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RESEARCH ARTICLE

Genome-wide association analyses identify candidate loci for amyloid imaging and plasma biomarkers in adults with Down syndrome

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Abstract

BACKGROUND: People with Down syndrome (DS) overproduce amyloid-beta (Aβ) due to triplication of the amyloid precursor protein (*APP*) gene on chromosome 21, and consequently accumulate brain amyloid load at younger ages. We conducted genome-wide association (GWA) analyses on amyloid imaging and plasma biomarkers to discern the genetic architecture of amyloid burden in DS.

METHODS: GWA analyses were performed on amyloid positron emission tomography (PET) and plasma biomarkers (Aβ40, Aβ42, Aβ42/40 ratio) in participants from the Alzheimer's Biomarker Consortium-Down Syndrome (ABC-DS) and on plasma Aβ biomarkers available in an independent DS cohort, followed by meta-analysis of plasma Aβ biomarker data.

RESULTS: Meta-analysis on plasma biomarkers identified four novel loci: two for Aβ42 (*PFKFB3*/rs147647642, $p = 2.83E-08$; *DLX3-PICART1*/rs12952028, $p = 9.31E-09$) and two for Aβ40 (*LINC01941-GYPC*/rs78338676, $p = 9.33E-09$; *PDE4D*/rs146261781,

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p = 9.97E-08). Five genome-wide signals were observed for amyloid-PET in the ABC-DS cohort that need confirmation in an independent DS dataset.

DISCUSSION: Despite the small sample, our findings highlight the unique genetic architecture of amyloid burden in DS.

KEYWORDS
AD biomarker, amyloid-PET, Centiloid, plasma Aβ, trisomy 21

Highlights

- Genetic markers for amyloid biomarkers in Down syndrome (DS) were identified.
- Meta-analyses identified four novel loci for plasma amyloid in two DS cohorts.
- Five loci associated with amyloid positron emission tomography levels were identified in the Alzheimer's Biomarker Consortium-Down Syndrome cohort.
- Multi-trait analysis revealed loci linking variants to amyloid biomarkers.

1 | BACKGROUND

Alzheimer's disease (AD) is a slowly progressing, irreversible, and multifactorial neurodegenerative disorder characterized by extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tau tangles.[1,2] AD predominantly affects older adults and accounts for almost 60%–80% of all dementia cases.[2,3]

Down syndrome (DS) is a genetic disorder resulting from the trisomy of chromosome 21, which leads to a spectrum of cognitive impairments and developmental delays, with a higher incidence of AD.[4] Dosage-dependent increased expression of the amyloid precursor protein (*APP*) gene located on chromosome 21 results in Aβ plaques, instigating a series of pathophysiological pathways such as tau hyperphosphorylation, neurofibrillary tangle formation, increased oxidative stress, neuroinflammation, and synaptic and neuronal loss leading to dementia.[5,6]

The cascade of pathophysiological pathways resulting in dementia onset in individuals with DS is similar to that evident in autosomal dominant AD in the neurotypical population due to the triplication of the *APP* gene in individuals with DS.[7] The prevalence of AD in DS individuals younger than age 40 is less than ≈5%, and doubles with each 5-year interval up to the age of 60.[8]

Amyloid precursor protein (APP) is an abundantly produced, rapidly metabolized, single-pass transmembrane protein that can undergo proteolytic cleavage in two different pathways (amyloidogenic and nonamyloidogenic) by α-, β-, and γ-secretases.[9] Amyloidogenic cleavage of APP produces Aβ40 and Aβ42 peptides, of which Aβ40 is the most common (≈80%–90%) and Aβ42 constitutes only 5%–10% of total Aβ. The longer peptide (Aβ42), being more hydrophobic and fibrillogenic, is specifically associated with AD pathogenesis.[10,11] In plasma, Aβ40, Aβ42, and Aβ42/40 ratio levels, and in brain, Aβ plaques have been shown to be reliable diagnostic biomarkers for AD, especially during the early stage of the disease.[12,13]

A common three-allele apolipoprotein E (*APOE*) polymorphism (*APOE2*, *APOE3*, and *APOE4*) determines six genotypes (2/2, 2/3, 2/4, 3/3, 3/4, and 4/4) that have a profound effect on amyloid burden in non-DS populations. As compared to *APOE 3/3* homozygotes, *APOE4* carriers have high and *APOE2* carriers have low amyloid burden.[14] Similar findings have been reported in cerebrospinal fluid (CSF).[15] Additional non-*APOE* loci have also been reported to affect amyloid load in the brain and CSF.[15,16] The *APOE2* allele has shown a protective effect against the development of dementia in individuals with DS,[17] whereas the *APOE4* allele was associated with an earlier onset of AD and cognitive decline in DS, as demonstrated by its correlation with earlier biomarker changes, including amyloid accumulation and hippocampal atrophy.[18]

Because AD-related biomarkers can detect AD pathology in DS,[19] we performed genome-wide association (GWA) analyses on Aβ neuroimaging and plasma biomarkers in adults with DS to investigate if the same genetic loci associated with these biomarkers in non-DS populations are also associated in DS, or if DS has a unique genetic architecture in affecting amyloid load in the background of trisomy of chromosome 21. GWA analyses were performed on plasma Aβ (Aβ40, Aβ42, and Aβ42/40 ratio) and brain Aβ positron emission tomography (Amyloid-PET) biomarkers available in the participants of the Alzheimer's Biomarker Consortium-Down Syndrome (ABC-DS)[20] and a subset of independent subjects with plasma Aβ data from the Multiomic Studies of Alzheimer's Disease in Adults with Down Syndrome (omicsADDS) study.[21]

2 | METHODS

2.1 | Participants

Participants were 375 and 133 non-Hispanic White (NHW) adults with DS from the ABC-DS and omicsADDS studies, respectively. The

ABC-DS is a multicenter study to explore the AD-related clinical, cognitive, imaging, genetic, and fluid biomarker data in adults (≥25 years of age) with DS. The detailed recruitment process and clinical diagnosis have been reported earlier.[22–24] There were an additional 45 healthy sibling controls that were not included in this study. Of the 375 NHW DS participants in the ABC-DS study, 259 were cognitively stable (CS) and 97 had AD dementia. Baseline demographic information from the ABC-DS participants is given in Table 1.

The omicsADDS is an offshoot of a larger, single-site, longitudinal observational study that included 612 karyotyped, confirmed DS adults enrolled in the longitudinal observational study.[21,23–25] All DS participants enrolled in study were ≥ 30 years of age. This study utilized a sample of 133 NHW DS participants who had plasma Aβ40, Aβ42, and Aβ42/40 ratio data available and were distinct from the ABC-DS participants. Of 133 omicsADDS participants, 128 were CS (*APOE4* carriers: *N* = 28 [21.87%]) and 5 had AD dementia (*APOE4* carriers: N = 1 [20%]). omicsADDS participants did not have amyloid-PET data available.

RESEARCH IN CONTEXT

1. **Systematic review**: Down syndrome (DS) is caused by chromosome 21 trisomy. Participants with DS are prone to high Alzheimer's disease (AD) risk. This study aimed to explore the genetic markers associated with amyloid beta (Aβ) biomarkers in participants with DS from two distinct cohorts.
2. **Interpretation**: Meta-analyses of plasma amyloid biomarkers in two DS cohorts identified four novel loci. Five genome-wide significant loci with an elevating effect on amyloid positron emission tomography levels were identified in the Alzheimer's Biomarker Consortium-Down Syndrome cohort. Multi-trait analysis revealed additional loci linking genetic variants to amyloid biomarkers.
3. **Future directions**: Validation in independent DS cohorts is needed to confirm these findings. Larger studies and functional analyses will help clarify the biological roles of identified loci in amyloid pathology.

2.2 | Plasma Aβ biomarkers

Building on recent advancements in ultra-sensitive measures for plasma biomarkers, automated single molecule array (Simoa) technology (Quanterix, Lexington, MA, USA) was used to quantify plasma amyloid (Aβ40 and Aβ42) in ABC-DS. The Aβ42/40 ratio was calculated by dividing Aβ42 by Aβ40.[20] A detailed description of the plasma Aβ40 and Aβ42 measurement methods for omicsADDS has been published previously.[24]

2.3 | Amyloid-PET imaging

Amyloid PET imaging was conducted in DS participants following the specified uptake periods and scanning durations for Aβ imaging using [C-11] Pittsburgh compound B (PiB) at four sites and florbetapir/([F-18]AV-45) at three sites. To ensure harmonization across sites, PET image reconstruction parameters were standardized for various PET scanner models. The Centiloid scale was employed to enable direct comparisons of different Aβ radiotracers, such as PiB and florbetapir, in both cross-sectional and longitudinal studies.[20,26–30]

2.4 | Genotyping, imputation, and quality control

Genome-wide genotyping was performed at the Center for Applied Genomics at Children's Hospital of Philadelphia using the Illumina Infinium General Screening Array (GSA) Version 2, with added disease markers for all samples, except for 25 participants for whom Version 3 was used, which resulted in a total of 759,993 and 654,027 variants in Version 2 and Version 3, respectively. Variants were mapped to the reference human genome assembly GRCh38. Imputation was performed on autosomes except chromosome 21 using the TOPMed imputation server with the TOPMed reference panel (Version r2) to enhance the resolution of the genomic information.[31–33] Variants with imputation quality scores (R^2) greater than 0.3 were retained, resulting in 22,466,993 variants. In addition, participants with a call rate below 95% were excluded, as were single-nucleotide polymorphisms (SNPs) that were not in the Hardy–Weinberg equilibrium (HWE $p < 1E\text{-}05$). For downstream analyses, we focused on SNPs meeting the criteria of an imputation quality score $R^2 > 0.3$ and a minor allele frequency (MAF) ≥ 1% (*N* = 6,160,269 SNPs). Principal components (PCs) were computed through PLINK v1.90 using a sliding window approach, with a window size of 2000 base pairs and 200 variants per window to assess population structure.

2.5 | Chromosome 21 genotyping

Chromosome 21 for DS participants was treated separately because the traditional genome analysis tools cannot handle chromosome 21 trisomy. Trisomic variants were treated as copy number variations (CNVs) and were called in using the cnvPartition CNV Analysis (v3.2.0) plug-in in GenomeStudio 2.0 with Genotyping module. Allele-specific genotypes were then exported using the CNV Region Report plug-in (v.2.1.2). In total, 9785 and 8446 variants were called on GSAv2 and GSAv3, respectively. The p-arm of chromosome 21 was dropped from analysis because it is known to be highly heterochromatic with numerous repeat sequences. In the current data, this attribute resulted in a high rate of variants called disomic among those karyotyped with DS and discrepancies between duplicates in this region. For these reasons, all variants genotyped on the p-arm were excluded from analysis.

TABLE 1 Baseline demographic information of ABC-DS non-Hispanic White DS.

	Total (*N* = 375)	Aβ40 (*N* = 275)	Aβ42 (*N* = 275)	Aβ42/40 ratio (*N* = 275)	Amyloid-PET Centiloid (*N* = 218)	All amyloid biomarkers[b] (*N* = 199)
Age, mean ± SD	45.13 ± 9.89	44.96 ± 9.84	44.96 ± 9.84	44.96 ± 9.84	42.45 ± 9.58	42.61 ± 9.28
Sex, *N* (%)						
Male	204 (54.40%)	148 (53.82%)	148 (53.82%)	148 (53.82%)	128 (58.7%)	118 (59.2%)
Female	171 (45.60%)	127 (46.18%)	127 (46.18%)	127 (46.18%)	90 (41.3%)	89 (44.7%)
***APOE* genotype, *N* (%)**						
2/2	2 (0.54%)	2 (0.73%)	2 (0.73%)	2 (0.73%)	2 (0.91%)	2 (0.73%)
2/3	47 (12.70%)	37 (13.45%)	37 (13.45%)	37 (13.45%)	30 (13.76%)	29 (14.5%)
2/4	9 (2.43%)	7 (2.55%)	7 (2.55%)	7 (2.55%)	4 (1.83%)	4 (2.01%)
3/3	232 (62.70%)	170 (61.82%)	170 (61.82%)	170 (61.82%)	136 (62.38%)	123 (61.8%)
3/4	73 (19.73%)	53 (19.27%)	53 (19.27%)	53 (19.27%)	39 (17.88%)	36 (18.1%)
4/4	7 (1.89%)	6 (2.18%)	6 (2.18%)	6 (2.18%)	6 (2.75%)	5 (2.51%)
Not Available	5 (1.35%)				1 (0.45%)	
Dementia status,[a] *N* (%)						
Cognitively stable	259 (69.07%)	204 (74.18%)	204 (74.18%)	204 (74.18%)	178 (81.60%)	163 (81.91%)
APOE4 carriers, *N* (%)	51 (19.69%)	40 (19.60%)	40 (19.60%	40 (19.60%	35 (19.66%)	31 (19.01%)
Dementia	97 (25.86%)	71 (25.82%)	71 (25.82%)	71 (25.82%)	40 (18.30%)	36 (18.09%)
APOE4 carriers, *N* (%)	35 (36.08%)	26 (36.61%)	26 (36.61%)	26 (36.61%)	14 (35.00%)	14 (38.88%)
Not available	19 (5.07%)					

Abbreviation: Aβ, amyloid-beta; ABC-DS, Alzheimer's Biomarker Consortium-Down Syndrome; SD, standard deviation.
[a]Dementia classifications were established through a clinical consensus process involving a multidisciplinary team that included a psychologist, a physician, and at least two specialists in Alzheimer's disease dementia in DS. These classifications were based on comprehensive assessments, including medical history, clinical evaluations, and cognitive testing. Participants with DS were categorized as cognitively stable (CS), diagnosed with mild cognitive impairment (MCI) specific to DS (MCI-DS), or dementia. For analysis purposes, MCI-DS and dementia were grouped together under the category "Dementia." Participants whose diagnosis could not be definitively determined were classified as "Not available."
[b]Total 199 DS subjects with all four on amyloid-PET and plasma biomarkers (Aβ40, Aβ42, and Aβ42/40 ratio).

2.6 | *APOE* genotyping

Genotypes for rs7412 (*APOE2*) and rs429358 (*APOE4*) single-nucleotide polymorphism (SNPs) were performed using the KASP genotyping platform from LGC Genomics.[34] Direct genotyping was chosen over array or imputation-based approaches because of the well-known challenges in accurately capturing the *APOE* genetic variation in this GC-rich genomic region due to probe hybridization issues and the structurally complex nature.

2.7 | Statistical analyses

2.7.1 | Phenotype data

Baseline amyloid-PET and plasma Aβ40, Aβ42, and Aβ42/40 ratio were utilized in all analyses. Denormalized values for amyloid-PET Centiloid, Aβ40, and Aβ42 were used throughout the analyses (Figure S1). Due to the skewness in the Aβ42/40 ratio, rank-based inverse normal transformation (INT) was applied to normalize the data via R (version 4.4.0). Basic demographics, including age at blood collection, sex, and dementia status at the time of enrollment, were collected for the analyses.

Baseline dementia status was determined by a clinical team, including a psychologist, a physician, and AD-DS specialists, using medical and cognitive testing data. Participants with DS were classified into three groups: cognitively stable (CS), mild cognitive impairment (MCI-DS), and persistent memory and functional decline (AD-DS). Those without a clear diagnosis were labeled as "unable to determine". For analysis, MCI-DS and AD-DS cases were grouped as "dementia," and five participants without a valid diagnosis were excluded.[20]

2.7.2 | Associations of *APOE* polymorphism,

Of the 375 DS participants, *APOE* genotype on two SNPs (*APOE4*/rs429358 and *APOE2*/rs7412) was available in 370. DS participants were categorized into six *APOE* genotypes (2/2, 2/3, 2/4, 3/3, 3/4, and 4/4) based on the *APOE2*, *APOE3*, and *APOE4* alleles. To estimate the dosage effect of the *APOE2* and *APOE4* alleles, linear regression was applied, adjusting for baseline age, sex, and dementia status. Due to the opposite effects of *APOE2* and *APOE4* on AD risk and Aβ levels, nine participants with the *APOE 2/4* genotype were excluded from analysis (Table 1).

2.7.3 | Genome-wide association analyses

Single-trait GWA analyses

Only NHW DS participants with both genome-wide genotyping data and each Aβ biomarker data were included in the analyses. Single-SNP analysis was performed using linear regression framework implemented in PLINK,[35] using the covariates of age, sex, dementia status, and the first four PCs of ancestry to capture the underlying population structures. SNPs on chromosome 21 were analyzed with MatrixEQTL (v2.3),[36] enabling up to four genotype calls per SNP, accounting for the chromosome 21 trisomy genotype structure.

A secondary GWAS with dementia status as the outcome was conducted on 330 ABC-DS participants (92 AD cases, 238 CS) using logistic regression in PLINK,[35] excluding the chromosome 21. The analysis incorporated age, sex, and the first four PCs of ancestry as covariates to adjust for potential confounding due to population stratification. Although not the primary focus of the study, these results were utilized to assess the relevance of top SNPs identified in amyloid-biomarker analyses within DS populations, examining their potential role in mediating AD risk. In addition, comparative analyses were performed to evaluate these findings in the context of non-DS populations.

Genome-wide significant (GWS) and suggestive significant thresholds were set at $p \leq$ 5E-08 and $p \leq$ 1E-05, respectively. Visualization of GWA results across all chromosomes was achieved with Manhattan and QQ plots generated using the R packages qqman (v0.1.9)[37] and Haplin (v7.3.2).[38] Fine-mapping and variant-level exploration were conducted with LDlinkR (v1.4.0).[39]

For plasma Aβ40, Aβ42, and Aβ42/40 ratio phenotypes that were available in both the ABC-DS and omicsADDS cohorts, meta-analyses were conducted by combining the summary statistics results from both cohorts for each individual phenotype using the standard error–based weighted model implemented in METAL (version 2020-05-05),[40] while keeping the genomic control option enabled.

Multi-trait GWA analysis

A total of 199 NHW DS participants from the ABC-DS cohort had information available for all four amyloid biomarkers. We combined these four phenotypes for multivariate regression analysis. Multivariate regression analysis has the potential to increase the power by considering the underlying correlation between variables.[41] Briefly, each amyloid biomarker was first regressed to age, sex, dementia status and the first four PCs. Then two types of residuals (Ɛ) of the regression were used as phenotype in the multi-phenotype analysis through the GEMMA software v0.94[42] with linear mixed models based on the model implemented in R version 4.4.1 (http://www.r-project.org). GEMMA linear model was:

$$= AW + \beta XT + G + E, G \sim MN\}\}\text{d} \times \text{n}''(0, Vg, K), E \sim MN\}\}\text{d} \times \text{n}''(0, Ve, I\}\}\text{n} \times \text{n}'')$$

where Y is a 4×199 matrix of four amyloid biomarkers for 199 individuals; W is a 199×1 matrix of a column of 1 s as covariate; A is a 1 by 4 matrix of the corresponding coefficients; X is a 199 vector of marker genotypes; and β is a 4 vector of marker effect sizes for the four amyloid biomarkers. The model was corrected for the relatedness with the centered relatedness matrix.

2.8 | Comparison of DS genetic loci with non-DS populations

Independent SNPs achieving a significant threshold at $p \leq$ 1E-03 for plasma biomarkers (Aβ40, Aβ42, and Aβ42/40 ratio) and amyloid-PET Centiloid in DS were compared to previously published large GWAS summary statistics in non-DS populations for plasma biomarkers[43] and amyloid-PET.[16]

In addition, SNPs with $p \leq$ 1E-03 in DS were cross-checked with the summary statistics of the largest AD case–control to date[44] along with reported top 99 AD SNPs.[44,45] This multi-layered comparison with non-DS populations aimed to evaluate the extent of generalization of the results obtained in DS as well as to determine its unique genetic architecture.

2.9 | Polygenic risk score

We used PRSice-2[46] to calculate the AD risk polygenic risk score (PRS) from the largest euploid AD case–control study (N = 788,898) to date[44] and examined its impact on DS amyloid phenotypes and dementia in DS. The weighted sum of the risk alleles found in the DS cohort was used to calculate PRS. Although we do not expect that a PRS developed in a euploid population would be directly relevant to a DS population, we do expect that many individual SNPs would be predictive. To concentrate on GWS variants, the traditional clumping and thresholding (C+T) approach was applied to GWS variants with $p \leq$ 5E-08. The variant with the most significant p-value in each region was retained after eliminating variants with $R^2 > 0.1$ within a 250 kb window using linkage disequilibrium (LD) clumping for PRS. Chromosome 21 variants were not included in the analysis. The population mean was used to standardize the obtained PRS. After controlling for age, sex, and the first four PCs, linear regression models were constructed to account for population stratification and potential confounding variables.

In addition, PRS associations were separately adjusted for well-established AD-associated alleles (*APOE2* and *APOE4*). Adjusting for these confounding factors has allowed the PRS to isolate the effect of other genetic variants on amyloid phenotypes while minimizing the influence of known genetic modifiers and demographic variables. In addition, we estimated AD PRS after excluding the *APOE* region (GRCh38, chr19:43,907,927–45,908,821) to examine the contribution of non-*APOE* variants to amyloid biomarkers.

2.10 | Functional annotations

To assess the biological relevance of the identified variants and genes, we utilized the functional mapping and annotation (FUMA)

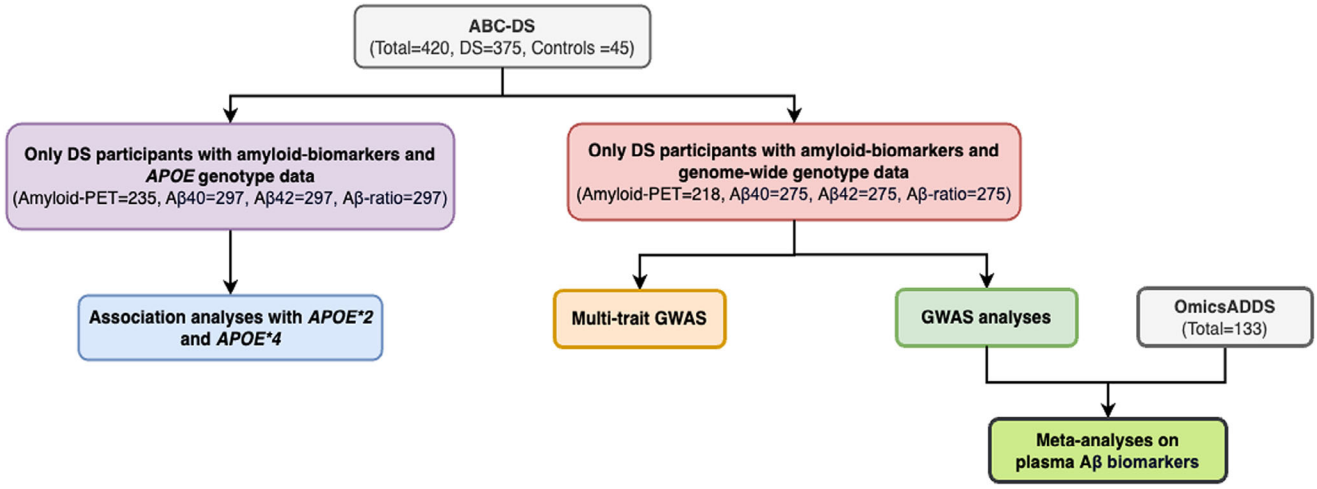

FIGURE 1 Flow chart depicting the various association analyses conducted in this study. Aβ, amyloid-beta; ABC-DS, Alzheimer's Biomarker Consortium-Down Syndrome; GWAS, Genome-wide Association Study; omicsADDS, Multiomic Studies of Alzheimer's Disease in Adults with Down Syndrome.

platform (https://fuma.ctglab.nl/). This web-based tool enables the annotation, prioritization, visualization, and interpretation of GWA study (GWAS) results, helping to explore the functional roles of genetic variants within a biological context.[47] SNPs were annotated for functional consequences on gene functions using Annotate Variation (ANNOVAR), pathogenicity using Combined Annotation-Dependent Depletion score (CADD score), potential regulatory functions using RegulomeDB rank (RDB rank), and effects on gene expression using expression quantitative trait loci (eQTL) and then mapped to genes based on their physical position on the genomes, eQTL associations, and three-dimensional (3D) chromatin interactions. In addition, mapped genes were used for conducting gene-set enrichment analysis using GENE2FUNC function by using Kyoto Encyclopedia of Genes and Genomes (KEGG) and Molecular Signature Database in FUMA. False Discovery Rate (FDR) adjusted *p*-value of 0.05 was defined as a significant threshold for the gene-set to be considered statistically significant.

3 | RESULTS

The 375 NHW DS participants from the ABC-DS cohort were between 25 and 81 years of age. Of the 375 participants, 69% were cognitively stable, and 26% had dementia with *APOE* genotypes available in 370 and genome-wide genotype data in 330. The available Aβ biomarker data for genotype–phenotype analyses varied from 218 to 275 for GWA analyses, with mean age of 42 and 45 years for amyloid-PET and plasma Aβ biomarkers, respectively (Table 1). The frequency of *APOE4* carriers was higher in individuals with DS with dementia as compared to DS without dementia in the ABC-DS cohort (36.1% vs 19.7%; $p = 2.1E-03$; Table 1). The second independent sample of DS participants ($N = 133$) was derived from the omicsADDS cohort having plasma Aβ biomarkers data that were included in the meta-analysis. The omicsADDS cohort is slightly older (50.85 vs 45.13 years) and has more female participants (64% vs. 46%) than the ABC-DS cohort. Figure 1 summarizes the various analyses conducted in this study.

3.1 | Amyloid biomarkers in ABC-DS participants with and without dementia

Dementia was associated with significantly higher amyloid-PET Centiloid values ($p = 5.22E-15$) (Figure S2A). A similar but non-significant trend was seen for plasma Aβ42 ($p = 6.6E-02$) and Aβ40 ($p = 1.51E-01$) levels (Figure S2B–S2C), as well as for the Aβ42/Aβ40 ratio ($p = 9.62E-01$, Figure S2D). Pearson's correlation showed a strong correlation only between plasma Aβ40 and Aβ42 (Pearson $r = 0.76$, Figure S2E).

3.2 | Association of two *APOE* SNPs

Although *APOE4* showed the lowering effect on plasma Aβ42/40 ratio ($p = 4.30E-03$, $\beta = -0.36$; Table S1), it did not show the expected effect on the deposition of Aβ plaques in the brain as measured by amyloid-PET ($p = 3.98E-01$). On the other hand, *APOE2* revealed the expected lowering effect on amyloid-PET ($p = 1.78E-03$, $\beta = -13.45$; Table S1).

3.3 | Genome-wide single-trait analyses

Standard error–based meta-analyses were conducted for plasma Aβ biomarkers by combining the genome-wide summary statistics from the ABC-DS (Figures S3–S5) and omicADDS datasets. Quantile–quantile plot (QQ-plot) did not demonstrate population stratification for single-trait GWAS on plasma Aβ40, Aβ42, and Aβ42/40 ratio, and amyloid-PET (λ = 1.02, 1.011, 1.055, and 0.992, respectively)

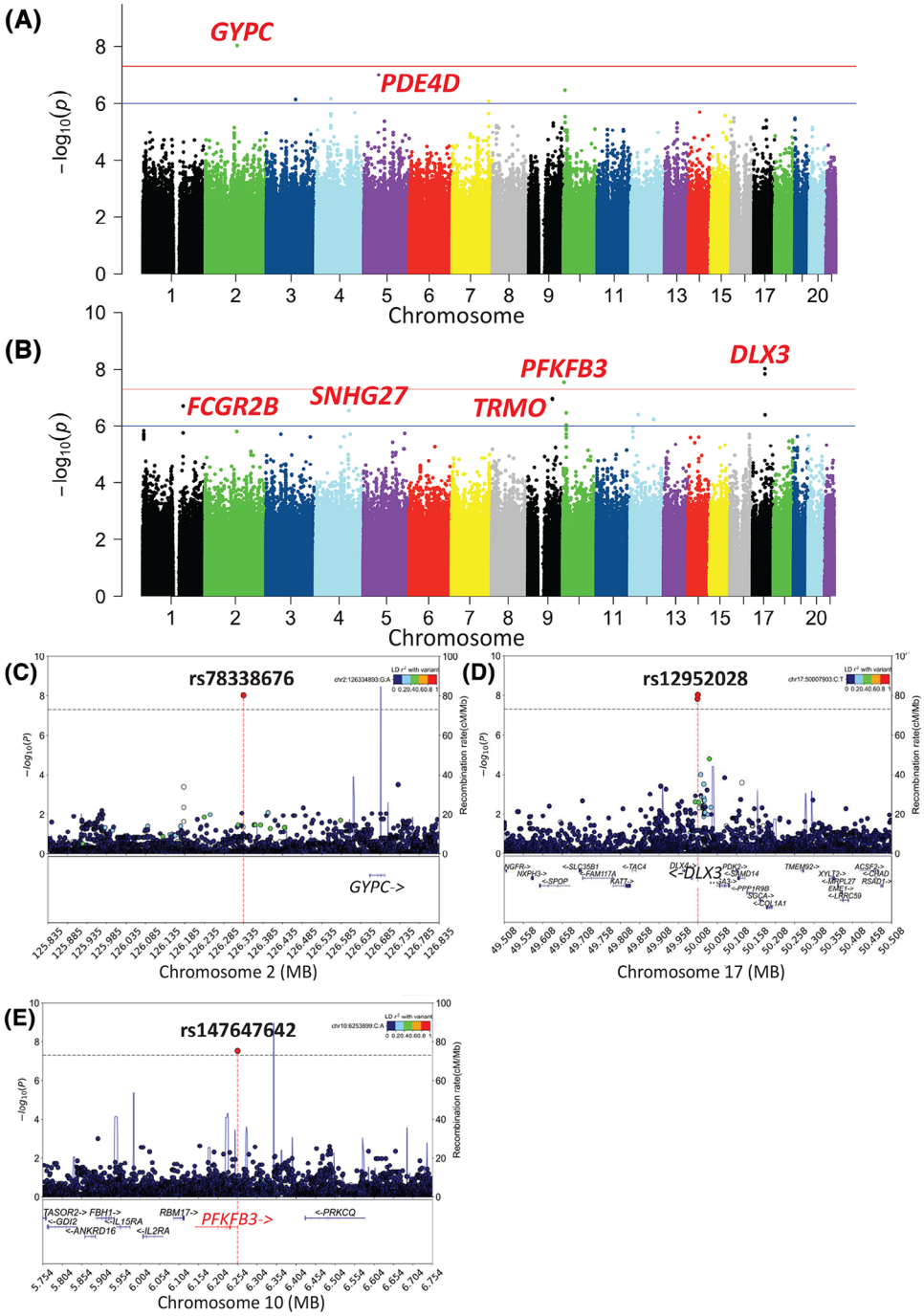

FIGURE 2 Manhattan plot illustrating the genome-wide *p*-values in Aβ40 (A) and Aβ42 (B). The red line depicts the genome-wide significance level ($p \leq 5E\text{-}08$), and the blue line represents suggestive associations ($p \leq 1E\text{-}06$). Regional plots of the association of plasma Aβ40 and Aβ42 meta-analyses on chromosomes 2, 10, and 17 (C–E). (C) Regional plot in the *LINC01941,GYPC* locus on chromosome 2 (Aβ40). (D) Regional plot in the *DLX3,ITGA3* locus on chromosome 17 (Aβ42). (E) Regional plot in the *PFKFB3* locus on chromosome 10 (Aβ42). Aβ, amyloid-beta.

(Figure S6). Manhattan plots in Figure 2A,B show the loci reaching genome-wide significance and subthreshold significance, and association results are summarized in Table 2 and Tables S2–S5. Meta-analysis for Aβ40 resulted in one GWS signal near *LINC01941,GYPC* on chromosome 2 (rs78338676, $p = 9.33E\text{-}09$, $\beta = -120.6$, MAF = 0.034) and one subthreshold GWS signal in the intronic region of *PDE4D* on chromosome 5 ($p = 9.97E\text{-}08$, $\beta = -143.4$, MAF = 0.013). Meta-analysis on Aβ42 showed two GWS loci. The strongest association was observed for rs12952028 located on chromosome 17 near *DLX3,PICART1* ($p = 9.31E\text{-}09$, $\beta = -1.28$, MAF = 0.491) followed by rs147647642 on chromosome 10 in an intron of *PFKFB3* ($p = 2.83E\text{-}08$, $\beta = -4.21$, MAF = 0.020). Meta-analysis on Aβ42/40 ratio did not result GWS sig-

TABLE 2 Novel loci associated with Aβ40, Aβ42, and Aβ42/40 ratio in meta-analyses of NHW DS participants.

								ABC-DS		omicsADDS		Meta-analysis	
CHR	Position (GRCh38)	Gene	MAF	Lead variant	Consequence	A1	A2	β	p	β	p	β	p
Aβ40													
2	126334893	*LINC01941, GYPC*	0.035	rs78338676	Intergenic	A	G	−130.2	1.49E-08	−49.06	1.12E-02	−120.6	**9.33E-09**
5	59376870	*PDE4D*	0.013	rs146261781	Intronic	A	G	−182.3	2.17E-07	−81.83	6.04E-02	−143.4	**9.97E-08**
Aβ42													
4	132567755	*SNHG27, LINC01256*	0.012	rs183216858	Intergenic	C	A	5.4	5.25E-05	8.44	1.54E-03	−6.01	2.95E-07
10	6253899	*PFKFB3*	0.020	rs147647642	Intronic	A	C	−3.92	2.41E-06	−6.22	3.96E-03	−4.21	**2.83E-08**
17	50007903	*DLX3, PICART1*	0.491	rs12952028	Intergenic	C	T	−1.28	2.11E-07	−1.34	3.40E-02	−1.28	**9.31E-09**
Aβ42/40 ratio													
17	72428504	*LINC00673*	0.407	rs2302740	ncRNA_intronic	T	C	0.339	6.42E-05	0.41	1.51E-03	0.36	2.42E-07
22	48212304	*LOC284930, MIR3201*	0.025	rs114630130	Intergenic	A	C	1.014	1.22E-04	0.88	1.62E-02	0.97	4.79E-06

Abbreviations: Aβ, amyloid-beta; A1, effect minor allele; A2, major allele; ABC-DS, Alzheimer's Biomarker Consortium–Down Syndrome; CHR, chromosome; MAF, minor allele frequency; NHW, non-Hispanic White; omicsADDS, Multiomic Studies of Alzheimer's Disease in Adults with Down Syndrome.

nal, but it did reveal two suggestive loci on chromosomes 17 and 22 (*p*-value range 4.70E-06–2.42E-07). Locus zoom plots for GWS signals for Aβ40 and Aβ42 are shown in Figure 2c–e.

GWA analysis for amyloid-PET Centiloid in the ABC-DS cohort identified five GWS loci on chromosomes 1, 7, 15, and 19. In addition, two subthreshold GWS loci were found on chromosomes 7 and 11. Of interest, all novel top signals were associated with elevating effects on amyloid-PET levels. The strongest novel signal, rs532620170, was present near *RHBDL2,AKIRIN1* on chromosome 1 (p = 2.90E-09, MAF = 0.012), followed by rs143578940 near *BAIAP2L1,NPTX2* on chromosome 7 (p = 4.92E-09, MAF = 0.012), *ZNF329,ZNF274*/rs148455801 on chromosome 19 (p = 5.86E-09, MAF = 0.019), *NEDD4,RFX7*/rs8024654 on chromosome 15 (p = 1.32E-08, MAF = 0.057) and *LOC107986794,POM121L12*/rs1880432 on chromosome 7 (p = 4.63E-08, MAF = 0.010). Although one subthreshold GWS signal was located near *LOC101927630,SNX13* on chromosome 7 (rs75431572; p = 6.35E-08, MAF = 0.013), the other signal was present in an intron of *CARD18* on chromosome 11 (rs7107383, p = 7.45E-08, MAF = 0.091) (Table 3, Figure 3, and Table S6). Locus zoom plots for GWS signals for amyloid-PET Centiloid are shown in Figure 4.

3.4 | Genome-wide multi-trait analysis

A multi-trait GWAS was performed on 199 DS participants from the ABC-DS cohort who had complete data on all plasma Aβ and amyloid-PET biomarkers. Plasma Aβ and amyloid-PET biomarkers were analyzed to identify pleiotropic loci that influence multiple biomarkers simultaneously. Variants in high linkage disequilibrium ($R^2 \geq 0.8$) with GWS signals were examined using the 1000 Genomes Project reference but revealed no overlapping variants, as they had low MAFs (ranging from 0.006 to 0.028 in 1000G and 0.010 to 0.019 in ABC-DS). QQ-plot did not demonstrate population stratification ($\lambda = 0.999$) (Figure S7). Two loci achieved GWS (Figure 5). The strongest association was observed for rs2033613 (p = 2.12E-10, MAF = 0.050) on chromosome 4 near *RNF150,ZNF330* (Table S7), which was also associated with plasma Aβ42/40 ratio in single-trait analysis (p = 6.22E-05, β = 0.767, MAF = 0.047). The next GWS signal, rs12796256, was observed on chromosome 11 near *IFTAP,LINC02760* (p = 2.23E-08, MAF = 0.050). Locus zoom plots for these two GWS signals are shown in Figure 6. It is noteworthy that one suggestive hit on chromosome 11, along with six linked-SNPs in and around *CARD18* (rs7107383, p = 1.86E-06, MAF = 0.075), was subthreshold GWS in single-trait analysis (p = 7.45E-08, β = 23.47, MAF = 0.091). One subthreshold GWS signal, rs538665664, was seen on chromosome 9 near *NFIL3,MIR3910-2* (p = 7.21E-08, MAF = 0.055), which also showed nominal associations in single-trait analyses (p = 1.71E-03, β = 15.84, MAF = 0.061), Aβ42/40 ratio (p = 2.23E-02, β = 0.40, MAF = 0.061), and Aβ40 (p = 4.4E-02, β = −33.1, MAF = 0.061). The multi-trait analysis has enabled us to identify pleiotropic loci for amyloid-PET and Aβ42/40 ratio.

3.5 | Comparison of amyloid-associated SNPs in DS with non-DS populations

We investigated the potential overlap of significant SNPs observed in DS at p < 1E-03 with the reported summary statistics for plasma Aβ[43] and amyloid-PET[16] biomarkers in non-DS populations. Only two

TABLE 3 Novel loci associated with amyloid-PET Centiloid in the ABC-DS NHW DS participants.

CHR	Position (GRCh38)	Gene	MAF	Lead variant	Consequence	A1	A2	β	p
1	38954124	*RHBDL2,AKIRIN1*	0.012	rs532620170	Intergenic	T	C	59.01	**2.90E-09**
7	98433501	*BAIAP2L1,NPTX2*	0.012	rs143578940	Intergenic	A	T	97.77	**4.92E-09**
7	51417431	*LOC107986794,POM121L12*	0.010	rs1880432	Intergenic	G	T	76.82	**4.63E-08**
7	17715281	*LOC101927630,SNX13*	0.013	rs75431572	Intergenic	G	A	58.09	6.35E-08
11	105138157	*CARD18*	0.091	rs7107383	Intronic	A	T	23.47	7.45E-08
15	56055077	*NEDD4,RFX7*	0.057	rs8024654	Intergenic	T	C	29.63	**1.32E-08**
19	58175781	*ZNF329,ZNF274*	0.019	rs148455801	Intergenic	G	A	44.75	**5.86E-09**

Abbreviations: A1, effect minor allele; A2, major allele; ABC-DS, Alzheimer's Biomarker Consortium–Down Syndrome; CHR, chromosome; MAF, minor allele frequency; NHW, non-Hispanic White.

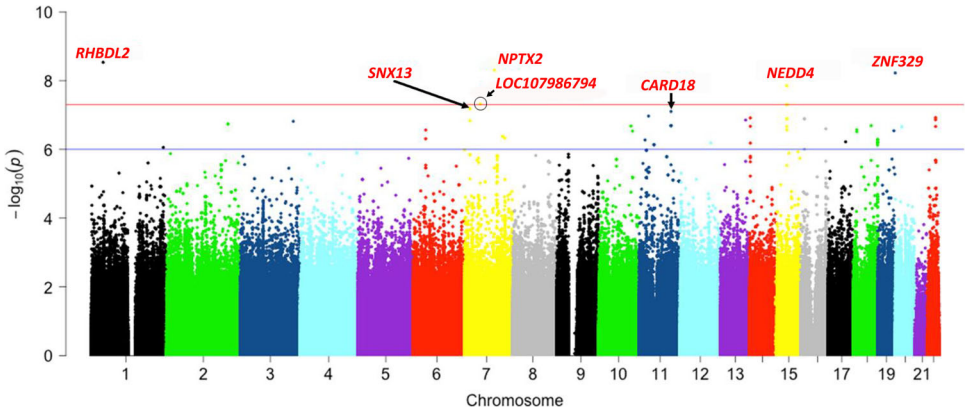

FIGURE 3 Manhattan plot illustrating the genome-wide *p*-values in amyloid-PET Centiloid. The red line depicts the genome-wide significance level ($p \leq$ 5E-08), and the blue line represents suggestive associations ($p \leq$ 1E-06). PET, positron emission tomography.

SNPs each for Aβ42, Aβ42/40 ratio and amyloid-PET in DS overlapped with non-DS populations (Table S8). This limited overlap of amyloid biomarkers between DS and non-DS populations suggested a partially shared, but vastly distinct genetic underpinnings of Aβ biology between DS and non-DS.

Next, we examined our top DS SNPs in the reported AD case–control data in non-DS and vice versa. The chromosome 2 top signal, *LINC01941,GYPC*/rs78338676, which showed association with Aβ40 in both single- (p = 9.33E-09) and multi-trait (p = 1.13E-04) (Table S2) analyses, was also associated with AD risk (p = 2.59E-02) (Table S9). Of the top 99 reported AD risk SNPs in the non-DS NHW population,[44,45] 77 were present in our ABC-DS dataset (Table S10); of which, nominal associations were seen with only 5 AD SNPs: *CR1*/rs6656401 on Chr1, *BIN1*/rs6733839 on Chr2, *LOC100996654,EGFR*/rs76928645 on Chr7, *MYO15A*/rs2242595 on Chr17, and *KLF16*/rs149080927 on Chr19. However, these associations of AD risk/protective alleles with amyloid biomarkers in DS were opposite from the expected directions and thus are not considered overlapping; for example, the AD risk allele of *CR1* was associated with lower amyloid-PET, and the AD protective allele of *EGFR* with higher amyloid-PET levels. Comparison of 77 AD SNPs with the ABC-DS case–control data found three significant associations with dementia in DS (*APOE4:* p = 5.76E-04, *MYO15A*/rs2242595: p = 9.95E-03, and *EGRFP*/rs76928645: p = 3.50E-02; Table S10); however, only the *APOE4* association was in the same direction as reported in non-DS. Furthermore, *APOE4* was not the top genetic risk factor for dementia in DS.

3.6 | Polygenic risk score

The PRS for AD in non-DS individuals was applied to our DS cohorts and was associated with higher amyloid-PET Centiloid level in DS (p = 1.7E-02; coefficient = 4.12), which remained significant after adjusting for the effect of *APOE4* (p = 3.9E-02; coefficient = 3.89) but became borderline non-significant after removing the *APOE* region (p = 0.1.13E-01; coefficient = 2.78) (Table S11). The PRS for AD was associated with protection against dementia in DS (p = 1.98E-02; coefficient = 0.37), which lost its significance after removing the *APOE* region, indicating that the euploid PRS as a whole was not predictive for dementia in the DS population.

3.7 | Functional annotations

To identify potential functional variants underlying Aβ biomarker levels, we screened both GWS and suggestively ($p \leq$ 1E-05) associated SNPs for evidence of functional relevance. This approach allowed us to capture additional variants that, although not reaching genome-wide

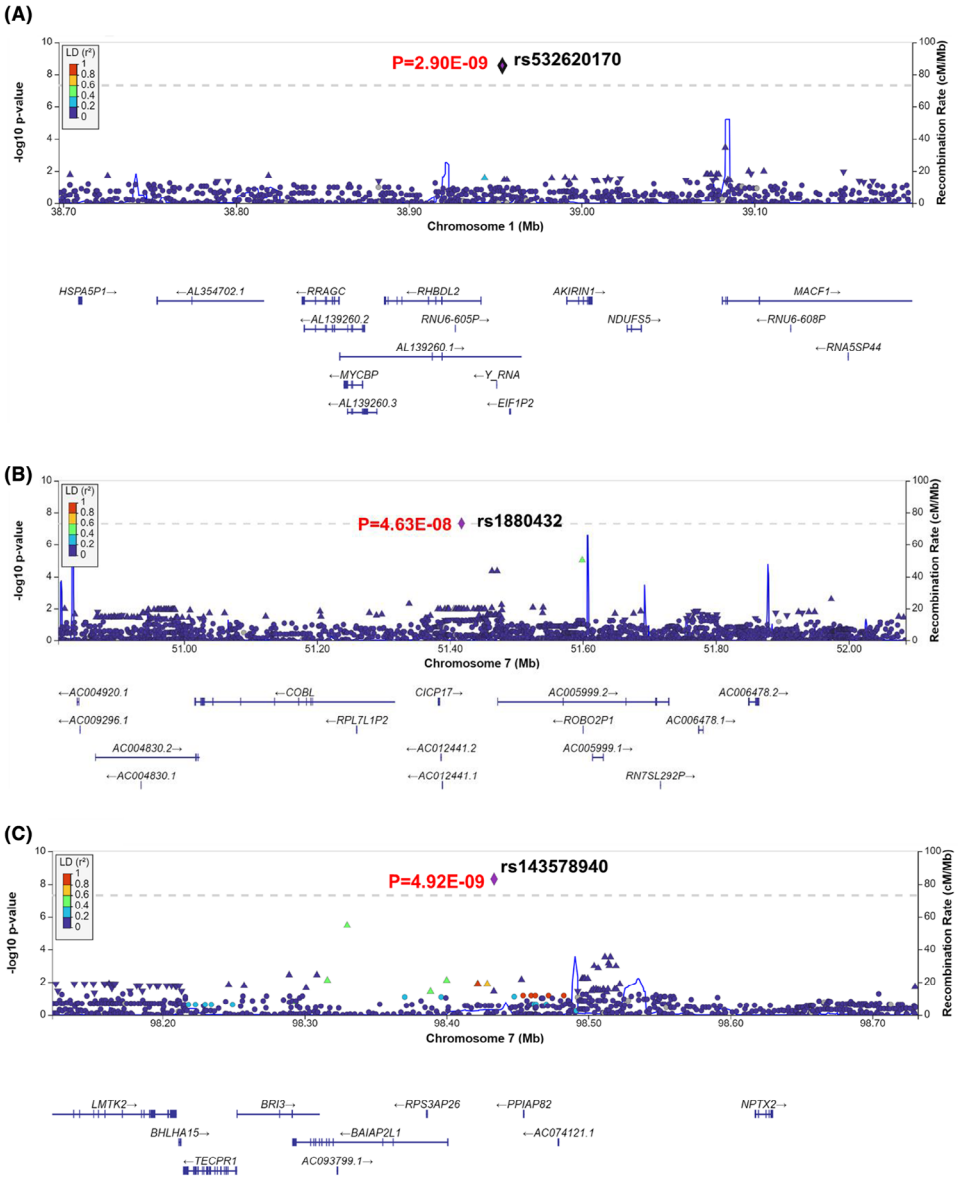

FIGURE 4 Regional plots of the association of amyloid-PET Centiloid on chromosomes 1, 7, 11, 15, and 19. (a) Regional plot in the *RHBDL2,AKIRIN1* locus on chromosome 1. (b) Regional plot in the *LOC107986794,POM121L12* locus on chromosome 7. (c) Regional plot in the *BAIAP2L1,NPTX2* locus on chromosome 7. (d) Regional plot in the *LOC101927630,SNX13* locus on chromosome 7. (e) Regional plot in the *CARD18* locus on chromosome 11. (f) Regional plot in the *NEDD4,RFX7* locus on chromosome 15. (g) Regional plot in the *ZNF329,ZNF274* locus on chromosome 19. PET, positron emission tomography.

significance, may play biologically meaningful roles based on functional annotations. The SNP rs79988196 on chromosome 19 (p = 3.7E-06), associated with Aβ40, has an RDB rank of 2b, suggesting it is likely to influence transcription factor binding or chromatin state. It also has a CADD score of 10.24, indicating that it may have mild functional effects. In addition, another exonic variant, rs1049948 (p = 5.70E-06), in the *PHC1* gene, which is suggestively associated with Aβ42/40, was found to have a CADD score of 28 and RDB rank of 2b, suggesting it could have a deleterious as well as regulatory effect. Gene-set enrichment analysis conducted using genes mapped for the Aβ42/40 ratio showed the enrichment of innate immunity and JAK/STAT kinase-related pathway (Table S12), highlighting the importance of innate immunity.

4 | DISCUSSION

The main objective of this study was to identify genetic markers that modulate plasma Aβ and amyloid-PET levels, key hallmarks of AD, in DS participants. To accomplish this goal, four main analyses were conducted. First, considering the established associations of two *APOE* SNPs (*APOE4*/rs429358 and *APOE2*/rs7412) with AD and Aβ plaques in the non-DS population, we investigated their associations with plasma and neuroimaging Aβ biomarkers in DS. Second, single-trait GWA analyses were conducted to identify novel loci mediating plasma and neuroimaging Aβ biomarker levels. Third, a multivariate regression approach was employed to enhance statistical power by accounting for the correlations between four amyloid phenotypes (amyloid-PET,

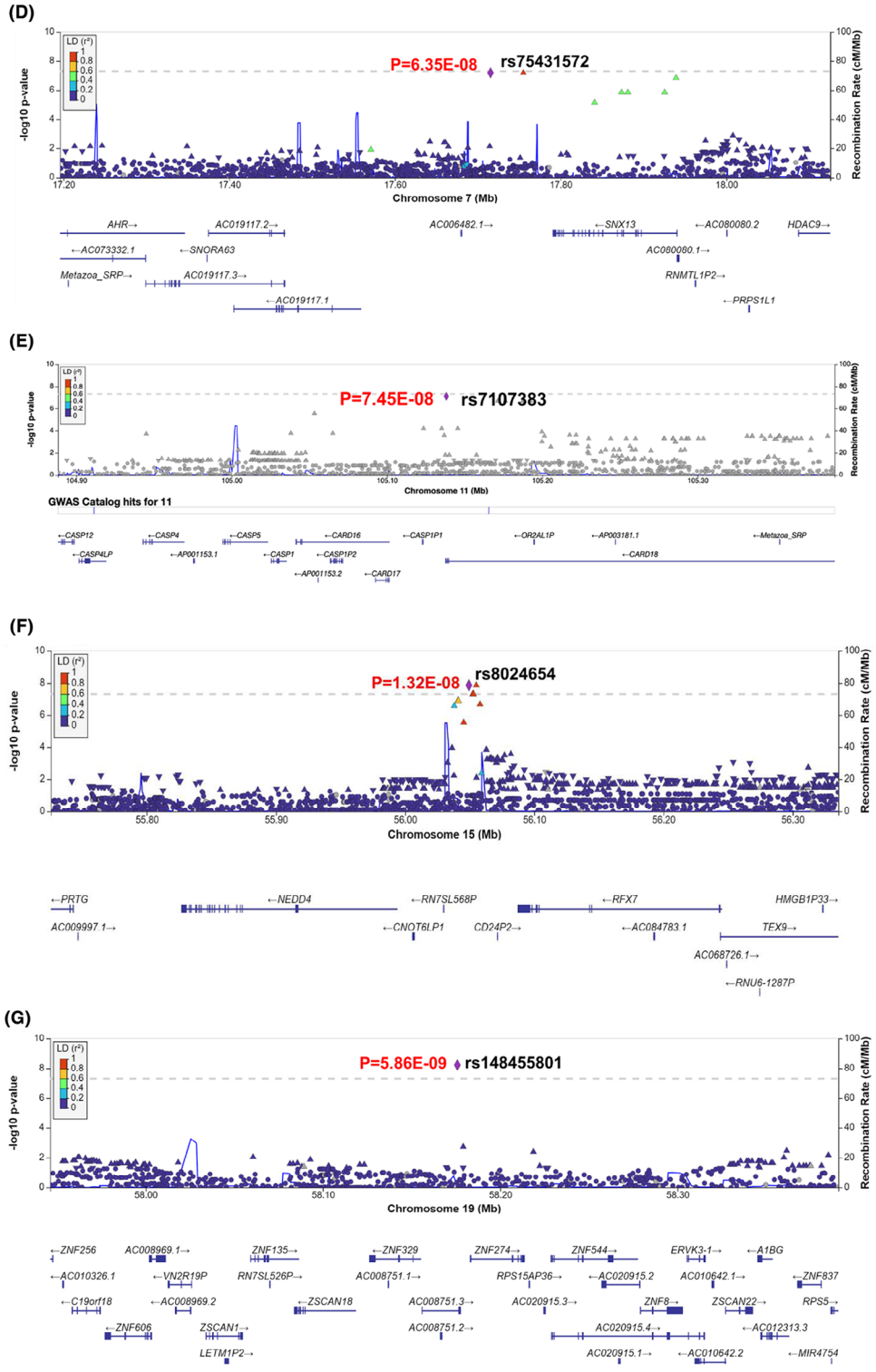

FIGURE 4 Continued

Aβ40, Aβ42, and Aβ42/40 ratio) to identify pleiotropic loci. Fourth, in silico functional analyses were performed on significant variants to identify potential biological processes underlying variation in Aβ biomarkers and consequently the dementia risk in DS. In addition, we investigated the contribution of Alzheimer's PRS in modulating plasma and neuroimaging Aβ biomarkers in DS and examined the potential correlations between genetic variants identified in DS to those reported in the non-DS population for Aβ biomarkers and AD risk.

Unlike the reported strong association of *APOE4* with amyloid-PET,[14,16] CSF Aβ42,[15] and plasma Aβ42 and Aβ42/40 ratio[48] in the non-DS population, it was not significant in our DS participants. On the other hand, *APOE2* showed the expected protective effect against the

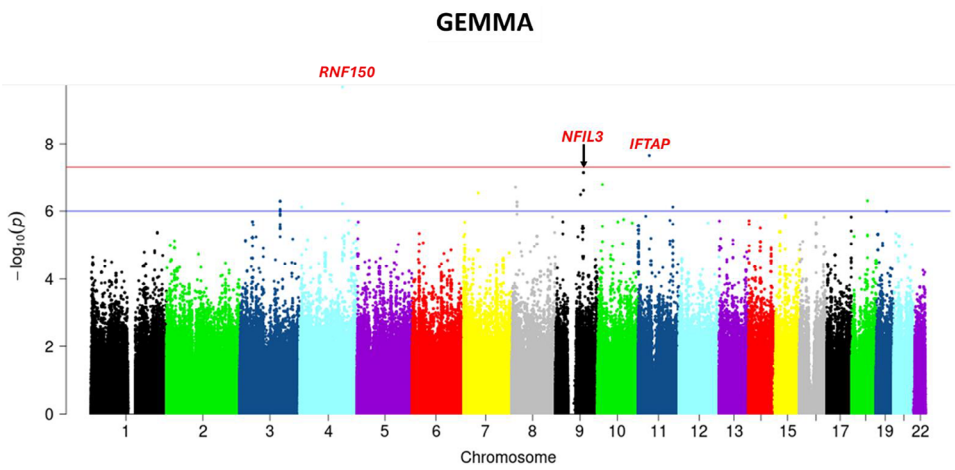

FIGURE 5 Manhattan plot illustrating the genome-wide *p*-values in GEMMA combining plasma Aβ40, Aβ42, Aβ42/40 ratio, and amyloid-PET. The red line depicts the genome-wide significance level ($p \leq$ 5E-08), and the blue line represents suggestive associations ($p \leq$ 1E-06). Aβ, amyloid-beta; PET, positron emission tomography.

deposition of Aβ plaques in DS brains, albeit with far less significance than shown in non-DS subjects.[14–16] Although *APOE4* is associated with dementia risk in DS, unlike in non-DS, where it is always the top SNP, its significance position (p = 5.76E-04) was 2462 among the selected SNPs of 5281, with $p <$ 1E-03. *APOE2*, which normally shows a highly significant protective effect against AD in non-DS, was not significant in DS (p = 2.16E-01). Similarly, some AD-associated SNPs that revealed an association with amyloid biomarkers were not in the expected direction. Overall, these data suggest that although some of the known AD genes overlap in DS, the genetic architecture of biomarkers and dementia in DS may be distinct.

Single-trait meta-analyses of plasma Aβ biomarkers from two independent DS datasets yielded three GWS ($p \leq$ 5E-08), one subthreshold GWS (p = 9.97E-08), and three suggestive ($p \leq$ 1E-05) signals, having the same directional allelic effects in both samples. The top SNP for Aβ40 (*GYPC* /rs78338676, p = 9.33E-09) was also associated with reduced plasma Aβ42 levels (p = 1.59E-06) and showed association in multi-trait analysis (p = 1.13E-04) and with AD dementia in DS (p = 4.74E-02, OR = 2.94). This finding was further corroborated by its nominal association (p = 2.59E-02; OR = 1.06) in the largest AD case–control data.[44] *GYPC* (glycophorin C) encodes for glycophorin C and glycophorin D.[49] Significant methylation changes in *GYPC* are observed in the plasma of patients with ovarian cancer compared to those without the disease, highlighting its potential role as a biomarker,[50] which needs to be evaluated in DS. Although direct evidence linking *GYPC* to AD-DS is limited, its role in maintaining red blood cell membrane integrity under oxidative stress conditions, as well as its involvement in cell adhesion processes, suggests potential implications in AD-DS pathology.[51,52] These functions highlight the importance of *GYPC* in cellular integrity and signaling, calling for further exploration of its potential contributions to neurodegenerative disorders. *PDE4D*/rs146261781, an intronic subthreshold GWS signal for Aβ40 (p = 9.97E-08), was also associated with all Aβ in multi-trait analysis (p = 8.10E-08). *PDE4D* (hosphodiesterase-4D) is involved in mediating memory processes and promoting hippocampal neurogenesis. This is a good candidate gene, as overexpression of *PDE4D* has been implicated in AD, where it contributed to cognitive impairments and disrupted neural regeneration in the hippocampus.[53]

One of the two GWS loci identified for Aβ42, *PFKFB3*/rs147647642 (p = 2.83E-08), which is also associated with lowering Aβ40 (p = 3.40E-07), seems to be directly implicated in modulation of Aβ levels. *PFKFB3* (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3) codes for one of the major pro-glycolytic enzymes PFKFB3, which is abundantly present in astrocytes and is involved in modulation of Aβ and neurodegeneration, and thus has been suggested to be a promising therapeutic target for AD.[53] Knockdown mouse models (Pfkfb3 ± mice) of *PFKFB3* demonstrate a protective effect against retinal pigment epithelium (RPE) disorders and retinal damage caused by Aβ-induced microglial activation. The partial loss of *PFKFB3* reduces microglial pro-inflammatory activity, mitigates RPE senescence, and preserves retinal structure and function. These findings suggest that targeting *PFKFB3*-mediated pathways in microglia could alleviate inflammation and protect against retinal degeneration in age-related macular degeneration.[54] The second GWS signal for Aβ42, rs12952028 (p = 9.31E-09), is intergenic between *DLX3* and *PICART1* and was also associated with Aβ40 (p = 6.71E-06). *DLX3*,*PICART1*/rs12952028 has an RDB rank of 1f, implying strong evidence of regulatory activity and possibly impacting gene regulation. rs12952028 is an eQTL for *DLX3* (Distal-Less Homeobox 3) (http://www.mulinlab.org/qtlbase). Dlx3 is crucial for placental development and embryonic survival, as its deletion disrupts the placental morphogenesis by downregulating Esx1 expression.[55] The top signal for Aβ42/40 ratio, rs2302740 (p = 2.42E-07), is an intronic variant in a long non-coding RNA, *LINC00673*, expressed in the brain.[56] *LINC00673* has been suggested to be a promising clinical diagnostic and prognostic biomarker in cancer treatment[57] and should be further investigated in AD and DS.

Our gene-set enrichment analysis conducted on genes mapped to AB42/40 ratio highlighted the role of innate immune response

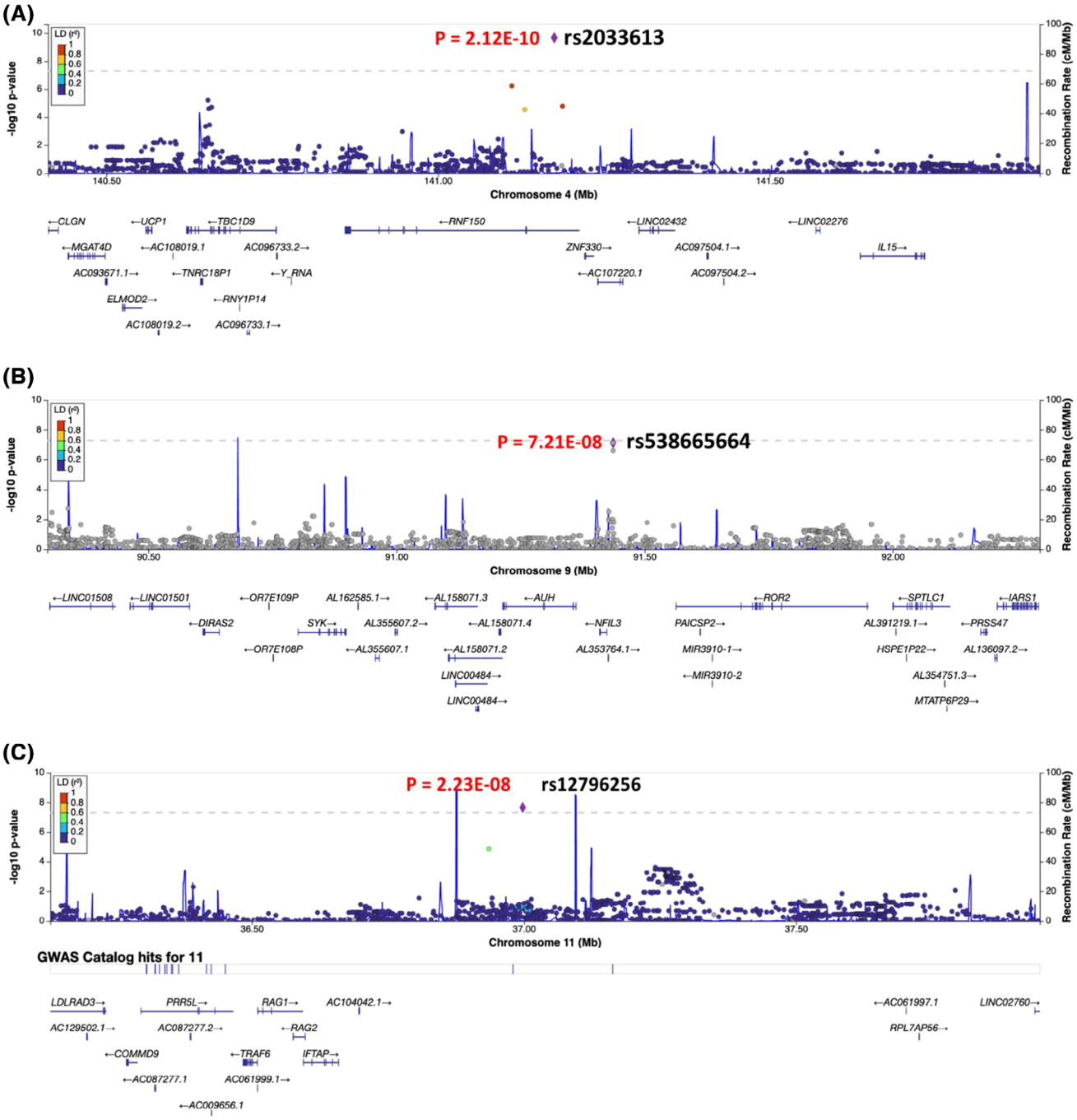

FIGURE 6 Regional plots of the association multi-trait GWAS on chromosomes 4 and 11. (a) Regional plot in the *RNF150,ZNF330* locus on chromosome 4. (b) Regional plot in the *NFIL3,MIR3910-2* locus on chromosome 9. (c) Regional plot in the *IFTAP,LINC02760* locus on chromosome 11.

along with JAK/STAT pathway, similar to findings from prior AD and DS studies.[44,58,59] JAK/STAT is a key signaling pathway involved in modulation of pro-inflammatory and inflammatory signals, which is also known to be involved in an array of functions including cell development and differentiation.[60] A recent study has shown that downregulation of JAK/STAT could reduce autoimmune burden in patients with DS.[61]

For amyloid-PET, no replication DS data were available and so the results should be considered provisional. As summarized in Table 2, we identified five GWS ($p \leq$ 5E-08) and two subthreshold GWS (p = 7.45E-08-6.35E-08) loci on chromosomes 1, 7, 11, 15, and 19, and all were associated with elevating amyloid-PET levels. *RHBDL2,AKIRIN1*/rs532620170, *BAIAP2L1,NPTX2*/rs143578940, and *ZNF329,ZNF274*/rs148455801 are novel intergenic variants with RDB ranks of 2b, 1b, and 1f, respectively, indicating their strong regulatory potential. NPTX2 supports synaptic development and plasticity, playing a critical role in maintaining neuronal circuit function. Higher CSF NPTX2 in early MCI shows synaptic compensation to AD pathology,

which diminished with progression. These findings suggest CSF NPTX2 as a biomarker for AD staging and progression.[62] Both *ZNF329* and *ZNF274* belong to the family of zinc finger protein coding genes, which seem to be relevant to brain tumors.[63]

Multi-trait analysis identified several pleiotropic loci with shared effects across multiple amyloid biomarkers. One of these loci, *RNF150*, is associated with chronic obstructive pulmonary disease,[64] gastric cancer,[65] and schizophrenia.[66]

From single-trait analyses, only two SNPs for each amyloid biomarker in DS, except for Aβ40, overlapped with non-DS populations. *LINC01339*/chr5:90234654:TA:T and *LINC01309,DAOA-AS1*/rs116934951 for Aβ42, *LINC01307,OLFM3*/rs59552580 and *ABCA6*/rs62082731 for Aβ42/40 ratio, and *MRAP2*/rs77779050 and *MRAP2*/rs78271721 for amyloid-PET Centiloid, were significant in both DS and non-DS populations with the same direction of effect. The limited overlap of Aβ-associated SNPs between DS and non-DS highlights partial, yet vastly distinct, genetic mechanisms influencing Aβ dynamics. However, the limited overlap suggests that the genetic landscape of AD in individuals with DS may be distinct, likely due to the trisomy of chromosome 21, which directly influences *APP* dosage. This implies that although certain risk loci are shared between DS and non-DS populations, the unique genetic and biological context of DS shapes Aβ-related pathways differently, emphasizing the need for tailored research in DS-specific AD risk.

This study is notable for its focus on individuals with DS, a population at a higher risk for AD due to trisomy 21. By integrating both amyloid-PET and plasma Aβ biomarkers in GWA analyses from two independent cohorts, we identified 14 potential novel loci linked to Aβ accumulation in individuals with DS. However, several caveats and limitations must be acknowledged. Novel genetic factors identified in this study may be unique to people with DS due to their unique genetic background of trisomy 21, and thus may not necessarily apply to non-DS individuals. A primary limitation is the small sample size, especially for amyloid-PET, which may reduce statistical power, increase the risk of false positives and false negatives, and limit the generalizability of our findings. Nonetheless, given the inherent challenges of recruiting individuals with DS for research, such as the rarity of the condition, specialized recruitment requirements, and ethical considerations, our study represents one of the largest DS cohorts. Furthermore, due to the limited sample size and the use of a MAF threshold of 1%, some identified variants may yield less reliable statistical estimates and be subject to potential biases. When variant carriers are few, even minor fluctuations in biomarker values can lead to overestimating effect sizes. Given these limitations, the interpretation of these results should be approached with caution. Future studies with larger sample sizes are needed to validate these associations. Finally, the study's inability to fully account for all potential confounding factors, such as environmental influences and other genetic variables, may limit the precision of the results.

In conclusion, we identified multiple novel loci associated with amyloid biomarkers in DS. These results emphasize the significance of studying the DS population as a promising avenue for identifying genetic factors involved in AD pathology.

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CONFLICT OF INTEREST STATEMENT

Bradley Christian receives positron emission tomography (PET) precursor and compounds from Avid Radiopharmaceuticals, and equipment from Cerveau Technologies. Benjamin Handen receives funding from National Institute of Child Health and Human Development, Autism Speaks, Roche Pharmaceuticals, and Patient-Centered Outcomes Research Institute (PCORI). Mark Mapstone is an inventor on patents related to biomarkers of neurodegenerative diseases owned by Georgetown University and the University of Rochester. The other authors declare no conflict of interest. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

All participants in the Alzheimer's Biomarkers Consortium-Down Syndrome (ABC-DS) cohort provided informed consent as required by

the grant proposal and approved by the relevant institutional review board. All study procedures were performed in accordance with the Declaration of Helsinki ethical principles. Data obtained from publicly available resources did not require consent, as these datasets contain no personal information and are limited to summary statistics.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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