HHS Public Access

Author manuscript

Mol Psychiatry. Author manuscript; available in PMC 2021 October 13.

Published in final edited form as:

Mol Psychiatry. 2021 July ; 26(7): 2680–2682. doi:10.1038/s41380-020-00903-3.

Leveraging aggression risk gene expression in the developing and adult human brain to guide future precision interventions

Ahra Kim, MPH[1], **Sarah L. Rader, BS**[2], **Thomas V. Fernandez, MD**[3], **Simon N. Vandekar, PhD**[1], **Alan S. Lewis, MD,PhD**[2,4,5,6,*]

[1]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN 37212, USA

[2]Department of Psychiatry and Behavioral Sciences, Vanderbilt University Medical Center, Nashville, TN 37212, USA

[3]Yale Child Study Center, Yale School of Medicine, New Haven, CT 06520, USA

[4]Department of Neurology, Vanderbilt University Medical Center, Nashville, TN 37212, USA

[5]Vanderbilt Kennedy Center, Vanderbilt University Medical Center, Nashville, TN 37212, USA

[6]Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN 37232, USA

Pathological aggressive behavior occurs in both clinical and non-clinical populations across the lifespan, with considerable prevalence in people with neurodevelopmental disorders (NDDs), including autism spectrum disorder[1] and attention-deficit/hyperactivity disorder.[2] Aggression significantly interferes with educational and social functioning, with negative interpersonal consequences far beyond childhood. Thus, an aspirational goal in caring for people with significant NDDs is risk stratification, mitigation, and prevention of aggressive behavior. Enhanced understanding of the genetic landscape contributing specifically to aggressive behavior in humans, and the possible existence of critical periods during which intervention may be most impactful will facilitate these goals. We thus read with interest a recent study from Zhang-James *et al.* in Molecular Psychiatry identifying 40 genes likely to play an important role in regulation of human aggression, synthesized from human genome-wide association studies (GWAS), rodent transcriptomic studies, and single-gene human disorders or knockout mouse studies.[3] These aggression risk genes (ARGs) were interconnected by functional network analysis, with multiple networks relevant to nervous system development. However, whether ARG expression is enriched in specific human brain regions during development or adulthood, or whether ARG expression in individual brain regions is distinctly regulated during development, is unknown. Such findings might further validate the ARGs as delineated by Zhang-James *et al.*, support the relevance of

[*]**Corresponding author:** Alan S. Lewis, MD, PhD, Department of Psychiatry and Behavioral Sciences, Vanderbilt University Medical Center, 465 21st Ave S., MRBIII Room 6140B, Nashville, TN 37240, Phone: 615-875-4027, Fax: 615-343-4622, alan.s.lewis@vumc.org.

Conflicts of interest

The authors declare no conflict of interest.

transcriptomic mechanisms for aggression, and narrow potential brain regions and time periods as interventional targets for aggression in children and adults.

We approached this question using developmental transcriptome data from the BrainSpan atlas,[4] which reports RNA sequencing data from cortical, subcortical and cerebellar brain regions from neurotypical human brains ranging from 8 weeks post-conception to 40 years of age with stringent quality controls. We first asked whether multiple sets of putative ARGs from Zhang-James *et al.*, identified by adult or child GWAS, online Mendelian inheritance in man (OMIM) reports, and a top 40 set of genes derived from cumulatively weighted human and animal studies, are more likely to be expressed in particular human brain regions during early development and young adulthood. We used Specific Expression Analysis (SEA), which uses BrainSpan data to define lists of transcripts enriched for a given brain region and timepoint, and at a given enrichment stringency (the Specificity Index threshold (pSI)), tests overlap significance using Fisher's exact test with Benjamini-Hochberg (BH) correction.[5, 6] We identified significant or near-significant enrichment in young adult cortex across all gene lists, while the child GWAS-derived ARGs was the only gene set with strong developmental enrichment occurring in cortex and cerebellum (Figure 1A,B). We next examined 16 brain regions from BrainSpan that had early fetal to young adulthood transcriptional data and asked, for each region, whether the expression of the top 40 ARGs over developmental time was regulated in a distinct manner as compared to *all* genes in that region reported in BrainSpan. Detection of brain regions in which ARGs are differentially developmentally regulated may support further investigation into these regions' roles in developmental aggression, even in the absence of SEA enrichment. To test this, we first established a cutoff at the 50^{th} percentile of raw gene expression from BrainSpan across all brain regions, genes, and timepoints to serve as a potential proxy for strong gene expression. We then used a generalized linear mixed effects model to predict the likelihood of mean gene expression exceeding this cutoff. The 50^{th} percentile split was chosen to attain better power by ensuring the proportion of enriched/nonenriched observations is approximately equal. Too low a cutoff might dilute signal amidst genes with limited biological relevance, while too high a cutoff would reduce power to detect significant effects. After BH correction for multiple comparisons, we found this outcome significantly depended on timepoint (increasing with age) and gene set (top 40 ARGs > total regional transcriptome) ($p < 10^{-3}$ for all brain regions for both main effects). For the interaction between timepoint and gene set, which would suggest a distinct developmental regulation program, we detected a significant interaction in 9 brain regions (7 cortical regions, amygdala, and cerebellar cortex) (Figure 1C, D). Repeating the analysis at the 75^{th} percentile expression cutoff again resulted in significant timepoint by gene interactions in amygdala and cerebellar cortex, as well as in 5 of the original 7 significant cortical regions. Orbitofrontal cortex and primary auditory cortex were no longer significant, while superior temporal cortex and striatum, which did not achieve significance at the 50^{th} percentile cutoff, were significant at the 75^{th} percentile cutoff. These data thus support the existence of a unique developmental trajectory of ARG expression in a cortical network, along with limbic and cerebellar regions for strongly expressed (and therefore potentially increasingly biologically impactful) ARGs.

Our findings from these two complementary analyses of BrainSpan extend the findings from Zhang-James *et al.*, suggesting that not only do ARGs participate in functional

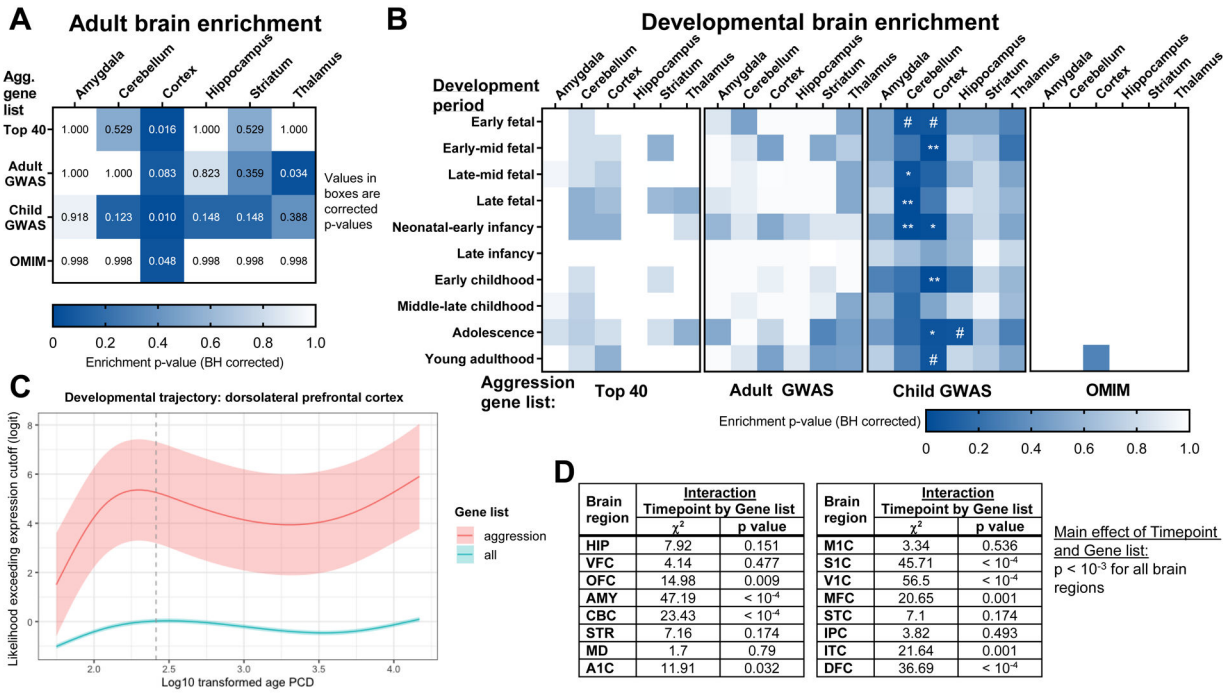
networks relevant to the nervous system, but also demonstrate transcriptional enrichment and unique developmental regulation within distinct brain regions. Because BrainSpan encompasses neurotypical individuals, it is possible that our findings may differ were our transcriptional reference to include individuals with NDDs. However, similar to our study, previous studies have mapped ASD-risk genes onto specific brain regions, cell types, and developmental time points using BrainSpan.[7] Encouragingly, most of the brain regions identified by our analysis, especially frontal, prefrontal, and primary sensory cortices, as well as the amygdala, have been strongly implicated in aggression regulation in clinical NDD populations, including ASD,[8] and schizophrenia.[9] Interestingly, there is mounting evidence supporting a role for the cerebellum in social behavior, including a recent study of bidirectional aggression modulation by Purkinje neurons in the cerebellar vermis.[10] Future cell-specific investigations from human brain or animal models will likely be useful in further narrowing to identify subregions and individual cell types relevant for aggression. Taken together, we believe this work illustrates a cause for optimism in future translational aggression studies that build upon convergent layers of evidence to explore increasingly precise interventions acting on distinct brain regions during key developmental timepoints, with a long-term goal to improve outcomes in clinical populations.

Acknowledgements

This work was supported by National Institutes of Health grants K23MH116339 (A.S.L.) and R01MH114927 (T.V.F.).

References

1. Kanne SM, Mazurek MO. Aggression in children and adolescents with ASD: prevalence and risk factors. Journal of autism and developmental disorders 2011; 41(7): 926–937. [PubMed: 20960041]
2. Saylor KE, Amann BH. Impulsive Aggression as a Comorbidity of Attention-Deficit/Hyperactivity Disorder in Children and Adolescents. Journal of child and adolescent psychopharmacology 2016; 26(1): 19–25. [PubMed: 26744906]
3. Zhang-James Y, Fernàndez-Castillo N, Hess JL, Malki K, Glatt SJ, Cormand B et al. An integrated analysis of genes and functional pathways for aggression in human and rodent models. Molecular Psychiatry 2018.
4. Miller JA, Ding SL, Sunkin SM, Smith KA, Ng L, Szafer A et al. Transcriptional landscape of the prenatal human brain. Nature 2014; 508(7495): 199–206. [PubMed: 24695229]
5. Dougherty JD, Schmidt EF, Nakajima M, Heintz N. Analytical approaches to RNA profiling data for the identification of genes enriched in specific cells. Nucleic Acids Res 2010; 38(13): 4218–4230. [PubMed: 20308160]
6. Specific Expression Analysis (SEA). http://genetics.wustl.edu/jdlab/csea-tool-2/, Accessed 04/07/2020.
7. Willsey AJ, Sanders SJ, Li M, Dong S, Tebbenkamp AT, Muhle RA et al. Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. Cell 2013; 155(5): 997–1007. [PubMed: 24267886]
8. Ibrahim K, Eilbott JA, Ventola P, He G, Pelphrey KA, McCarthy G et al. Reduced Amygdala-Prefrontal Functional Connectivity in Children With Autism Spectrum Disorder and Co-occurring Disruptive Behavior. Biol Psychiatry Cogn Neurosci Neuroimaging 2019.
9. Hoptman MJ. Impulsivity and aggression in schizophrenia: a neural circuitry perspective with implications for treatment. CNS Spectr 2015; 20(3): 280–286. [PubMed: 25900066]
10. Jackman SL, Chen CH, Offermann HL, Drew IR, Harrison BM, Bowman AM et al. Cerebellar Purkinje cell activity modulates aggressive behavior. Elife 2020; 9.



D

Brain region	Interaction Timepoint by Gene list χ^2	p value	Brain region	Interaction Timepoint by Gene list χ^2	p value
HIP	7.92	0.151	M1C	3.34	0.536
VFC	4.14	0.477	S1C	45.71	$< 10^{-4}$
OFC	14.98	0.009	V1C	56.5	$< 10^{-4}$
AMY	47.19	$< 10^{-4}$	MFC	20.65	0.001
CBC	23.43	$< 10^{-4}$	STC	7.1	0.174
STR	7.16	0.174	IPC	3.82	0.493
MD	1.7	0.79	ITC	21.64	0.001
A1C	11.91	0.032	DFC	36.69	$< 10^{-4}$

Main effect of Timepoint and Gene list:
$p < 10^{-3}$ for all brain regions

Figure 1. Aggression risk gene brain region enrichment and developmental regulation.

A,B Four aggression risk gene lists from Zhang-James *et al.* (Top 40 risk genes from cumulative weighting strategy, Adult genome-wide association study (GWAS), Child GWAS, and online Mendelian inheritance in man (OMIM)) were subjected to Specific Enrichment Analysis to test for enrichment in brain regions from adult brain only (A) or from developing brain (B). Overlap between aggression risk gene lists and transcripts enriched in a specific brain area was tested by Fisher's Exact Tests with Benjamini-Hochberg (BH) correction, and p values depicted as a heat map. Lists of enriched transcripts for overlap analysis were taken at a Specificity Index threshold (pSI) of 0.05.[5, 6] P values for each overlap are shown in each box in (A) but omitted in (B) for clarity. #, $p < 0.10$, *, $p < 0.05$, and **, $p < 0.01$. **C**. Example plot showing distinct developmental regulation trajectory of top 40 aggression risk genes (red) in the dorsolateral prefrontal cortex (DFC) as compared to all DFC genes in BrainSpan (blue). A generalized linear mixed effects model was used to predict whether mean raw expression value exceeded a cutoff of greater than 50th percentile of the entire BrainSpan transcriptome. The Y axis depicts the probability of exceeding this cutoff on a logit scale for each gene list. Vertical dashed line depicts time of birth. Shaded region depicts 95% confidence intervals. PCD, post-conception day. **D.** Results of generalized linear mixed effects model described in (C) examining how timepoint (early development to early adulthood), gene list (top 40 aggression risk genes versus all genes in that brain region in BrainSpan), and their interaction predict mean gene expression exceeding a raw expression cutoff of greater than 50th percentile of entire BrainSpan transcriptome. Main effects and interaction from Type II Wald Chi-square tests are shown after BH correction for multiple comparisons across each main effect and the interaction. For space considerations, only details of the interaction are shown, however, for all brain regions, the main effect of timepoint and gene list were highly significant, with higher probability of exceeding the expression cutoff at older developmental age and for top

40 aggression genes. Analyses shown in (C,D) are from R version 3.5.3. Abbreviations in (D): HIP, hippocampal formation; VFC, ventrolateral prefrontal cortex; OFC, orbitofrontal cortex; AMY, amygdala; CBC, cerebellar cortex; STR, striatum; MD, mediodorsal thalamus; A1C, primary auditory cortex; M1C, primary motor cortex; S1C, primary somatosensory cortex; V1C, primary visual cortex; MFC, anterior (rostral) cingulate (medial prefrontal) cortex; STC, posterior (caudal) superior temporal cortex; IPC, posteroventral (inferior) parietal cortex; ITC, inferolateral temporal cortex; DFC, dorsolateral prefrontal cortex.