Data supplement for Parellada et al., In Search of Biomarkers to Guide Interventions in Autism Spectrum Disorder: A Systematic Review. Am J Psychiatry (doi: 10.1176/appi.ajp.21100992)

Supplemental Methods

Literature search

The search was performed on April 2, 2020, on three databases.

Pubmed/Medline was searched using the terms:

(PDD OR ASD OR autism*) AND (biomarker* OR marker* OR endophenotype*)

The entire resulting search was downloaded by clicking 'Send To', selecting 'File', and selecting 'CSV'.

Embase was searched using Emtree terms:

(biological marker OR endophenotype OR marker) AND (autism)

In addition, language of article was set to English, and date record added to Embase was set to 01-01-1900 to 29-02-2020. From the search results, all records were selected and the 'Export' button was clicked. Format was selected as 'CSV - Fields by Column' and for output, 'Full Record' was selected.

Scopus was searched using the terms:

TITLE-ABS-KEY ((pdd OR asd OR autism*) AND (biomarker* OR marker* OR endophenotype*)) AND (LIMIT-TO (LANGUAGE , "English")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (SRCTYPE , "j"))

There is a 2,000 record limit on downloads, therefore the results were split into three batches using the 'Limit to' filters and selecting independent groups by year. To download, we clicked 'Select all', clicked 'Export', selected 'CSV Export', added 'Pubmed ID' and 'Abstract', and clicked Export. The three batches were combined into a single file using a text editor.

The results from the three searches were merged to identify overlapping records. This task was complicated by differing conventions on capitalization, punctuation, abbreviation, and format. We assessed matching PMIDs, similar year of publication, and Damerau Levenshtein distance of the first seven words of the title (lowercase and stripped of punctuation), the first, second, and last author names and first initial, and the first three letters of each word in the journal name.

Statistical analysis

Cohen's *d* was calculated from the stated *t* statistic and samples sizes using the 't2d' function in the 'psych' R library or from the stated mean and standard deviations of cases and controls using the equation:

$$d = \frac{\bar{x}_1 - \bar{x}_2}{s}$$

Where $\bar{x}_1$ is the mean value from cases, $\bar{x}_2$ is the mean value from controls, and *s* is the pooled standard deviation calculated by:

$$s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}}$$

Where, n_1 is the number of cases, n_2 is the number of controls, s_1 is the standard deviation of the cases, and s_2 is the standard deviation of the controls.

To correct for small sample sizes, Hedges' *g** was estimated from the Cohen's *d* value using the equation:

$$g^* = d\left(1 - \frac{3}{4(n_1 + n_2 - 2) - 1}\right)$$

The confidence intervals of *g** were estimated by:

$$g^* \pm 1.96\sqrt{\frac{(n_1 - n_2)}{n_1 n_2} + \frac{g^{*2}}{2(n_1 - n_2)}}$$

Where, n_1 is the number of cases, n_2 is the number of controls.

Supplemental tables

****** Tables S1 and S3–S7 are in separate Excel files. ******

Table S1. Initial search
Table S3. Interventional studies
Table S4. Non-interventional studies
Table S5. Molecular biomarkers
Table S6. Non-molecular biomarkers
Table S7. Glutathione effect sizes

Tables S2 and S8 appear below.

TABLE S2. PRISMA Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Methods and Fig. 1
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Methods, Fig. 1, and Supplementary methods
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Supplementary methods and Supplementary Table S1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Methods and Fig. 1
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Methods
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Methods
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Methods
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Methods
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Methods and Fig. 3

Continued

Section and Topic	Item #	Checklist item	Location where item is reported
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Methods
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Methods and Supplementary methods
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Methods, Fig. 3, and Supplementary methods
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Methods
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Methods
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Methods, Results, Fig. 2
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Methods, Fig. 3, and Supplementary methods
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Methods, Fig. 3, and Supplementary methods
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Methods and Fig. 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Supplementary Table S1
Study characteristics	17	Cite each included study and present its characteristics.	Supplementary Table S1, S2, and S3
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	NA

Continued

Section and Topic	Item #	Checklist item	Location where item is reported
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Data analysis and statistical methods, Fig. 3, Supplementary methods, and Supplementary Table S6
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Methods
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Data analysis and statistical methods, Fig. 3, Supplementary methods, and Supplementary Table S6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Data analysis and statistical methods, Fig. 3
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Methods, Data analysis and statistical methods, Fig. 3, Supplementary methods, Supplementary Table S6, and discussion
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Discussion
	23b	Discuss any limitations of the evidence included in the review.	Discussion
	23c	Discuss any limitations of the review processes used.	Discussion
	23d	Discuss implications of the results for practice, policy, and future research.	Discussion

Continued

Section and Topic	Item #	Checklist item	Location where item is reported
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Methods
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	NA
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Acknowledgements
Competing interests	26	Declare any competing interests of review authors.	Conflicts of Interest
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Supplementary Tables and Supplementary Methods

TABLE S8. Most cited biomarkers in animal interventional studies[a]

BM	Reference	Modality	Animal Model	Intervention	Age (postnatal days)	Biomarker Outcome	ASD Behavior Measures	Behavioral Improvement	Biomarker x Behavior Correlation	PMID
GSH	Sandhya et al., 2012	Brain	VPA	Bacopa monniera	30–40	Post > Pre	Social behavior	Yes	Not tested	22322665
	Pragnya et al., 2014	Brain	VPA	Bacopa monniera	13–40	Not different	Social behavior	Yes	Not tested	24803211
	Al-Amin et al., 2015	Brain	VPA	Bacopa monniera	26–55	Post > Pre	Social behavior	Yes	Not tested	25732953
	Rani et al., 2017	Brain	Terbutaline	Minocycline and doxycycline	15–29	Post > Pre	Nociception	Yes	Not tested	29074392
IL-6	Hsiao et al., 2013	Colon	MIA	Bacteroides fragilis	21-42	Not different	Social behavior	Yes	No	24315484
	Kirsten et al., 2018	Plasma	LPS	Pioglitazone	21-29	Pre > Post	Play, ultrasonic vocalizations	Yes	No	29791472
BDNF	Segal-Gavish et al., 2015	Brain, hippocampus	BTBR	Human mesenchymal stem cells	42-64	Post > Pre	Stereotyped behavior	Yes	Not tested	26257137
	Kirsten et al., 2019	Plasma	LPA	Pioglitazone	21-29	Pre > Post	Cognitive inflexibility	Yes	No	31036753
Serotonin	Sandhya et al., 2012	Brain, hippocampus	VPA	Bacopa monniera	21-35	Not different	Social behavior	Yes	Not tested	22322665
	Hsiao et al., 2013	Colon	MIA	Bacteroides fragilis	21-42	Pre > Post	Social behavior	Yes	Not tested	24315484
	Pragnya et al., 2014	Brain, hippocampus	VPA	Piperine	13	Post > Pre	Social behavior	Yes	Not tested	24803211
	Zhang et al., 2015	Brain	BTBR	Tryptophan depletion	90-120	Pre > Post	Social behavior	No	No	25445490
	Zhang et al., 2015	Brain	BTBR	Tryptophan addition	90-120	Post > Pre	Social behavior	Yes	No	25445490
	Tanaka et al., 2018	Brain, striatum	SERT	Tryptophan-free diet	90-180	Pre > Post	Social behavior	Yes	Not tested	30498565
Oxytocin	Pujol et al., 2018	Brain, RNA	MOR	Behavioral reward intervention	49-70	Post > Pre	Social behavior	Yes	Not tested	30242222
	Kirsten et al., 2019	Plasma	LPA	Pioglitazone	21-29	Not different	Social behavior	Yes	Not tested	31036753

[a] BDNF=brain-derived neurotrophic factor; BM=biomarker; BTBR=Black and Tan Brachyury (T1tf/J); GSH=glutathione; IL-6=interleukin-6; LPA=lysophosphatidic acid; LPS=lipopolysaccharide; MIA=maternal immune activation; MOR=mu-opioid receptor (Oprm1-/-) knock-out; PMID=PubMed reference number; RNA=ribonucleic acid; SERT=serotonin transporter knock-out; VPA=valproic acid.