The effects of mosaicism on biological and clinical markers of Alzheimer's disease in adults with Down syndrome

Laura Xicota,[a] Lam-Ha T. Dang,[a,b] Alice Lee,[c] Sharon Krinsky-McHale,[d] Deborah Pang,[d] Lisa Melilli,[a] Sid O'Bryant,[e] Rachel L. Henson,[f] Charles Laymon,[g] Florence Lai,[h] H. Diana Rosas,[h] Beau Ances,[f] Ira Lott,[i] Christy Hom,[i] Bradley Christian,[j] Sigan Hartley,[j] Shahid Zaman,[k] Elizabeth Head,[l] Mark Mapstone,[i] Zhezhen Jin,[m] Wayne Silverman,[i] Nicole Schupf,[a,b] Benjamin Handen,[c] and Joseph H. Lee,[a,b,*] Alzheimer's Biomarker Consortium – Down Syndrome (ABC-DS)

[a]Sergievsky Center, Taub Institute, Department of Neurology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA
[b]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA
[c]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA
[d]Department of Psychology, NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA
[e]Institute of Translational Research, University of North Texas Health Sciences Center, Fort Worth, TX, USA
[f]Department of Neurology, Washington University in St. Louis, St. Louis, MO
[g]Departments of Radiology and Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA
[h]Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA
[i]Department of Neurology, UC Irvine, Irvine, CA, USA
[j]Waisman Center and Department of Human Development and Family Studies, University of Wisconsin–Madison, Madison, WI, USA
[k]Department of Psychiatry, University of Cambridge, Cambridge, UK
[l]Department of Pathology & Laboratory Medicine and Department of Neurology, UC Irvine, Irvine, CA, USA
[m]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY, USA

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Summary

Background Individuals with Down syndrome (DS) are at high risk of early-onset Alzheimer's disease (AD); yet, some 20 percent do not develop any signs of dementia until after 65 years or in their lifetime. Mosaicism could contribute to this phenotypic variation, where some disomic cells could lead to lower levels of gene products from chromosome 21.

Methods We examined longitudinal neuropsychological and biomarker data from two large studies of DS: the Alzheimer Biomarker Consortium–Down syndrome study (ABC-DS) (n = 357); and a legacy study (n = 468). We assessed mosaicism using karyotyping or GWAS data. Participants had data on plasma AD biomarkers ($A\beta_{40}$, $A\beta_{42}$, tau, and NfL) and longitudinal cognitive measures. A subset had cerebrospinal fluid biomarkers ($A\beta_{40}$, $A\beta_{42}$, tau, ptau181, and NfL) and amyloid and tau PET data.

Findings For both cohorts, the prevalence of mosaicism was <10% (ABC-DS: 7.3%; Legacy: 9.6%), and those with mosaicism had lower plasma $A\beta_{40}$ and $A\beta_{42}$ concentrations. For the older legacy cohort, when compared to those with full trisomy, those with mosaicism had significantly smaller decline in total and annualized neurocognitive scores, and lower incidence and prevalence of dementia.

Interpretation Mosaicism in DS was associated with lower concentrations of plasma Aβ peptides, possibly leading to lower AD risk. However, its clinical impact was less clear in the younger ABC-DC cohort, and a follow-up study is warranted.

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*Corresponding author. Sergievsky Center, P&S Unit 16, 630 West 168th Street, New York, NY, 10032, USA.
E-mail address: JHL2@cumc.columbia.edu (J.H. Lee).

Research in context

Evidence before this study

Adults with Down syndrome show evidence of neuropathology for Alzheimer's disease (AD) by their mid 40s, and the majority develop AD by their 60s, due to an extra copy of the *APP* gene as well as interactions with other genes genome-wide. Given the importance of gene dosage, it is necessary to examine the role of mosaicism on the phenotypic variation in individuals with Down syndrome. In support, studies have shown that mosaicism in DS is associated with lower severity of congenital heart disease and cognitive impairment. Moreover, we previously have shown that the rate of mosaicism starts increasing in their 40s when AD neuropathology is apparent in about one-half of the individuals with Down syndrome. Thus, an investigation of mosaicism on AD risk is strongly warranted to understand the complex relationship between gene dosage leading to an increased risk of AD by measuring endophenotypes that are sensitive to actual physiological changes.

Added value of this study

This study provides biological insight into our understanding of the natural history of neurodegenerative process in adults with Down syndrome, a group of high-risk individuals who are most likely to develop AD during their lifetime. This study provides molecular and clinical insight by examining the effects of gene dosage on protein levels in the blood. This study characterizes how mosaicism is associated with lower concentrations of amyloid plasma biomarkers and with slower cognitive decline and potentially lower risk of AD over time in two independent cohorts of adults with Down syndrome.

Implications of all the available evidence

This study identified mosaicism as a potential source of variation in AD risk in adults with Down syndrome by characterizing potential physiological alterations in blood- and image-based endophenotypes. Findings from the present study not only enhance our understanding of amyloid biomarkers in Down syndrome, but they may also provide insight into the role of amyloid biomarkers in the general population. Further research is needed to assess the impact of mosaicism at birth vs. age-acquired mosaicism.

Introduction

Down syndrome (DS) is the most common genetic cause of intellectual disability and is associated with multiple comorbidities, including the development of Alzheimer's disease (AD), typically before the age of 60 years.[1] In most cases, DS is caused by full trisomy of chromosome 21. However, approximately 1.3–5%[2,3] of individuals with DS are born with mosaicism, where some cells carry only two copies of chromosome 21, instead of three, potentially leading to a wide range of phenotypic variation from few signs of DS to full syndromic presentation.[2] However, the impact of mosaicism on ageing phenotypes in individuals with DS has not been well-studied. Some studies have shown that, when compared to individuals with full trisomy, individuals with mosaicism at birth had, on average, a lower risk of having congenital heart disease (CHD),[3] a higher IQ score,[3] and a longer lifespan.[4,5]

In contrast to these gene-dosage effects, Lai and Williams did not observe any differences in the age at onset, duration, or clinical features of dementia between individuals with mosaicism and full trisomy.[6] We note that two competing risks are working against one another. That is, with age, the risk of dementia is increased, but at the same time, the prevalence of mosaicism is increased,[7,8] which could lower the levels of gene expression of the genes on chromosome 21, including the *APP* gene.

Although the underlying mechanisms for the age-related changes in prevalence remain unclear, one may postulate that it may be the consequence of age-related chromosome loss in trisomic cells,[9] or it may be associated with better survival rates of individuals with mosaicism.[4,5]

The present study will examine the effects of mosaicism in a comprehensive manner from levels of AD associated protein biomarkers to neuropsychological performance to AD status in two independent DS cohorts with longitudinal assessments. Given the observed phenotypic variations in DS, it is critically important to characterize the molecular profile in these high-risk cohorts who will inevitably experience dementia-related symptoms. Considering the presence of multiple AD-related genes on chromosome 21, including *APP*, the characterization of genotype–phenotype relation is unlikely to be straightforward.

Methods

Cohorts

We studied two independent cohorts of adults with DS. The first cohort, the Alzheimer's Biomarker Consortium-Down Syndrome (ABC-DS)[10] study, assessed AD biomarkers in adults with DS (ages 25 and older) from multiple sites across the United States and from one site in the United Kingdom. All participants underwent neuropsychological testing and blood sampling (biomarker and genetic analyses). In addition, those who agreed underwent magnetic resonance imaging (MRI) and positron emission tomography (PET) for amyloid and tau, as well as lumbar puncture for collection of cerebrospinal fluid (CSF). Participants were followed for

two cycles with visits occurring approximately every 16 months.[10] The second cohort is a study on ageing and dementia in adults with DS (participants with ≥30 years of age with the mean of 51.3 at the baseline), henceforth named as "legacy" cohort, which was a single-site study of adults with DS recruited from voluntary and state service provider agencies in New York, New Jersey, Connecticut, and eastern Pennsylvania between 1987 and 2017 (PI: Silverman).[11–14] All "legacy" participants were followed, every 14–18 months on average, for 5 years, up to a maximum of 15 years. Information from clinical records, assessment of select cognitive functions, and informant interviews were obtained at each visit. Blood samples were also obtained from all participants; biomarker and genetic analyses were performed on a subset of participants. For both cohorts, we excluded participants, if they: (1) lacked both karyotyping and genotyping information; or (2) had translocations. Table 1 shows demographic and clinical information for both cohorts. Longitudinal analyses were performed only on those individuals that had more than one visit (see Supplementary Table S1 for number of participants at each visit for each cohort), for ABC-DS 72.8% had more than one visit, for the legacy cohort 95.9% had more than one visit. With respect to lost to follow up in this longitudinal study, the ABC-DS study experienced delays in recruitment and loss of participants due to the COVID-19 pandemic, as individuals with DS are an at-risk population. For the legacy cohort, 53% of loss at follow-up were due to death of the participant. No differences in age were observed between those who were lost to follow up and those who remained in the study. Male participants were more likely to be lost to follow-up when compared with female participants, thereby increasing the proportion of females with longer follow-up periods. Sensitivity analyses were performed to assess the effect of loss at follow-up, with the results showing effect sizes in the same direction for the cognitive longitudinal analyses.

	ABC-DS[a] (N = 357)	Legacy[a] (N = 468)	p-value
Age (baseline)			
Mean (SD)	**45.7 (9.91)**	**51.3 (7.1)**	**<0.0001**
Range	**25–81**	**30.3–78.1**	
Sex assigned at birth (% Female)	**46.2%**	**63.2%**	**<0.0001**
Intellectual impairment (% Mild or higher)	**51.8%**	**31%**	**<0.0001**
Self-reported race (% White)	95.5%	91.9%	0.29
APOE			
APOE2 allele frequency	7.99%	8.5%	0.83
APOE4 allele frequency	13.5%	11.6%	0.41
% mosaic (all sources)	7.3%	9.6%	0.24
% mosaic with 50% or more disomic cells	1.4%	0.85%	0.51
AD Status			
AD at baseline (%)	**12.3%**	**8.8%**	**0.02**
Converted to AD dementia in follow-up (%)	19.1%	22.9%	0.21

[a]Values are either means with standard variations and range for continuous variables, or percentages for categorical variables.

Table 1: **Demographic descriptions of both analyzed cohorts.**

Ethics

All recruitment, informed consent, and study procedures were approved by each site's institutional review board: AASU0596 (Columbia University IRB and Advarra), AAAS1560 (Columbia University IRB and WCG IRB), and NYPSI7348 (NYSPI).

Sample-size estimation

Based on the total sample size of 825 (357 from ABC-DS and 468 from the legacy study), this study has 80% power to detect an effect size between 2 groups of 0.349–0.428 for continuous traits and of 0.132 for categorical traits. Independently, ABC-DS has 80% power to detect an effect size of 0.575–0.61 for continuous traits (cognition and plasma biomarkers) and of 0.175 for discrete traits, while the legacy study has 0.441–0.602 and 0.153 for 80% power. Power calculations were performed after statistical analysis as the sample was dependent on already available data.

SNP genotyping data

When karyotyping was absent, we used a custom Illumina Infinium General Screening Array V2 (Illumina, San Diego, CA, USA) to assess level of mosaicism. This custom SNP microarray had additional disease markers at the Center for Applied Genomics at Children's Hospital of Philadelphia. Genotype calling was carried out using Illumina's GenomeStudio v2.0.5 with Genotyping module. A total of 759,993 variants were genotyped for 656 genomic DNA samples. The standard quality control protocol was applied. Genotyping data was used to confirm self-reported sex/sex assigned at birth.

Mosaicism assessment

Karyotyping data was used as the primary source to assess mosaicism status. Karyotype was obtained either at enrolment or from medical records if karyotyping at enrolment was not available. In the case of karyotyping at enrolment, karyotype was obtained through standard procedures from peripheral blood, i.e., 20–30 cells were used for karyotyping. For those with karyotyping data obtained from medical records, the age at karyotyping or mosaicism proportion were not available. When karyotyping data were unavailable but SNP microarray data were (n = 22), we used GenomeStudio (Illumina, San Diego, CA, USA) to assess mosaicism, this was also done for individuals who had karyotyping data from medical records, without karyotyping at inclusion. The B allele frequency for each participant was plotted against position on chromosome 21, and participants that deviated from the expected frequencies (0, 0.3, 0.6, 1) were classified as mosaic, the rest were classified as full

trisomy.[15] To ensure classification, we compared B allele frequency plots of participants who had been karyotyped at inclusion as mosaic with those who did not have karyotyping data or for which the data was obtained from medical records. We observed that detection of mosaicism by karyotyping was more sensitive than that by SNP microarray, when restricted to those with both karyotyping and genotype data.

Proteomic biomarkers

Protein biomarkers for AD were measured as previously described.[16] Briefly, 500 µL of the earliest visit plasma samples available (i.e., baseline plasma) underwent thawing and a subsequent centrifugation step at 10,000 g for 5 min before being measured using Single Molecule Array (Simoa) HD-1 technology (Simoa; Quanterix, Lexington, MA, USA) for the following markers: Aβ42, Aβ40, total tau (t-tau), and neurofilament light chain (NfL). Analyses were performed in duplicates and control samples from pooled plasma were included on each plate. Coefficients of variance, lower limits of detection, and higher limits of detection for each marker have been previously reported.[16]

CSF biomarkers

CSF was available for the ABC-DS cohort only. The protocol for CSF collection was described previously.[17,18] Samples were thawed and biomarkers were measured using the Lumipulse G1200 platform (Fujirebio, Malvern, PA) for amyloid peptides and tau, and with a commercial ELISA for NfL (UmanDiagnostics, Umeå, Sweden).[17,18] Control and pooled samples were included for quality control.[18]

Neuroimaging

Neuroimaging data was available for the ABC-DS study only and included 3T structural and functional MR imaging, tau PET imaging using the tracer [F-18] AV1451, and beta-amyloid (Aβ) imaging using [C-11] PiB or [F-18]florbetapir. The T1-MRI scans were processed using FreeSurfer 5.3 or FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu/) to determine volumes of targeted brain regions. PET images were processed as described previously[19] to determine an SUVr outcome. To provide an amyloid index that could be compared across the two different radiotracers (PiB and florbetapir), radiotracer uptake was transformed on the Centiloid scale using the procedure described by Klunk et al.[20]

Each subject's tau PET image was registered to the corresponding T1 MRI. Regional uptake of [F-18]AV-1451 was expressed as SUVr, the average radiotracer concentration within a FreeSurfer region divided by the cerebellar cortex concentration. SUVr within the six Braak[21] regions described by Schöll et al.[22] (with the exception that the striatum was not included in the Braak region 5) was computed from FreeSurfer component-region SUVr. Neuroimaging statistical analyses were restricted to participants 40 years of age and older as those below that age were more likely to have low uptake of radiotracers.

Cognitive assessment and clinical diagnosis

All participants received comprehensive evaluations at approximately 16 to 18-month intervals.[10,23] Testing was conducted either at a participant's day program or residence (legacy) or at a study site (ABC-DS). These assessments included review of clinical records (only for our legacy participants and for one of the ABC-DS sites), informant interviews, direct assessment of select cognitive functions, and neurological examinations.[23] Procedures were harmonized for the different sites in ABC-DS.[10]

For the current analyses, we examined performance on an enhanced version of the Down Syndrome Mental Status Examination (DSMSE)[24] which had been administered to both cohorts. The DSMSE is an omnibus measure of neuropsychological functioning that assesses a broad range of skills, including recall of personal information, orientation to season and day of week, immediate and delayed memory, language, visuospatial function, and praxis. Several measures were generated from this test, a sum of the memory items (DSMSE-memory, maximum = 24), a sum of the non-memory items (DSMSE-nonmemory, maximum = 79), and a total score (maximum = 103). Reliability of the DSMSE, using Cronbach's α, was examined in a previous study[23] and was found to be high (0.97). It was also determined to be sensitive to changes association with prodromal AD[23] and clinical dementia in adults with DS.[25]

Diagnostic status based upon the case consensus review procedures

The dementia status of each participant was rated based upon consideration of all information available, including evidence of decline over the 16- to 18-month period that elapsed between our cycles of data collection. Procedure for clinical case consensus process that has been described in earlier publications.[10,23,25] Clinical status was classified into the following categories: *a) Cognitively-Stable (CS),* indicating with reasonable certainty that significant impairment was absent; *(b) Mild Cognitive Impairment-Down Syndrome (MCI-DS),* indicating that there was some indication of cognitive and/or functional decline over and above what would be expected with ageing alone, though of insufficient severity to suggest the presence of dementia; and *(c) AD-Dementia,* indicating that multiple indications of significant declines were evident that could not be explained by circumstances unrelated to AD neuropathology (such as a traumatic life event or severe illness) or other underlying progressive neuropathology. These procedures were equivalent for both cohorts. For the purpose of these analyses, individuals with MCI were

considered to be non-demented. In addition, individuals who could not be categorized into these three groups were excluded from the analysis (Twenty-two participants in total combining both cohorts).

Statistics

Statistical analyses were performed on R (version 4.2.1).[26] Normality was checked using Shapiro–Wilk test, if the variable did not conform to normality, it was log10 transformed unless log transformation further skewed the distribution. Linear regression models were used to assess the association of mosaicism with biomarkers, adjusting for age, sex assigned at birth, AD status (AD-Dementia vs. non-demented), and severity of premorbid intellectual disability, these analyses were performed independently for each cohort. Similar analyses were performed for cognitive variables, for changes in DSMSE (difference between the value for the first visit and the value for the last visit) also included baseline DSMSE and the time difference between the first and the last visit. For categorical variables (Premorbid intellectual disability, AD at baseline, conversion to AD) binomial models were performed, and odds ratio (OR) was calculated. For demographic variables, any continuous variables were compared by Student's t-test, and categorical variables were compared by Chi- Squared test or Fisher exact test, when appropriate.

Meta-analysis

Meta-analysis was performed for the plasma biomarker variables, as they presented either significant or trends for significant associations with mosaicism, using an adapted METAL formula and a fixed effects model.[27] The choice of a fixed effects model was done under the assumption that the studies share the same direction of effect focusing on mosaicism.

Role of funders

Study sponsors did not play a role in manuscript design, data collection, data analysis, interpretation, nor writing of the manuscript.

Results

Demographic description of the cohorts

Participants from both cohorts were included in the analyses if they were mosaic for DS or had full trisomy 21, and they were excluded if they had a translocation. Mosaicism was determined using karyotype data at inclusion when available. When missing or when karyotyping was obtained from medical records, SNP microarray genotype data from a blood sample was used to assess mosaicism. Thus, we could not determine whether mosaicism was present at birth or was acquired later in life. In comparison to participants in the ABC-DS cohort, participants in the legacy cohort were more likely to: be older ($p < 0.0001$, Student's t-test), be female ($p < 0.0001$, Chi-Square; for investigation of women's health issues and aging), have severe intellectual impairment as measured by level of functioning ($p < 0.0001$, Chi Square), and be unaffected at baseline ($p = 0.02$, Chi Square). Table 1 shows the full demographic and clinical details of both cohorts.

As shown in Table 1, no significant difference in the levels of mosaicism was observed between the two cohorts. However, among those with mosaicism, the participants in the legacy cohort were older (Table 2, $p = 0.021$, Student's t-test), mirroring what was observed for the whole cohort.

The frequency of mosaicism increased with age

We first assessed the age-specific prevalence of mosaicism by dividing the cohorts into age groups of 10 years. For the ABC-DS cohort, there were no mosaic participants in those 30 or younger. The legacy cohort did not have any participants younger than 31, and no participants 40 or younger were mosaic (Table 3). The frequency of participants with mosaicism increased from 40 years old in the ABC-DS cohort (<40 vs. 41–50: $p = 0.019$, Fisher's Exact test) and from 50 years in the legacy cohort (41–50 vs. 51–60: $p = 0.014$, Fisher's Exact test) (Table 3 and).

Mosaicism was associated with lower concentrations of plasma amyloid peptides

Because the prevalence of both mosaicism and AD increases with age, we analyzed the effects of mosaicism on AD and its endophenotypes, including the AD-related biomarkers. We hypothesized that participants with mosaicism would have lower concentrations of AD-related biomarkers, particularly of amyloid biomarkers due to a potential reduction in expression of *APP* which would decrease the concentrations of amyloid peptides. Although not all participants, particularly the legacy participants, had biomarker data, we observe some differences. In the ABC-DS cohort, participants with mosaicism had significantly lower concentrations of amyloid proteins (both $A\beta_{40}$ (11.5% lower) and $A\beta_{42}$ (9.4% lower); Table 4). In the legacy cohort, a similar direction was observed, especially for $A\beta_{40}$ (9.4% lower), although it failed to reach significance. When performing meta-analysis, significant differences for $A\beta_{40}$ ($p = 0.011$, linear regression model) and $A\beta_{40}$ ($p = 0.038$, linear regression model) between individuals with mosaicism and individuals with full trisomy were observed, reinforcing the impact of mosaicism on amyloid peptide production.

Mosaicism was not associated with different concentrations of CSF biomarkers

To determine whether the observed decrease in amyloid concentrations was occurring systemically from lowering of *APP* expression, we analysed CSF biomarker concentrations from the initial visit in a subset of 50 participants

	ABC-DS[a] (N = 26)	Legacy[a] (N = 45)	p-value
Age (baseline)			
Mean (SD)	**50.5 (8.9)**	**55.64 (8.4)**	**0.021**
Range	**33–65**	**43.01–78.08**	
Sex assigned at birth (% Female)	38.5%	57.7%	0.12
Intellectual impairment (% Mild or higher)	53.8%	36.4%	0.15
Race (% White)	100%	91.1%	0.29
APOE			
APOE2 allele frequency	14%	11.1%	0.71
APOE4 allele frequency	16%	10%	0.71
AD Status			
AD at baseline (%)	19.2%	8.88%	0.27
Converted to AD dementia in follow-up (%)	14.3%	21%	0.74

[a]Values are either means with standard variations and range for continuous variables, or percentages for categorical variables.

Table 2: **Demographic description of individuals with mosaicism for each cohort.**

Age (years)	ABC-DS[a] (N = 357)	Legacy[a] (N = 468)
≤40	1.8% (2/111)	0% (0/18)
41–50	9.4% (11/117)	5.3% (10/190)
51–60	9% (10/111)	12.3% (26/211)
61–70	21.4% (3/14)	11.9% (5/42)
>70	0% (0/2)	57.1% (4/7)

[a]Values are presented as percentages and the total of mosaic participants over total participants in the age group.

Table 3: **Descriptive Statistics for Mosaic Participants by 10-year age ranges.**

from ABC-DS since the legacy study lacked CSF data. We did not observe significant differences among the six participants with mosaicism and the 44 people with full trisomy (Table 5).

Mosaicism was not associated with differences in amyloid or tau deposition in the brain

We subsequently examined the effects of mosaicism on accumulation of amyloid and tau in the brain as measured by PET imaging, even though concentrations of CSF biomarkers did not differ significantly. Both amyloid (n = 129) and tau (n = 54) PET imaging from the first visit were evaluated after restricting to those who were 40 years of age or older to reduce floor effects. PET amyloid values are presented as centiloid values, while tau is presented as a composite index of radiotracer uptake. There were no significant differences in amyloid brain accumulation between those with mosaicism and those with full trisomy (Table 6). No significant differences were observed between participants with mosaicism and those with full trisomy for tau accumulation either (Table 6), although only a very small number of adults with mosaicism had tau PET scans available.

Mosaicism did not have a significant impact on cognition

We lastly examined the potential effect of mosaicism on neuropsychological performance and AD status. Given this phenotype represents the most downstream phenotype from karyotype based on the central dogma of molecular biology, it is expected that the genetic influence might be more moderate compared with other endophenotypes (e.g., proteomics). For ABC-DS, at time of analysis, there were only two observations available, for the legacy cohort, there were up to nine. Due to the longer follow-up in this somewhat older legacy cohort, we were able to observe longitudinal differences only in this cohort. In particular, the changes in DSMSE, a test of overall cognitive function, from the baseline to the last observation in participants with mosaicism were significantly smaller than in full trisomy participants ($p = 0.014$, linear regression model), additionally, mosaicism was protective for both AD at baseline ($p = 0.02$, logistic regression model) and conversion to AD ($p = 0.0015$, logistic regression model) after adjusting for covariates. Compared with full trisomy, those with mosaicism showed a slower annualized change after adjusting for covariates. However, this phenotypic change did not reach statistical significance (Table 7).

Discussion

Upon examining the influence of mosaicism on the full spectrum of AD phenotypes in adults with DS, the present study confirmed that the prevalence of mosaicism in DS increased with age, starting in their 40s.[7,8] Although the prevalence of mosaicism was low (<10%) in the present study, adults with DS with mosaicism had significantly lower concentrations of plasma amyloid β peptides (in the ABC-DS cohort), a slower decline in cognitive performance, and a lower incidence and prevalence of AD (in the legacy cohort). When CSF and PET amyloid and tau data were examined, however, those with mosaicism did not show a significant difference, most likely due to a limited number of individuals who underwent these procedures. Although this study supports the hypothesis that mosaicism could lead to lower phenotypic expressions AD phenotypes through the amyloid pathway, further studies are needed to understand the mechanisms.

We confirmed the two previous studies that described an age-related increase of mosaicism in DS.[7,8] That is, the percentage of participants with mosaicism increased after 40 years of age and continued to increase in older individuals. This age-related increase in mosaicism, of any chromosome, has also been observed in the general population.[28,29] For disomic individuals, mosaicism leads to an alteration of dosage, rather than a normalization of dosage; therefore, it is associated with AD.[30–33] To fully examine the role of mosaicism on AD, it would be necessary to also consider germline

Biomarker[a]	ABC-DS (N = 309)			Legacy (N = 265)			Meta-analysis
	Full trisomy (N = 286)	Mosaic (N = 23)	β(p)	Full trisomy (N = 241)	Mosaic (N = 24)	β(p)	β(p)
$A\beta_{40}$							
Mean (SD)	450.5 (93)	412 (98.3)	**−51.6 (0.013)**	332.7 (101.7)	295 (126.2)	−31.21 (0.16)	**−42.19 (0.011)**
Range	60.9–759	187–617		28.8–703	94.9–640		
$A\beta_{42}$							
Mean (SD)	15.3 (3)	14.3 (3.3)	**−1.44 (0.027)**	14.6 (5)	13.3 (5.5)	−1.13 (0.30)	**−1.30 (0.038)**
Range	2.2–24.5	6.7–21.3		1.1–30.2	5.3–24.9		
Tau							
Mean (SD)	2.7 (1.9)	2.7 (1.8)	−0.04 (0.39)	3.3 (7.4)	3 (1.2)	*0.08 (0.086)*	0.02 (0.13)
Range	0.13–18.4	0.18–9.8		0.6–115	1–6.2		
NfL							
Mean (SD)	21.4 (20.1)	26.4 (24.8)	−0.031 (0.51)	34.9 (31.6)	43.1 (27.7)	0.06 (0.26)	0.01 (0.34)
Range	4.1–233	6.6–122		4.9–267	12.5–134		

Only participants with data for the biomarkers were included. Models were adjusted for sex assigned at birth, age, dementia status, and level of premorbid intellectual disability. Tau and NfL linear models were calculated after log10 transformation of the biomarker data. [a]Pg/mL.

Table 4: **Associations of mosaicism with Alzheimer's Disease blood-based biomarkers for each of the cohorts.**

mosaicism, which may have a lifelong influence on AD risk later in life. However, it is most likely that acquired mosaicism is the primary driver since the prevalence of mosaicism at birth is quite low at 1.3–5%.[2,3] In addition, we speculate that the cellular mechanisms for age-related increase in mosaicism might be attributed to an increase in chromosomal instabilities and the formation of micronuclei, which increases with age in trisomic cells.[9] If this hypothesis of chromosomal instabilities was correct, chromosomal instabilities would not have been restricted to chromosome 21 but would have been observed in all chromosomes, as observed (data not shown). Another mechanism that could explain the increase in prevalence of mosaicism is a better survival of individuals with mosaicism.[4,5] However, as stated, we cannot confirm this hypothesis, since we were unable to identify individuals with mosaicism at birth.

Studies have shown amyloid peptides can be produced by blood cells as well as other peripheral tissues.[34] In the ABC-DS cohort and in the meta-analysis, we observed that the plasma concentrations of $A\beta_{40}$ and $A\beta_{42}$ were lower in those with mosaicism after adjusting for age, sex assigned at birth, and premorbid ID level. This association might lead one to conclude that the lower production of these two biomarkers resulted from a lower expression of the *APP* gene in those with mosaicism. Since this study has not measured three

Biomarker	Full trisomy (N = 44)	Mosaic (N = 6)	β(p)
$A\beta_{40}$ (pg/mL)			
Mean (SD)	10,618 (3429.5)	11,449 (2870.4)	107.77 (0.94)
Range	4921–19,337	8597–16,723	
$A\beta_{42}$ (pg/mL)			
Mean (SD)	537.4 (192.7)	577.3 (107.0)	0.03 (0.93)
Range	220–1101	470–730	
Total tau (pg/mL)			
Mean (SD)	581.1 (460.1)	626.8 (273.9)	0.106 (0.43)
Range	86–2000	204–975	
p-tau181 (pg/mL)			
Mean (SD)	68.8 (65)	73.1 (45.6)	0.096 (0.43)
Range	8.7–323.7	24.6–146.9	
NfL (pg/mL)			
Mean (SD)	1080.3 (1001.7)	1268.7 (827.1)	0.10 (0.29)
Range	203–6056	246–2674	

[a]Only participants with data for the biomarkers were included. Models were adjusted for sex assigned at birth, age, dementia status, and level of premorbid intellectual disability. All biomarkers except $A\beta_{40}$ were log normalized before analysis.

Table 5: **Associations of mosaicism with CSF biomarkers.**[a]

	Full trisomy (N = 116)	Mosaicism (N = 13)	β(p)
Centiloid			
Mean (SD)	50.55 (36.84)	61.88 (34.6)	−0.687 (0.94)
Range	−19.19–156.71	7.13–144.15	
	Full trisomy (N = 50)	**Mosaic (N = 4)**	**β(p)**
Tau (Mayo Composite)			
Mean (SD)	1.3 (0.33)	1.49 (0.36)	0.259 (0.097)
Range	0.99–2.3	1.1–1.96	

Analyses were restricted to participants older than 40 years. Models were adjusted for sex assigned at birth, age, dementia status, and level of premorbid intellectual disability.

Table 6: **Comparison of PET Centiloid between adults with full trisomy vs. mosaic.**

pertinent factors (specifically, the concentrations of sAPPα, total concentrations of APP, or the expression levels of the *APP* gene), it is difficult to conclude whether this is due to: (1) reduced *APP* expression; (2) normalization of the gene dosage on chromosome 21, which would have weakened the gene dosage effects on the amyloidogenic pathway; or (3) both. The results from the legacy cohort suggested that participants with mosaicism had lower concentrations of plasma amyloid biomarkers. We note that other indicators such as CSF biomarkers and imaging biomarkers were less than informative due to small sample sizes. Although, the ratio of $A\beta_{42}/A\beta_{40}$ is usually used as biomarker for AD risk,[35] we did not see a significant difference in those with mosaicism (data not shown). One likely explanation is that mosaicism may have lowered the expression of *APP* gene such that the concentrations of all amyloid peptides may have been decreased; thus, the ratio will be unaffected. This dosage hypothesis for mosaicism is exemplified by a number of reports of individuals who had a very mild DS phenotype and were diagnosed with mosaic DS after an early onset of AD (<60 years).[36–39] We further note that our finding of a relatively low degree of mosaicism in this high risk individuals ameliorating some AD related phenotypes does not contradict the finding by Nuebling and colleagues,[36] who showed that a low degree mosaic trisomy in a person with disomy can lead to early onset AD. Both studies illustrate the importance of gene-dosage effects on AD related complex phenotypes.

Mosaicism may result in reduced accumulation of amyloid due to the decreased expression of the APP protein as previously described in partial trisomy cases that did not include an extra copy of the *APP* gene.[40,41] However, in our sample, we did not observe any differences in brain amyloid or tau accumulation by PET imaging for those with mosaicism. Although the data from tau PET show only marginal, but not significant, association for higher accumulation in those with mosaicism, the sample size was too small to draw definitive conclusions. Moreover, as our assessment of

Cognitive characteristic	ABC-DS (N = 357)			Legacy (N = 468)		
	Full trisomy (N = 331)	Mosaic (N = 26)	p or β(p)	Full trisomy (N = 423)	Mosaic (N = 45)	OR/β(p)
Premorbid intellectual disability (% Mild)	51.7%	53.8%	0.87 (0.73)	30.5%	35.6%	0.53 (0.07)
% AD baseline	11.8%	19.2%	1.26 (0.23)	9.0%	8.9%	**0.13 (0.02)**
% AD converters	10.4%	14.3%	0.87 (0.85)	32.9%	21%	**0.24 (0.0015)**
Age at onset						
Mean (SD)	54.5 (5.8)	53.5 (7.8)	0.09 (0.87)	57.1 (5.3)	63.7 (9.4)	−0.32 (0.81)
Range	45–68	48.0–59.0		43.3–75.3	51.4–80.4	
DSMSE baseline						
Mean (SD)	59 (16)	52.8 (20.9)	−1.10 (0.66)	52.3 (24.9)	49.6 (25.3)	0.65 (0.84)
Range	4–83	9.5–84.0		0–97	0–94	
DSMSE at last visit						
Mean (SD)	59 (16.9)	55.1 (20)	−3.72 (0.26)	37.0 (29.4)	43.2 (26.7)	4.62 (0.2)
Range	0–82	11.5–83.5		0–94.5	0–95	
Change baseline –last visit DSMSE[a]						
Mean (SD)	−1.58 (8.2)	−3.7 (8.5)	−1.78 (0.35)	−16.7 (21.5)	−10.9 (14.6)	**8.26 (0.014)**
Range	−32.5–28.0	−18.5–15.5		−83.0–33.5	−64.5–6.0	
Annualized change						
Mean (SD)	−1.06 (6.9)	−1.57 (6.1)	−0.76 (0.64)	−3.3 (4.8)	−3.29 (5.7)	1.1 (0.17)
Range	−23.5–28.0	−9.3–15.5		−29.1–16.4	−31.7–2.5	

For continuous traits, linear models were applied, adjusting for sex assigned at birth, dementia status at visit, age at visit, and level of premorbid intellectual disability. For dementia status logit binomial models were applied, adjusting for sex assigned at birth, age at visit/age at onset, and level of premorbid intellectual disability. A similar model was used for premorbid intellectual disability, adjusting for sex assigned at birth and age at visit. Changes from the baseline were adjusted for sex assigned at birth, dementia status at the last visit, age at last visit, time difference between first and last visit, level of premorbid intellectual disability, and baseline values of DSMSE. For the age at onset of AD, linear models were corrected for age at first visit, sex assigned at birth, and premorbid intellectual impairment.
[a]Difference between the last visit and the baseline measure.

Table 7: **Relations between mosaicism and Alzheimer's disease and cognitive phenotypes.**

mosaicism is based on blood samples, this observation may not be extrapolated to other tissues such as the brain.

For mosaicism, it is also necessary to take into account which tissues were involved in chromosomal rescue. In chromosomal rescue during cell division, one of the daughter cells loses one of the trisomic chromosomes to become disomic and this occurs differentially by tissue type; therefore, it is necessary to contrast mosaicisms occurring at birth vs. age-acquired mosaicism. For age-acquired mosaicism, the question remains whether all mitotic cells have a similar likelihood for chromosomal rescue or whether certain cells do not. While most cells in the brain are post-mitotic (or divide very rarely), microglia are an exception as they have been shown to divide and share embryonic origin with peripheral immune cells. As such, further examinations are needed.

The contributing reasons for the observed differences between the ABC-DS and legacy datasets are as follows: (1) the eligibility criterion for age was ≥25 years (with the mean age of 45.7 at the baseline) for the ABC-DS cohort and ≥30 years of age (with the mean age of 51.3 at the baseline) for the legacy cohort; and (2) the period of follow up was far longer for the legacy cohort than for the ABC-DS cohort. Consequently, the proportion of participants who might be susceptible to dementia was far smaller for the ABC-DS cohort than for the legacy cohort. Consequently, the legacy study had statistical power to detect the difference in AD risk and cognitive decline between mosaic vs. full trisomy. Indeed, we observed slower cognitive decline in participants with mosaicism in the legacy cohort, as well as lower incidence and prevalence of clinical dementia. With a longer follow in ABC-DS, we might be able to confirm the findings from the legacy study.

Limitations

Due to the low frequency of mosaicism and its rare occurrence, our analyses are limited by a small sample size for the CSF and PET biomarkers. In addition, we were unable to characterize the effects of varying levels of mosaicism on AD risk, as the number of participants with a higher percentage of disomic cells over trisomic cells was limited in both cohorts (8.8% of total mosaic participants in the legacy cohort and 19.2% of total mosaic participants in ABC-DS). Lastly, we measured mosaicism in peripheral blood, which may not represent mosaicism in the target organ and tissues.[3,33] Studies of postmortem fetal and adult mosaic brains might have shed light on the effects of mosaicism on both brain development and neurodegeneration in DS; however, this is beyond the scope of this study. Our measure of mosaicism reflects cross-sectional assessment at age 30 or older for the legacy cohort and 25 or older for the ABC-DS cohort and does not measure the lifelong exposure that might be responsible for the observed gene dosage and AD-related phenotypes in these adults with DS. With follow-up assessments of karyotype data in the ABC-DS study might allow us to better examine age-related mosaicism on chromosome 21. A future collaborative study with a pediatric DS dataset may enhance our understanding of how lifelong mosaicism may influence individuals with DS from neurodevelopment to neurodegeneration. In addition, our study is generalisable to the general population in the US and Western Europe on most accounts (e.g., sex-assigned at birth, levels of intellectual disability, etc.), but it is limited in ethnic diversity. To this end, the ABC-DS study is actively working on recruiting participants from more diverse populations. In this longitudinal cohort study, we did not observe indicators that suggest bias in loss-to-follow up.

In sum, the present study shows a possible beneficial effect of mosaicism on AD and its endophenotypes, potentially lowering the risk of AD in DS, a genetically high-risk population. The lower concentrations of plasma Aβ suggest a direct effect of the lower *APP* dosage. If that is the case, that would also explain the effects on cognition observed in the legacy cohort, and therefore part of the variability in AD presentations seen in the population with DS. This study offers one explanation for the observed wide phenotypic variations associated with AD in adults with DS, emphasizing the need for further examination of differing genomic contributions toward AD in adults with DS.

Contributors

Study design and conceptualization of the study: LX, BH, JHL.

Data collection: SKM, DP, LM, SO'B, RLH, CL, FL, HDR, BA, IL, CH, BC, SH, SZ, EH, MM, WS, NS, BH, JHL, ABC-DS study group.

Analysis, including accessing and verifying the underlying data, or Interpretation: LX, L-HTD, AL, BH, JHL, ABC-DS study group.

Drafting and Revisions of the Manuscript: LX, L-HTD, AL, SKM, DP, LM, SO'B, RLH, CL, FL, HDR, BA, IL, CH, BC, SH, SZ, EH, MM, ZJ, WS, NS, BH, JHL.

All authors read and approved the final version.

Data sharing statement

All available data for the ABC-DS study, including demographic, clinical, genomic, proteomic, metabolomic/lipidomic, and imaging data are available in the LONI repository (https://ida.loni.usc.edu/login.jsp) upon approval of an analysis plan. In addition, legacy data including demographic, clinical, genomics, proteomic and metabolomic data are available in the AD Knowledge Portal (https://adknowledgeportal.synapse.org/Explore/Studies/DetailsPage/StudyDetails?Study_Name=(omicsADDS) upon request.

Declaration of interests

BA is a member of VCID Advisory Board without payment. BH receives funding from National Institute of Child Health and Human Development, Autism Speaks, and Roche Pharmaceuticals. EH has consulted for Alzheon and Cyclotherapeutics and received royalties from Elsevier Press. JHL is part of the external advisory board for the Alzheimer's Disease Resource Center for Minority Aging Research, University of Texas, and for the Center of Life Science, Nazarbayev University, Astana, Kazakhstan. MM is an inventor on patents related to biomarkers of neurodegenerative diseases owned by Georgetown University and the University of Rochester. SH is the vice-chair of the ISTAART Down syndrome PIA. SKM is an employee for the New York State Office for

People with Developmental Disabilities (OPWDD) and is a consultant for the NIH grant R01-HD098179. SZ is the chair of the scientific committee of the T21 Research Society receiving paid registration to the biannual meeting. The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105433.

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