excluded from the statistical analysis due to the onset of a psychiatric disease after T1 in TD controls, or to achieve a gender-balanced design across groups (see Supplementary

Table S1). Hereby we excluded 12 female controls to receive an approximate gender ratio of ~ 4:1 in both samples. As the focus of the study was on repetitive behaviors, we further excluded participants with missing RBS-R data. The final data set thus contained usable MRI data of n= 70 participants.

Model fitting using nested model comparison

The employed general linear model (GLM) was selected after we initially calculated vertexwise nested model comparisons (i.e. F-tests) to examine the goodness-of-fit of distinct model versions in the total sample, i.e. individuals with ASD and TD controls. The identification of the most parsimonious model was guaranteed by the use of a step-up model selection procedure. In this stepwise approach, a reduced model is compared to a more complex model with results indicating whether the addition of a new model term significantly increased the goodness-of-fit at each vertex. While the inclusion of the quadratic age term yielded an improved goodness-of-fit relative to the basic (i.e. reduced) model, all other examined variables, namely AgeT1-by-group and $AgeT1^2$-by-group, did not increase the goodness-of-fit.

Gene decoding analysis

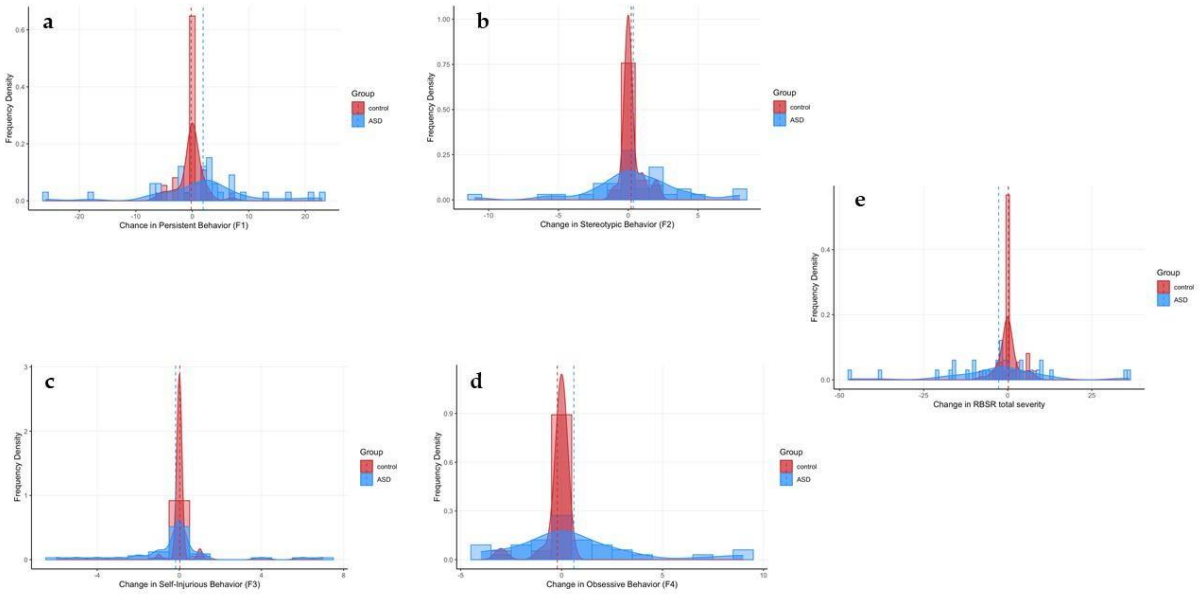
In order to decode the unthresholded *t*-maps of the main effect of group (Fig. 1a) and the main effect of change in RBS-R total severity from T1 to T2 (ΔRBS-R$_{(T2-T1)}$) in individuals with ASD on measures of spc (Figure 3b), derived from Matlab, we initially uploaded them to the NeuroVault server (https://neurovault.org). The gene decoding analysis (GEDA) was subsequently performed in R using code embedded in NeuroVault and Neurosynth (https://neurosynth.org). All 20,787 protein coding genes from the Allen Human Brain Atlas (AHBA; [14]) were tested for spatial gene expression that resembles our neuroimaging difference maps [15]. More specifically, the analysis constructs a linear model for each of the six donor brains from the AHBA, where the slopes encode how similar each gene's spatial expression pattern is to the input imaging map. In line with the input maps, these analyses are restricted to cortical tissue. The slopes are then subjected to a one-sample *t*-test to identify genes whose spatial expression patterns consistently, i.e. across the donor brains, show high resemblance to the imaging maps.

Enrichment analysis

To elucidate the biological function of the genes derived from the GEDA and their relevance in ASD, a gene enrichment analysis was computed using the GeneOverlap package in R (10.18129/B9.bioc.GeneOverlap). Here, we tested our GEDA gene-list, derived from the *t*-map displaying between-group differences (thresholded at $p < 0.05$), for enrichment with various gene-sets associated with ASD at genetic and transcriptomic level. For enrichment testing for genetic associations with ASD, we used the 102 rare and de novo protein truncating variants identified in the largest exome sequencing study of ASD to date [16]. We also included an ASD-related gene list compiled by SFARI (SFARI.ASD.genes; categories S, 1, 2, & 3 downloaded in November 2020 from https://gene.sfari.org/). A list of differentially expressed genes (DEGs) (up-/downregulated) in post mortem tissue was further utilized to test for gene enrichment at the transcriptomic level [17], as well as for genes that are differentially expressed in specific cell types in ASD (astrocytes, excitatory and inhibitory neurons; [18]). Moreover, we included genes from differentially expressed co-expression modules in ASD that map onto specific biological processes, i.e. synaptic transmission [19].
For enrichment analysis of the gene list we derived by the GEDA of the *t*-map with the main effect of RBSR change in ASD (thresholded at $p < 0.05$) we employed a gene set that has been linked to Repetitive Sensory Motor behavior (RSM) and Insistence on Sameness (IS) in a genome-wide association study (GWAS; [20]). The study identified a significant association between RSM and IS with various Single Nucleotid Polymorphisms (SNPs), which are part of gene locus 8p21.2-8p21.1.

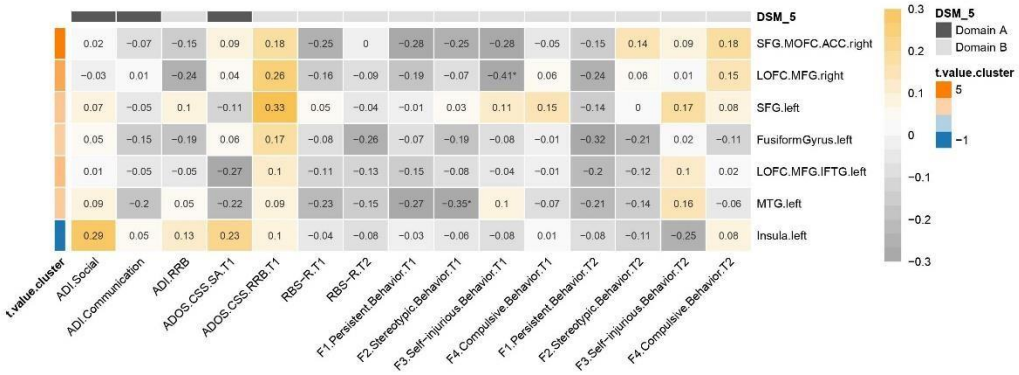
Supplementary Figures

Supplementary Figure S1. Distribution of longitudinal changes in RBS-R subdomain and total severity across groups



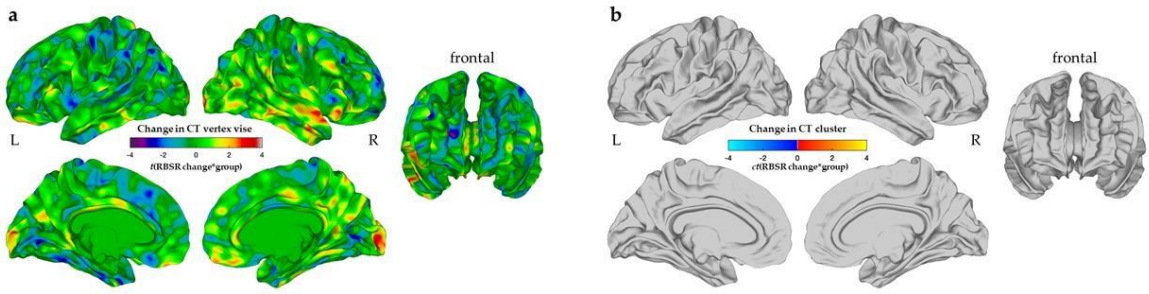
Histograms displaying the distribution of longitudinal changes in subscale (i.e. persistent (A), stereotyped (B), obsessive (C), and self-injurious (D) behavior) and total severity raw scores in the German version of the Repetitive Behavior Scale-Revised (RBS-R) in individuals with autism spectrum disorder (ASD; blue) and typically developing controls (red).

Supplementary Figure S2. Correlation between symptom severity domains and group-differences in symmetrized percent change (spc) of CT



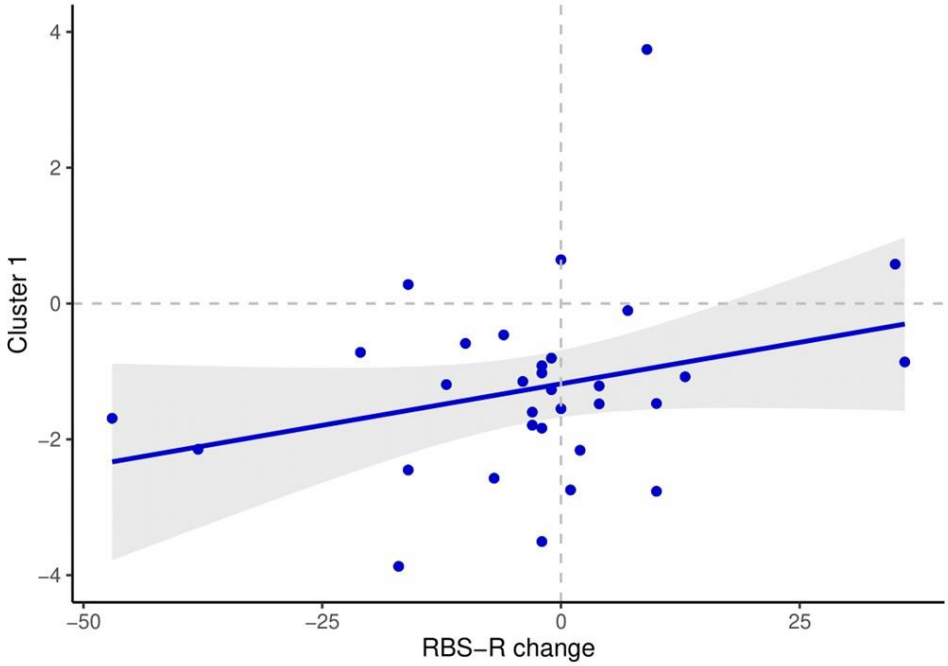
Brain-behaviour correlations between clusters with significant differences in symmetrized percent change of cortical thickness (CT_{spc}) and measures of symptom severity subdivided into DSM-5 Domain A and B symptoms within the ASD group. We observed a significant negative correlation (*) between CTspc in the right lateral orbital frontal cortex and right rostral middle frontal gyrus and a change in self-injurious behavior as measured by the RBS-R. We also observed a significant negative correlation between CTspc and a change in stereotypic behavior in the left middle temporal gyrus. Right: right hemisphere, left: left hemisphere, SFG: superior frontal gyrus, MOFC: medial orbital frontal cortex, ACC: anterior cingulate cortex, LOFC: lateral orbital frontal cortex, MFG: middle frontal gyrus, IFTG: inferior temporal gyrus, MTG: middle temporal gyrus, tAIs: subject-level total neuroanatomical abnormality index, ADI: Autism Diagnostic Interview-Revised, RRB: repetitive/restricted behaviour, ADOS.SA/ADOS.RRB: Autism Diagnostic Observation Schedule Calibrated Severity Score for Social Affect (SA) and restricted and repetitive behaviours (RRB), SRS-2: Social Responsiveness Scale-2, RBS-R: Repetitive Behaviors Scale – Revised.

Supplementary Figure S3. Interaction effect of longitudinal RBS-R changes-by-group on longitudinal changes in cortical thickness



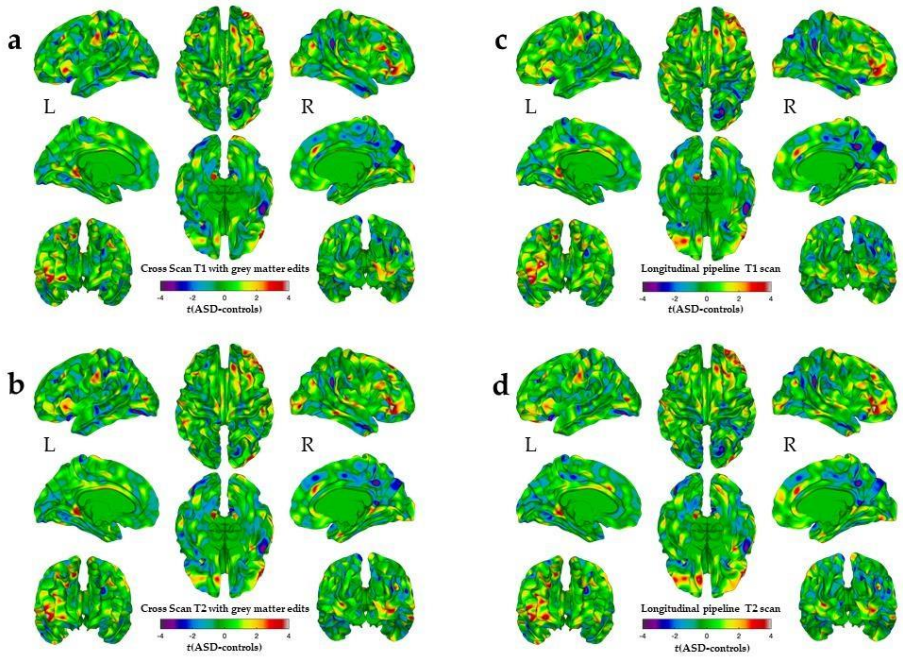
Interaction effect of longitudinal changes in total severity on the German version of the Repetitive Behavior Scale-Revised (RBS-R)-by-group (i.e. individuals with autism spectrum disorder vs. typically developing controls) on longitudinal changes in cortical thickness, quantified by the symmetrized percent change (spc; https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalTwoStageModel). Displayed are the un-thresholded *t*-maps (a) and the random field theory (RFT)-based cluster corrected ($p<0.05$, 2-tailed) difference maps following multiple comparisons (b). *Abbreviations:* L: left hemisphere, R: right hemisphere.

Supplementary Figure S4. Correlation between symmetrized percent change in CT (CT_{spc}) in ASD and change in restricted and repetitive behavior (RRB).



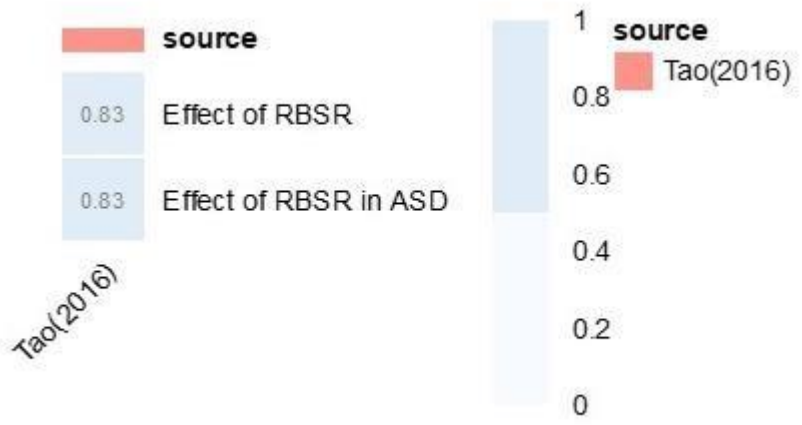
Correlation plot of CT_{spc} in significant Cluster 1 (y-axis; right superior temporal gyrus, middle temporal gyrus, inferior parietal cortex, banks superior temporal sulcus, lateral occipital cortex and transverse temporal cortex) and change in RRB (x-axis) measured by the Restricted and Repetitive Behavior Scale Revised (RBSR) total score. Negative CT_{spc} values indicating cortical thinning are most strongly correlated with decreasing RBSR total scores reflecting an improvement in RRB symptom severity.

Supplementary Figure S5. Significant between-group differences in cortical thickness estimates in scans that did not vs. did undergo the longitudinal stream in FreeSurfer



Vertex-wise between-group differences in cortical thickness (CT) estimates in individuals with autism spectrum disorder (ASD) relative to typically developing (TD) controls in crosssectionally processed MRI data at timepoint 1 (T1) (a) and timepoint 2 (T2) (b) compared to cross-sectional scans at T1 (c) and T2 (d) that were processed using longitudinal stream in FreeSurfer [21]. Displayed are the un-thresholded *t*-maps (a-d), where increased CT in individuals with ASD compared to TD controls is marked in yellow to red and reduced CT is marked in blue to cyan. *Abbreviations:* L: left hemisphere, R: right hemisphere.

Supplementary Figure S6. Genomic underpinnings of the main effect of RBSR



Genomic underpinnings of the effect of the Restricted and Repetitive Behavior Scale-Revised (RBSR) total score on symmetrized percent change in cortical thickness (CT_{spc}) based on the *t*map of (1) the main effect of RBSR in the total sample (n=70) and (2) in the ASD sample (n=33). For the enrichment analysis a gene list with gene expression patterns known to be associated with restricted and repetitive behavior of Tao et al. (2016) was used [20]. No Significant Oddsratios (OR) at an FDR-rate *p*-value <0.05 resulted for genes expressed in the *t*-maps.

Supplementary Tables

Supplementary Table ST1. Overview of exclusion reasons

	ASD	controls
N	10	25
Psychiatric diagnosis[1]	0	4
Premature birth	0	1
Missing RBS-R data	6	8
Abnormalities in brain anatomy[2]	2	0
Bad qualitiy of MRI scans[3]	2	0
Matching samples[4]	0	12

Note: [1]Comorbid diagnosis were allowed in individuals with autism spectrum disorder (ASD), e.g. Attention-deficit/hyperactivity disorder (ADHD), functional eneuresis, mild depressive episode, mixed obsessional thoughts and acts; [2]cysts; [3]motion artefacts; [4]exluding 12 female controls, to achieve genderbalanced sample; *Abbreviations:* RBS-R: Repetitive Behavior Scale-Revised; MRI: magnetic resonance imaging

Supplementary Table ST2. Intra-individual differences in RBS-R total severity scores over time

	Increase (RBSR total score) total				Decrease (RBSR total score)				Stable in RBSR
	Mean	**Standard deviation**	**%**	**MaxI**	**Mean**	**Standard deviation**	**%**	**MaxD**	**%**
ASD	+ 11	± 12	33.3	+ 35	- 11	± 12	60.1	- 47	6
controls	+ 4	± 2	21.2	+ 8	- 4	± 2	21.6	- 8	57

Note: Based on the total sample (n=70); [%] = percentage of individuals in the ASD or control group with an increase/decrease or stable values of RBSR-total score; [MaxI] = maximum increase in RBSR total score; [MaxD] = maximum decrease in RBSR total score; RBSR: Restricted and repetitive-Behavior-Scale-Revised

Supplementary Table ST3. Main effect of longitudinal change in RBS-R total severity on longitudinal change in measures of cortical thickness

Contrast	Cluster	Region Labels	Hemisphere	BA	Vertices	Talairach			t_{max}	$p_{cluster}$
						x	y	z		
ΔRBS-R$_{(T2-T1)}$										
1	precuneus cortex, isthmus-cingulate cortex		R	7, 26, 29-31	2335	9	-49	23	3.36	6.76 x 10^{-4}
2	supramarginal gyrus		R	40	2192	47	-17	19	3.79	6.78 x 10^{-4}
	3	superior frontal gyrus, caudal anterior-cingulate cortex	R	4, 6, 8, 10, 24, 33	1933	7	33	15	4.48	1.01 x 10^{-3}
	4	precuneus cortex, isthmuscingulate cortex	R	7, 26, 29-31	1465	21	-53	10	3.53	3.46 x 10^{-3}
	5	superior temporal gyrus, middle temporal gyrus	R	20-22, 42	2223	48	-17	-7	3.41	4.29 x 10^{-3}
	6	lingual gyrus, fusiform gyrus	R	17-19, 37	1039	21	-68	0	2.84	3.70 x 10^{-2}

Note: Based on the total sample (n= 70), i.e. individuals with autism spectrum disorder (ASD) and typically developing (TD) controls. Hemisphere: *L*: Left, *R*: Right; *BA*: approximate Brodmann area(s); Vertices: number of vertices within the cluster; t_{max}: maximum *t*-statistic within the cluster; *p*-cluster: cluster-corrected *p*-value.

Contrast	Cluster	Region Labels	Hemisphere	BA	Vertices	Talairach			t_{max}	$p_{cluster}$
						x	y	z		
ΔRBS-R$_{(T2-T1)}$										
	1	superior temporal gyrus, middle temporal gyrus, inferior parietal cortex, banks superior temporal sulcus, lateral occipital cortex, transverse temporal cortex	R	5, 7, 17-22, 41, 42	5948	65	-26	6	4.00	4.28 x 10^{-5}
	2	lateral orbital frontal cortex, precuneus cortex, isthmuscingulate cortexortex, rostral middle frontal gyrus	R	7, 11, 26, 29-31, 46	4283	6	-39	30	4.25	6.52 x 10^{-5}
	3	supramarginal gyrus, postcentral gyrus, insula	R	1-3, 13, 40	3391	43	-20	19	3.61	6.40 x 10^{-4}

Note: Based on individuals with autism spectrum disorder (ASD) only (n= 33). Hemisphere: *L*: Left, *R*: Right; *BA*: approximate Brodmann area(s); Vertices: number of vertices within the cluster; t_{max}: maximum *t*-statistic within the cluster; *p*-cluster: cluster-corrected *p*-value.

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