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RESEARCH ARTICLE

Common genetic signatures of Alzheimer's disease in Down Syndrome [version 1; peer review: 2 approved, 2 approved with reservations]

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Abstract

Background: People with Down Syndrome (DS) are born with an extra copy of Chromosome (Chr) 21 and many of these individuals develop Alzheimer's Disease (AD) when they age. This is due at least in part to the extra copy of the *APP* gene located on Chr 21. By 40 years, most people with DS have amyloid plaques which disrupt brain cell function and increase their risk for AD. About half of the people with DS develop AD and the associated dementia around 50 to 60 years of age, which is about the age at which the hereditary form of AD, early onset AD, manifests. In the absence of Chr 21 trisomy, duplication of *APP* alone is a cause of early onset Alzheimer's disease, making it likely that having three copies of *APP* is important in the development of AD and in DS. In individuals with both DS and AD, early behavior and cognition-related symptoms may include a reduction in social behavior, decreased enthusiasm, diminished ability to pay attention, sadness, fearfulness or anxiety, irritability, uncooperativeness or aggression, seizures that begin in adulthood, and changes in coordination and walking.

Methods: We investigate the relationship between AD and DS through integrative analysis of genesets derived from a MeSH query of AD and DS associated beta amyloid peptides, Chr 21, GWAS identified AD risk factor genes, and differentially expressed genes in DS individuals.

Results: Unique and shared aspects of each geneset were evaluated based on functional enrichment analysis, transcription factor profile and network analyses. Genes that may be important to both disorders: *ACSM1*, *APBA2*, *APLP1*, *BACE2*, *BCL2L*, *COL18A1*, *DYRK1A*, *IK*, *KLK6*, *METTL2B*, *MTOR*, *NFE2L2*, *NFKB1*, *PRSS1*, *QTRT1*, *RCAN1*, *RUNX1*, *SAP18*, *SOD1*, *SYNJ1*, *S100B*.

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Conclusions: Our findings indicate that oxidative stress, apoptosis, and inflammation/immune system processes likely underlie the pathogenesis of AD and DS.

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article can be found at the end of the article.

Keywords

Alzheimer's Disease, Down Syndrome, Behavior, Memory, Learning

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Introduction

Amyloids are peptide or protein aggregates that form from the misfolding of normally soluble proteins, which then stick together due to their chemical properties and accumulate in extracellular compartments and organs¹. Amyloids form fibrous structures and plaques that are highly insoluble, resistant to degradation, and are involved in several diseases such as Alzheimer's disease (AD), Down syndrome (DS), spongiform encephalopathies, and type II diabetes^{2,3}. The amyloid plaques associated with AD are formed from peptides derived from the mis-processing of APP, a protein that typically resides around nerve cells². The toxic peptide fragments are called beta amyloids. In AD, the amyloid plaques deposit in brain tissue, destroy neuronal connectivity, disrupt signaling at synapses, and eventually result in nerve cell death, tissue loss, and a reduction in brain mass². Smaller aggregates of beta-amyloid and not the plaques themselves trigger the immune system and inflammatory processes⁴.

Early onset AD that runs in families is linked to the *APP* and *PSEN1/PSEN2* genes⁵. A mutation in one of these three genes may cause AD to develop early whereas the more general form of the disease, late onset, is typically linked to the *APOE* gene⁶. *PSEN1/PSEN2* are transmembrane proteins that are the catalytic subunit of gamma secretase, the enzyme responsible for cleaving APP. Mutations in the *PSEN* genes may result in the abnormal cleaving and processing of APP to smaller toxic beta amyloid fragments which aggregate and accumulate^{7,8}.

People with Down Syndrome (DS) are born with an extra copy of chromosome 21 (Chr 21) and many of these individuals develop AD as they age⁹. This is due at least in part to the extra copy of the *APP* gene located on Chr 21. By the age of 40, most people with DS have amyloid plaques which disrupt brain cell function and increase their risk for AD³. About half of the people with DS develop AD and the associated dementia around 50 to 60 years of age, which is about the age at which the hereditary form of AD, early onset AD, manifests¹⁰. Duplication of *APP* alone, in the absence of Chr 21 trisomy, is another cause of early onset AD^{11,12} making it likely that having three copies of *APP* is important in the development of AD in DS.

In both early and late onset AD the clinical symptoms include dementia, memory decline, and the inability to retain recent information or store new memories¹³. As the disease progresses, AD individuals may exhibit problems with language, reasoning, decision making, executive function, mood swings, aggressive behavior, and apathy. Late stage symptoms of AD may result in seizures, hypertonia myoclonus, incontinence, and mutism¹⁴. **Death commonly occurs from general inanition, malnutrition, and pneumonia.**

Memory loss and forgetfulness, which is typical in AD individuals, is less pronounced in people with both DS and AD. This may in part be a floor-effect due to the memory deficit already present in DS individuals¹⁵⁻¹⁷. Studies report an impairment in verbal short-term memory (example: serial order of a list

of words) relative to visuo-spatial memory (manual selection in serial order of locations) and deficits in explicit long-term memory¹⁸. Also in individuals with DS there is evidence of hippocampal dysfunction and deficits in prefrontal systems as compared with mental age-matched controls¹⁹. In DS individuals, the hippocampal volume is reduced prior to the onset of dementia, and these reductions were found to relate to memory mainly due to the loss of neurons and neuronal volume as a result of neurofibrillary tangle formation²⁰.

In this study we investigate the relationship between AD and DS through integrative geneset analysis of genes derived from peptides associated with amyloid plaques found in AD and DS individuals, Chr 21 genes, AD risk factor genes, and differentially expressed genes (DEX) identified through a transcriptome analysis of DS individuals for both the dorsal frontal cortex (DFC) and cerebellar cortex (CBC).

Methods

Geneset characteristics

All genesets used in this study are presented in *Extended data Workbook 1*²¹. The Chr 21 geneset was obtained from NCBI Gene. A total of **250 unique gene IDs** were obtained at the time of manuscript preparation (September 1st, 2020). The AD-DS geneset, consisting of 292 genes, was obtained from **GeneWeaver** using "Alzheimer's Down Syndrome" as the search term. The geneset was originally generated via **gene2mesh** v.1.1.1 (updated: 2019-01-07) from Medical Subject Headings (MESH Terms) GS236695 • [MeSH] Amyloid beta-Peptides: **D016229**. The AD risk factor geneset is comprised of 279 genes, many of which were identified and/or confirmed through a large scale GWAS of 71,880 clinically diagnosed AD and AD-by-proxy cases and 383,378 controls²².

The DEX genesets for the DFC (842) and CBC (570) were obtained from **The Down Syndrome Developmental Brain Transcriptome database**. Human Brain Transcriptome, Department of Neurobiology Yale University School of Medicine which is a publicly accessible database containing searchable differential gene expression information of transcriptome data in developing and adult DS versus control human brains. The data was generated from 15 sets of a DS and a matched control brain each. The specimens ranged from embryonic development to adulthood²³.

Geneset overlap

Common genes among the AD-DS, Chr 21, DEX DFC, DEX CBC and AD risk factor genesets were assessed using venn diagram analysis (<http://www.interactivenn.net/>) and visualized with the UpSet Library in **RStudio**, R Version 4.0.2.

Keyword categories

Keyword categories were used to evaluate the genesets. The keyword categories were chosen based on the major phenotypes associated with AD and DS. The terms used were: aging, Alzheimer's disease, amyloid; apoptosis, behavior cholesterol, circadian, cognition; Down Syndrome, face, fibril, immune, inflammation, insulin, learning, leptin, memory, muscle, myelin; obesity, sleep, speech, and tau.

Functional analyses

Gene ontology characterization of the genesets was performed in both DAVID and the Gene Ontology database for Biological Process (BP). The Benjamini corrected P-value was used to determine enrichment significance. Functional information based on GO annotations for the genes associated with a keyword search term related to AD and DS were identified and noted.

Gene Ontology pathway enrichment was used to further characterize the AD-DS and Chr21 genesets in order to obtain a broader overview of collective gene function. The Benjamini-corrected P-value was used to determine significance.

APP protein interaction network

The APP protein-protein interaction network was built in STRING (version 11.0), based on experimentally validated interactions. The combined scores for the interactions are computed by combining the probabilities from the different evidence channels and corrected for the probability of randomly observing an interaction. First and 2nd shell interactions are included in the network. The network was exported from STRING and analyzed in Cytoscape (version 3.7). Network bottlenecks and clusters were identified with Cytoscape plugins CytoHubba (version 0.1) and MCODE (version 1.6.1), respectively.

Results

Geneset overlap

The number of common genes among all of the 5 genesets (AD-DS, Chr 21, AD risk factors, DEX DFC, and DEX CBC) along with the gene names and Gene Ontology classifiers are shown in Figure 1 and Extended data Workbook 2²⁴. The AD-DS, Chr 21 and AD risk factor genesets overlap by eight genes: *APP*, *BACE2*, *COL18A1*, *DYRK1A*, *RCAN1*, *SOD1*, *SYNJ1*, and *S100B* (Figure 1). *BACE2* encodes an integral membrane glycoprotein that cleaves the APP protein into amyloid- β , a critical step in the cause of AD and DS. *COL18A1* encodes the alpha chain of type XVIII collagen. It is associated with vascular deposits and senile plaques in AD brains²⁵. The *DYRK1A* gene product can phosphorylate APP and alter the protein's stability and the formation of amyloid- β ^{26,27}. Increased *RCAN1* expression is associated with neuronal death and Tau hyperphosphorylation, as well as neurofibrillary tangle formation in DS and AD individuals²⁸.

SOD1 is the only gene present in all of the genesets. *SOD1* is associated with apoptosis and oxidative stress²⁹. The extra copy of *SOD* on Chr 21 results in increased gene expression and increased production of H_2O_2 which is believed to underlie many of the DS-related pathologies²⁹. *SOD1* is also associated with neurodegeneration in amyotrophic lateral sclerosis and AD^{30,31}. *SYNJ1* encodes a lipid phosphatase

Figure 1. GeneSet overlap. UpSet plot showing geneset overlap highlighting gene content similarity between the AD-DS, Chr21, AD risk factors DEX DFC, and DEX CBC genes..

that is involved in autophagosomal/endosomal trafficking and synaptic vesicle recycling. Its dysfunction has been linked to several neurodegenerative diseases, including AD and DS³². *S100β* belongs to a family of cytokines that are strongly associated with activity underlying AD related pathologies such as APP processing, protein inclusion formation, and Tau post-translational modifications. *S100β* is also linked to DS. *S100β* levels are increased in neuronal progenitor cells of DS patients³³ and in human induced pluripotent stem cells derived from DS patients³⁴. Two additional genes, *KLK6* and *BCL2L*, are shared among the AD-DS, AD risk factors, DEX DFC and DEX CBC genesets. *KLK6* has been proposed as a biomarker for AD³⁵. *BCL2L* is located on the outer mitochondrial membrane and is a negative regulator of apoptosis³⁶.

Keyword enrichment

Each of the genesets were evaluated for association with AD and DS related phenotypes (Figure 2 and Extended data, Workbook 3³⁷). The keyword categories shared among all genesets are muscle, immune, insulin, glucose, behavior, oxidation and heart. The AD-DS geneset has a high frequency of genes associated with most of the keyword categories. The largest represented categories are: AD, muscle, inflammation/immune system, insulin, amyloid, behavior, aging, learning/memory, circadian processes and face/facial features. There were no genes directly associated with DS. For the Chr 21 geneset, unlike the AD-DS geneset, there were very few genes associated with the keyword categories. The highest frequency categories are immune, muscle, aging, behavior and insulin. Three genes are connected to AD (*NDUFV3*, *APP* and *BACE2*) and one with DS (*DSCAM*).

The enriched keyword categories for the DEX DFC are very similar to the results obtained for the AD-DS geneset: muscle, inflammation/immune system, insulin, aging, face/facial features, behavior, AD, and learning/memory. There are 13 genes

directly associated with AD (*NDUFS2*, *APAF1*, *BACE1*, *CACNA1F*, *COX5B*, *COX6A2*, *GRIN1*, *GRIN2A*, *LPL*, *PLD3*, *PSEN1*, *RYR3*, *UQCRC1*) and one gene associated with DS (*DSCR9*). For the DEX CBC geneset the most representative categories are again similar to the AD-DS geneset as well as the DEX DFC geneset: muscle, immune/inflammation, insulin, behavior, face/facial features, aging and amyloid. There are four genes directly linked to AD (*ATP5H*, *APOE*, *APAF1*, *RYR3*). There are no genes directly associated with DS.

Behavior-related genes

Given that behavioral phenotypes are highly shared between AD and DS, the specific types of behaviors identified from the keyword enrichment were evaluated more in depth. The AD-DS geneset has a large number of behavior related genes and genes related to learning and memory: (Behavior 33, Learning 26, Memory 21). This observation is based on the GO results obtained for three random genesets of the same size: Behavior 7,2,1; Learning 0,1,1; Memory: 0,0,1. The behavior gene categories are diverse and include fear, locomotion, eating and feeding, addiction related (nicotine, cocaine, ethanol), social, and others such as circadian, mating, and response to pain. The learning categories include visual learning, associative learning, and also olfactory, motor, and nonassociative learning. The memory related categories are short-term and long-term memory, and in one instance, susceptibility to memory impairment (Figure 3).

Functional analyses

Many of the significant BP enrichment classifiers for the AD-DS geneset are associated with cell death ($P=3.01E-83$), apoptosis ($P=1.30E-70$) and inflammation/immune system ($P=1.65E-36$). For the Chr21 geneset, the significant BP enriched terms are linked to keratin (keratinization, $P=1.04E-37$), skin (skin development ($P=2.83E-29$) and epithelium processes ($P=3.19E-15$) as well as tissue ($P=3.56E-14$), organ ($P=3.40E-09$)

Figure 2. Keyword enrichment. Identification of genes associated with terms relating to AD and DS based on gene ontology term classification. AD-DS genes: blue, Chr 21 genes: orange, DEX DFC genes: green, DEX CBC genes: gray, X-axis, keyword categories; Y-axis, frequency of occurrence in the geneset.

Figure 3. Evaluation of GO classification terms based on behavioral, memory, and learning categories. Comparison of frequencies of behavior related genes in the AD-DS, Chr 21, DEX DFC, and DEX CBC genesets. X-axis, geneset; Y-axis, frequency of occurrence (percentage of total genes) in the geneset.

and anatomical structure development ($P=8.66E-09$). The significant pathways associated with the AD-DS geneset are related to neurodegenerative disorders (AD $P=3.1E-23$, Parkinson's disease ($P=1.39E-04$) and Huntington's disease $P=1.36E-07$) as well as many signaling pathways linked to insulin ($P=1.86E-09$) and inflammation (Jak/Stat $P=9.49E-04$, Toll receptor ($P=4.04E-10$), Interferon-gamma signaling ($P=8.90E-06$). There were no significant pathways associated with Chr 21. All pathways are listed in *Extended data Workbook 4*³⁸.

Transcriptional profile

The transcription profiles of the AD-DS and Chr 21 genesets were evaluated here and compared with the DEX genesets which were previously evaluated by Olmos-Serrano *et al.*²³ (*Extended data, Workbook 5*³⁹). There are 64 transcription factors present in the AD-DS geneset. Several of these are directly associated with AD (*GSK3B*, *IL1B*, *MAPK3/8/10/14*, *WNT1*, *WNT3A*, *KAT5*, *NOTCH1* and *TNF*), Tau (*GSK3B* and *CLU*) and amyloid (*CD36*, *NLRP3*, *CLU*, *FOXO3*, *PARP1*, *PRNP*). Of these, many are related to mitochondria processes (*AKT1*, *CLU*, *GSK3B*, *HIF1A*, *MAPK3,8,10,14*, *MTOR*, *NFKB1*, *PPARGCIA*, *PARP1*, *PRNP*, *PRKCA*, *SIRT1*, *STAT3*, *SREBF2*, *UBB*) and also inflammation, oxidative stress, and aging (*TP53*, *STAT1/3*, *NFKB1*, *HIF1A*, and *NEF2L2*).

For the Chr 21 geneset, 18 transcription factors were identified. *RUNX1* which is associated with ossification⁴⁰ and nervous system development⁴¹ observed comparable expression in a study comparing AD and DS brains. Gene variants of *RUNX1* are associated with both AD and DS⁴². The *OLIG1/OLIG2* transcription factors regulate oligodendroglial

differentiation and myelination and neuron fate commitment⁴³. In DS, due to the gene triplication, *OLIG1/OLIG2* causes alterations in brain development⁴⁴. *OLIG2* is associated with the psychotic symptoms of AD and also schizophrenia⁴⁵. Of the Chr 21 transcription factors, only one is associated with mitochondria—*GABPA*— which is involved in the activation of cytochrome oxidase expression and nuclear control of mitochondrial function⁴⁶.

There is one common transcription factor between the AD-DS and DEX-DFC genesets: *NFE2L2* (also known as *NRF2*), which is associated with the oxidative stress response with aging, spatial learning, memory, and neuro-inflammation via regulation of antioxidant response elements^{47,48}. *NFE2L2/NRF2* regulates *BACE1*, the rate-limiting enzyme for amyloid- β peptide ($A\beta$) generation. *NRF2* activation decreases production of *BACE1* and *BACE1* antisense RNA (*BACE1-AS*) transcripts and $A\beta$ production and ameliorates cognitive deficits in animal models of AD⁴⁹. Depletion of *NFE2L2/NRF2* increases *BACE1* and *BACE1-AS* expression and $A\beta$ production and worsens cognitive deficits⁵⁰.

There are two transcription factors common between the AD-DS and DEX-CBC genesets. *MTOR* has been identified as a key target for therapeutic intervention in AD because of its regulation of several key signaling pathways: phosphoinositide 3-kinase (PI3-K)/protein kinase B (Akt), glycogen synthase kinase 3 [GSK-3], AMP-activated protein kinase (AMPK), and insulin/insulin-like growth factor 1 (IGF-1)⁵¹. Both upstream and downstream components of mTOR signaling are associated with AD progression and pathogenesis.

MTOR inhibits autophagic processes and contributes to amyloid β -peptide generation and/or clearance⁵². *MTOR* activation also contributes to aberrant hyperphosphorylated tau⁵³. The other common TF is *NFKB1* which is a key regulator of innate immunity and strongly associated with the inflammatory response involving cytokines and chemokines⁵⁴. *NFKB1* is also linked to aging and AD^{55,56}.

APP protein-protein interaction network

An APP protein-protein interaction network was created to identify genes from the genesets evaluated in this study that are connected to APP through 1st and 2nd shell interactions.

A total of 362 proteins make up the network (*Extended data Workbook 6*⁵⁷).

The APP protein interaction network overlaps by 48 genes with the AD-DS geneset, 41 with the AD risk factor geneset, 21 with the DEX DFC, 12 with the DEX CBC geneset and four with the Chr 21 geneset. The shared genes are highlighted in the network to visualize and forecast additional genes that are potentially involved in APP signaling and that are relevant to both AD and DS (*Figure 4A*). The top proteins that bridge (bottlenecks) the different sections of the network and that may signify information flow are: APP, ENSG00000259680

Figure 4. APP protein-protein interaction network. (A) Geneset overlap between 1st and 2nd shell interactions and the AD-DS, Chr 21, DEX DFC, and DEX CBC genesets. AD-DS genes unique: red; Chr 21 genes unique: gray; DEX DFC genes unique: purple; CBC genes unique: orange; AD risk factors (RF) and AD-DS genes shared: green; DEX DFC genes shared with RF & AD-DS genes: green oval; CBC and DFC shared genes: dark blue V; CBC genes shared with RF: green triangle; APP: yellow rectangle. (B) Interaction network gene clusters. Cluster 1: red – COP subunits, signalosome complex, development; Ubiquitin, Cluster 2: yellow – Tubulin, microtubules, motors, intracellular transport; Cluster 3: green – apoptosis, insulin signaling, ubiquitin, VEGFR growth factor signaling; Cluster 4: blue – Ubiquitin, autophagy; and Cluster 5: black – APP processing (PSEN, gamma secretase complex). (C) Distribution frequency for interaction score.

(a novel protein coding gene with similarity to immunoglobulin heavy chain variable region.), SHC1, DLG4, STUB1, KLC1, GFA1, CENPJ, and GNO1.

The APP network contains six major clusters (Figure 4B). Cluster 1: COP subunits, signalosome complex, development, ubiquitin; Cluster 2: TUBULIN, microtubules, motors, intracellular transport; Cluster 3: apoptosis, insulin signaling, ubiquitin, VEGFR growth factor signaling; Cluster 4: UBIQUITIN, autophagy; Cluster 5: APP processing (PSEN, gamma secretase complex); and Cluster 6: TUBULIN, microtubules.

The AD risk factor genes, Chr 21, and AD-DS genes are mostly dispersed throughout the network but a couple of areas in the network contain several connected AD risk factor genes. Predicted genes of interest based on their connectivity to these areas are *METTL2B* (tRNA methylation), *IK* (immune response), *SAP18* (RNA splicing), *QTRT1* (tRNA modification), *APLP1* (Prion pathway), *PRSSI* (proteolysis, extracellular matrix digestion), *ACSM1* (lipid metabolism), *APBA2* (binds beta amyloid, synaptic transmission, and nervous system development). The validity of all of the interaction scores range from 0.4–1.00 and, for the most part, are uniformly distributed with 695 of the interactions falling in the low to mid-range of 0.4 and 0.7 and 617 falling in the mid to high-range of 0.7 and 1.0 (Figure 4C).

Conclusion

Genesets associated with AD, DS, and Chr 21 were evaluated to identify genes, transcription factors, and pathways that may shed light on the relationship between AD and DS. Genes common to multiple genesets are either directly involved in APP processing or in TAU post translational modification. Many of the genes associated with the amyloid plaques in AD and DS function in learning and memory. A network analysis of APP protein-protein interactions was used to analyze the topology and connectivity of the genesets and, based on interactions with common AD-DS genes and AD risk factor genes, provide the foundation to predict potential genes of interest. Genes that connect the network and represent information flow as well as regions of high interconnectivity are also of interest for follow up studies. Given the central role of APP

related processes in the pathology of AD and DS, all of the proteins in the APP interaction network are either potential risk factors for AD or may contribute to disease progression in both AD and DS. Taken together, our findings indicate that oxidative stress, cell death/apoptosis, and inflammation/immune system processes likely underlie the pathogenesis of both AD and DS.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Figshare: Extended Data Workbook 1. Genesets: AD-DS, Chr 21, AD risk factors, DEX DFC and CBC, <https://doi.org/10.6084/m9.figshare.13106693.v1>²¹.

Figshare: Extended Data Workbook 2. Common Genes: Gene overlap between the AD-DS, Chr 21, AD risk factors, DEX DFC and CBC genesets, <https://doi.org/10.6084/m9.figshare.13106741.v1>²⁴.

Figshare: Extended Data Workbook 3. Keyword Gene Enrichment: Enrichment of the AD-DS, Chr 21, AD risk factors, DEX DFC and CBC genesets, <https://doi.org/10.6084/m9.figshare.13106750.v1>³⁷.

Figshare: Extended Data Workbook 4. GO Terms and Pathways: Gene Ontology Biological Process terms and pathways associated with the AD-DS and Chr 21 genesets, <https://doi.org/10.6084/m9.figshare.13106762.v1>³⁸.

Figshare: Extended Data Workbook 5. Transcription Factors: TFs present in the AD-DS, Chr 21 genesets, <https://doi.org/10.6084/m9.figshare.13106774.v1>³⁹.

Figshare: Extended Data Workbook 6. APP Network File: APP protein-protein interaction network, <https://doi.org/10.6084/m9.figshare.13106777.v1>³⁷.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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This paper by Delprato, Sharma, and colleagues examined genetic and transcriptomic factors that may explain Alzheimer's disease in Down syndrome by putting together five genesets: genes on chromosome 21; genes identified from the search term "Alzheimer's Down syndrome" using GeneWeaver; genes identified from a large-scale genome-wide association study (GWAS); genes identified from the differential gene expression study of the dorsal frontal cortex (DFC) and cerebellar cortex (CBC) using the Down Syndrome Developmental Brain Transcriptome database. Subsequently, they performed a series of bioinformatic analyses to generate associated genes and concluded that oxidative stress, apoptosis, and inflammation/immune system processes were important biological processes in AD in DS. This paper is timely as an increasing number of genomic and transcriptomic studies are being carried out to better understand the biology of AD in adults with DS and the non-DS population. However, there are a few points that need to be clarified:

- The title, "[Common genetic signatures of Alzheimer's disease in Down Syndrome](#)," suggests that this paper deals with genes that are likely to be involved in the neurodegenerative disease process in adults with Down syndrome. However, it is stated that the differential gene expression geneset was obtained from the Down Syndrome Developmental Brain Transcriptome Database, which included 15 sets of DS brains and matched control brains, where some of the brain specimens in the database were in embryonic stages. It is unclear how well these DS brain samples from embryonic stages would represent the neurodegenerative processes associated with AD in DS. It would be informative to repeat the analysis after excluding tissues that were from embryonic stages, if the sample size is sufficient.
- In the Introduction (page 3, lines 4-8), it is stated that "Amyloids form fibrous structures and plaques ... are involved in several diseases, such as Alzheimer's disease (AD), [Down Syndrome](#)

(DS), ..." Most would agree that adults with DS have a high risk of developing AD. However, it is unclear whether the formation of excess amyloids is biologically involved in the Down Syndrome itself, not just AD in DS. It should be clarified whether the wide range of DS phenotypes shown in Figure 2 can be attributed to the amyloid form fibrous structure and plaques.

- Comparisons of the genes identified from AD-DS vs. DEX DFC and AD-DS vs. DEX CBC were interesting. It would be equally interesting to present DEX DFC vs. DEX CBC comparisons as they may represent genes involved in cognitive vs. motor processes.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Human genetics, Genetic epidemiology, Alzheimer's disease, Down syndrome, Autosomal dominant forms of Alzheimer's disease.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 19 Oct 2021

Anna Delprato, PO Box 352, Wakefield, USA

Reviewer 4:

APPROVED WITH RESERVATIONS

This paper by Delprato, Sharma, and colleagues examined genetic and transcriptomic factors that may explain Alzheimer's disease in Down syndrome by putting together five genesets: genes on chromosome 21; genes identified from the search term "Alzheimer's Down syndrome" using GeneWeaver; genes identified from a large-scale genome-wide

association study (GWAS); genes identified from the differential gene expression study of the dorsal frontal cortex (DFC) and cerebellar cortex (CBC) using the Down Syndrome Developmental Brain Transcriptome database. Subsequently, they performed a series of bioinformatic analyses to generate associated genes and concluded that oxidative stress, apoptosis, and inflammation/immune system processes were important biological processes in AD in DS. This paper is timely as an increasing number of genomic and transcriptomic studies are being carried out to better understand the biology of AD in adults with DS and the non-DS population.

However, there are a few points that need to be clarified:

- The title, “Common genetic signatures of Alzheimer’s disease in Down Syndrome,” suggests that this paper deals with genes that are likely to be involved in the neurodegenerative disease process in adults with Down syndrome. However, it is stated that the differential gene expression geneset was obtained from the Down Syndrome Developmental Brain Transcriptome Database, which included 15 sets of DS brains and matched control brains, where some of the brain specimens in the database were in embryonic stages. It is unclear how well these DS brain samples from embryonic stages would represent the neurodegenerative processes associated with AD in DS. It would be informative to repeat the analysis after excluding tissues that were from embryonic stages, if the sample size is sufficient.

Response:

The aim of the study was not to investigate and compare the neurodegeneration process in DS and AD. Our aim was to analyze gene expression patterns associated with both disorders in the context of genes located on Chr 21 because individuals with DS have a third copy of this chromosome which contains the APP gene as well as other relevant genes.

- In the Introduction (page 3, lines 4-8), it is stated that “Amyloids form fibrous structures and plaques ... are involved in several diseases, such as Alzheimer’s disease (AD), Down Syndrome (DS), ...” Most would agree that adults with DS have a high risk of developing AD. However, it is unclear whether the formation of excess amyloids is biologically involved in Down Syndrome itself, not just AD in DS. It should be clarified whether the wide range of DS phenotypes shown in Figure 2 can be attributed to the amyloid form fibrous structure and plaques.

Response:

We have addressed this point in the introduction by stressing that APP may account for some of the DS associated phenotypes but is most likely not solely responsible for the wide range of phenotypes associated with DS..

APP may account for some of the phenotypes but given the widespread alteration throughout the DS genome, an extra copy of APP is most likely not solely responsible for the multiple and varied phenotypes associated with DS.

- Comparisons of the genes identified from AD-DS vs. DEX DFC and AD-DS vs. DEX CBC were interesting. It would be equally interesting to present DEX DFC vs. DEX CBC comparisons as they may represent genes involved in cognitive vs. motor processes.

Response:

The DFC and CBC comparison of differentially expressed genes was performed in the

original study (Olmos-Serrano et al. 2016) on which the DS developmental transcriptome database was generated. These results are reported in this Table S5 Summarized here. The DFC Gene ontology enrichment analysis indicated potential dysregulated biological categories including transmission of nerve impulse (Benjamini-Hochberg-adjusted (BHA) $p=2.9 \times 10^{-8}$), synaptic transmission (BHA $p=1.4 \times 10^{-5}$), cell morphogenesis (BHA $p=1.4 \times 10^{-5}$), cell-cell signaling (BHA $p=6.7 \times 10^{-5}$), and axon ensheathment (BHA $p=4.8 \times 10^{-2}$) (Table S5).

For the CBC, enriched terms for DNA, RNA processing as well as endocytosis and vesicle transport and budding.

Also of interest, for the DFC the number of differentially expressed (DEX) genes increased in an age-dependent manner, with significantly more DEX genes emerging over postnatal development and adulthood (periods 8-14) (Figure 1D, and Table S4) whereas for the CBC the number of DEX genes remained constant.

Competing Interests: No competing interests were disclosed.

Reviewer Report 15 February 2021

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Ann-Charlotte Granholm

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This is an excellent genetic study of comparisons between AD and DS-related AD by Delprato and collaborators. Overall, this is an interesting and timely publication. The GeneSet overlay in Figure 1 is an excellent demonstration of the findings. There are only a few comments to be made and these are listed below.

1. In the abstract, last sentence, the authors state that "Our findings indicate that oxidative stress, apoptosis, and inflammation/immune system processes likely underlie the pathogenesis of AD and DS". However, these contributors to AD in DS have already been known for quite some time, albeit maybe not at the genetic level. It would be better to reformulate this sentence to something like this: "Our findings confirm that oxidative stress, apoptosis, and inflammation/immune system processes likely contribute to AD in DS – these processes have been investigated in animal models and post mortem human tissues previously but not at the human genetic level". Or something like that.
2. Introduction, first sentence: "stick together" should be replaced with "aggregate".
3. First paragraph of Introduction: "a protein that typically resides around nerve cells" should

be replaced with “an integral membrane protein that is concentrated in synapses of neurons”.

Introduction first paragraph: “Smaller aggregates of beta-amyloid and not the plaques themselves...” This could be more specific. For example “Oligomeric forms of the beta-amyloid, and not the beta-pleated sheets in plaques” or something like that.

4. Second paragraph of Introduction: “About half of the people with DS develop AD and the associated dementia around 50 to 60 years of age”. It is actually thought by most people now that 90% or more of those with DS developed AD – this should be referenced and corrected.
5. Throughout the text, “AD individuals” or “DS individuals” should be replaced with “people or individuals with AD or DS” – those who have either condition do not wish to be defined by their disease. This is very important.
6. Generally, people refer to DS-related AD as DS-AD and not AD-DS, since the DS condition came first.
7. Last sentence of Conclusions: it would not hurt to follow this statement with some literature citations that, indeed, confirm that these same processes have been proposed by others using both post mortem human tissue and animal models for DS. This would place these findings in the light of previous literature and acknowledge that these processes have been proposed by others and are here confirmed by gene expression studies.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Down syndrome and Alzheimer's disease.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 19 Oct 2021

Anna Delprato, PO Box 352, Wakefield, USA

Reviewer 3:

This is an excellent genetic study of comparisons between AD and DS-related AD by Delprato and collaborators. Overall, this is an interesting and timely publication. The GeneSet overlay in Figure 1 is an excellent demonstration of the findings. There are only a few comments to be made and these are listed below.

- In the abstract, last sentence, the authors state that “Our findings indicate that oxidative stress, apoptosis, and inflammation/immune system processes likely underlie the pathogenesis of AD and DS”. However, these contributors to AD in DS have already been known for quite some time, albeit maybe not at the genetic level. It would be better to reformulate this sentence to something like this: “Our findings confirm that oxidative stress, apoptosis, and inflammation/immune system processes likely contribute to AD in DS – these processes have been investigated in animal models and post mortem human tissues previously but not at the human genetic level”. Or something like that.

Response:

Done.

- Introduction, first sentence: “stick together” should be replaced with “aggregate”.

Response:

Done.

- First paragraph of Introduction: “a protein that typically resides around nerve cells” should be replaced with “an integral membrane protein that is concentrated in synapses of neurons”.

Response:

Done.

Introduction first paragraph: “Smaller aggregates of beta-amyloid and not the plaques themselves...” This could be more specific. For example “Oligomeric forms of the beta-amyloid, and not the beta-pleated sheets in plaques” or something like that.

Response:

Done.

- Second paragraph of Introduction: “About half of the people with DS develop AD and the associated dementia around 50 to 60 years of age”. It is actually thought by most people now that 90% or more of those with DS developed AD –this should be referenced and corrected.

Response:

We have added this information to the introduction.

- Throughout the text, “AD individuals” or “DS individuals” should be replaced with “people or individuals with AD or DS” – those who have either condition do not wish to be defined by their disease. This is very important.

Response:

Thank you for the correction. We have edited the text accordingly.

- Generally, people refer to DS-related AD as DS-AD and not AD-DS, since the DS condition came first.

Response:

To the best of our knowledge each instance of the “AD-DS” designation refers to the datasets and figures generated in the study and not the condition of AD in people with DS.

- Last sentence of Conclusions: it would not hurt to follow this statement with some literature citations that, indeed, confirm that these same processes have been proposed by others using both post mortem human tissue and animal models for DS. This would place these findings in the light of previous literature and acknowledge that these processes have been proposed by others and are here confirmed by gene expression studies.

Response:

The Conclusion section has been modified and references have been added.

Competing Interests: No competing interests were disclosed.

Reviewer Report 08 February 2021

<https://doi.org/10.5256/f1000research.29930.r76772>

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William C. Mobley

Department of Neurosciences, University of California San Diego School of Medicine, La Jolla, CA, USA

Sharma and colleagues have examined data for genetic contributions to the emergence of Alzheimer's disease (AD) in those with Down syndrome (DS). Their methods include the creation and/or mining of various genesets, including one for AD and DS derived from a MeSH query, as well as genesets representing Chromosome 21, AD risk factors from GWAS data, and differentially expressed genes in the DS cortex and cerebellum. Overlaps between genesets were examined to

identify genes of interest, including tissues and biological processes, functional analyses, behavior and transcription as well as an APP protein-protein interaction network. Overall, the findings confirm some expected associations and point to possible novel associations that may prove useful to those exploring the biology of AD in DS (AD-DS).

Having stated the positives, additional comments may prove useful to enhance the appreciation of the work. First, it should be clarified that *APP* is expressed in neurons wherein it is processed to A β peptides, some of which are found in plaques. It is not true that A β peptides are due to misprocessing of APP but instead that one of its products the A β 42 peptide is preferentially deposited in plaques. An increase in the relative levels of A β 42 to shorter A β species is cited as playing a role in pathogenesis, as well it might, but A β 42 is nevertheless a normal product of APP. Note, however, also, that A β 42 and other A β species are present in toxic oligomeric complexes that are now viewed as more significant for pathogenesis than are the amyloid plaques. A second comment concerns the section examining transcriptional profiles wherein a listing of differentially regulated genes is given. While the corresponding RNA levels corresponding to these genes differ in AD-DS versus controls only some of the genes listed encode transcription factors. I suspect the authors meant to convey that the products of these genes may impact transcription.

In the end, this paper will be important to the extent that it drives the testing of specific hypotheses. The large amount of data that comes from such analyses may not readily suggest follow-on studies. Given the extensive work invested here, the authors might choose to highlight a small subset of findings and predict relationships that, if further supported, would more incisively speak to the biology of AD in DS.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Studies on the pathogenesis of AD in DS.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Author Response 19 Oct 2021

Anna Delprato, PO Box 352, Wakefield, USA

Reviewer 2:

Sharma and colleagues have examined data for genetic contributions to the emergence of Alzheimer's disease (AD) in those with Down syndrome (DS). Their methods include the creation and/or mining of various genesets, including one for AD and DS derived from a MeSH query, as well as genesets representing Chromosome 21, AD risk factors from GWAS data, and differentially expressed genes in the DS cortex and cerebellum. Overlaps between genesets were examined to identify genes of interest, including tissues and biological processes, functional analyses, behavior and transcription as well as an APP protein-protein interaction network. Overall, the findings confirm some expected associations and point to possible novel associations that may prove useful to those exploring the biology of AD in DS (AD-DS).

Having stated the positives, additional comments may prove useful to enhance the appreciation of the work.

First, it should be clarified that APP is expressed in neurons wherein it is processed to A β peptides, some of which are found in plaques. It is not true that A β peptides are due to misprocessing of APP but instead that one of its products the A β 42 peptide is preferentially deposited in plaques. An increase in the relative levels of A β 42 to shorter A β species is cited as playing a role in pathogenesis, as well it might, but A β 42 is nevertheless a normal product of APP. Note, however, also, that A β 42 and other A β species are present in toxic oligomeric complexes that are now viewed as more significant for pathogenesis than are the amyloid plaques.

Response:

We thank the reviewer for the clarification concerning the processing of A β peptides. We have included this information in the introduction of the revised manuscript.

A second comment concerns the section examining transcriptional profiles wherein a listing of differentially regulated genes is given. While the corresponding RNA levels corresponding to these genes differ in AD-DS versus controls, only some of the genes listed encode transcription factors. I suspect the authors meant to convey that the products of these genes may impact transcription.

Response:

We have clarified this point in the revised manuscript.

In the end, this paper will be important to the extent that it drives the testing of specific hypotheses. The large amount of data that comes from such analyses may not readily suggest follow-on studies. Given the extensive work invested here, the authors might choose to highlight a small subset of findings and predict relationships that, if further supported, would more incisively speak to the biology of AD in DS.

Response:

There is a subset of findings highlighted in the Results section of the abstract. We have added more context to emphasize the significance of these findings.

Competing Interests: No competing interests were disclosed.

Reviewer Report 24 November 2020

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This is a well-written paper that adds useful information to the literature. The connection between Down syndrome and Alzheimer's disease is clinically well established and is currently not preventable, leading to increased difficulty in Down syndrome management. Genetic examination as in this paper should stimulate further molecular research in Down syndrome including the genetic influences on obesity, common in Down syndrome and a risk factor in Alzheimer's disease. I suggest further research by this group in adipokines such as leptin and adiponectin as well as interactions between Wnt, BACE, Notch, BCL and DYRK. This could lead to better understanding of the relationships between Down syndrome, Alzheimer's disease, leukemia and solid tumors. Down syndrome is associated with an increased risk of leukemia and a decreased risk for solid tumors while Alzheimer's disease is associated with a decreased risk for various solid tumors.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 19 Oct 2021

Anna Delprato, PO Box 352, Wakefield, USA

Reviewer 1:

This is a well-written paper that adds useful information to the literature. The connection between Down syndrome and Alzheimer's disease is clinically well established and is currently not preventable, leading to increased difficulty in Down syndrome management. Genetic examination as in this paper should stimulate further molecular research in Down syndrome including the genetic influences on obesity, common in Down syndrome and a risk factor in Alzheimer's disease. I suggest further research by this group in adipokines such as leptin and adiponectin as well as interactions between Wnt, BACE, Notch, BCL and DYRK. This could lead to better understanding of the relationships between Down syndrome, Alzheimer's disease, leukemia and solid tumors. Down syndrome is associated with an increased risk of leukemia and a decreased risk for solid tumors while Alzheimer's disease is associated with a decreased risk for various solid tumors.

Response:

Thank you for the encouraging review and the information pertaining to cancer and solid tumor formation in the context of AD and DS. We will keep this in mind for future studies.

Competing Interests: No competing interests were disclosed.

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