https://doi.org/10.1093/brain/awac247 BRAIN 2022: 145; e92–e94 | e92

BRAIN
LETTER TO THE EDITOR

Reply: Methodological drawbacks in the alleged association between foetal sonographic anomalies and autism

Idan Menashe,[1,2] Ohad Regev,[2,3] Amnon Hadar,[4,5] Gal Meiri,[2,6] Analya Michaelovski,[2,7] Ilan Dinstein[2,8] and Reli Hershkovitz[5]

1 Department of Public Health, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
2 Azrieli National Center for Autism and Neurodevelopment Research, Ben-Gurion University of the Negev, Beer Sheva, Israel
3 Joyce and Irving Goldman Medical School, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
4 Division of Obstetrics and Gynecology, Clalit Health Services, Beer Sheva, Israel
5 Division of Obstetrics and Gynecology, Soroka University Medical Center, Beer Sheva, Israel
6 Preschool Psychiatric Unit, Soroka University Medical Center, Beer Sheva, Israel
7 Child Development Center, Soroka University Medical Center, Beer Sheva, Israel
8 Psychology and Brain and Cognition Departments, Ben-Gurion University of the Negev, Beer Sheva, Israel

Correspondence to: Idan Menashe, PhD
Department of Public Health, Faculty of Health Sciences
Ben-Gurion University of the Negev, Beer Sheva 8410501, Israel
E-mail: idanmen@bgu.ac.il

We have carefully read the letter of Sagi-Dain *et al.*[1] entitled: 'Methodological drawbacks in the alleged association between foetal sonographic anomalies and autism' that severely criticized our recent publication in *Brain*. First, we believe that the language used by the authors is offensive and uncalled for. Scientists should be able to have discussions and raise concerns about methodology without using terms such as 'astonishment' and 'unfounded allegations'. It is also fine for scientists to disagree about methodological issues, respectfully.

To the point, the concerns raised by Sagi-Dain *et al.* are either incorrect or trivial and have already been addressed in the original publication. In fact, we are confident that our paper would not have been published in *Brain* if these concerns had merit. Below are listed the concerns of Sagi-Dain *et al.*, each followed by our response:

(i) Sagi-Dain *et al.* state that: 'The most prominent shortcoming of the study is the striking absence of any description of the methodology and rationale for the selection of the 229 controls from the general population.'

This is simply not true. In the study design section, we clearly indicate that the typically developing (TDP) controls 'were matched to cases by year of birth, sex (male/female) and ethnicity (Jewish/Bedouin)', as required by the STROBE guidelines for case-control studies.[2]

(ii) Sagi-Dain *et al.* suggest that 'the disparity between numerous significant differences in the rates of specific sonographic anomalies in children with ASD compared to general population cohort, to the prominent lack of such differences between children with ASD to neurotypical siblings, except echogenic intracardiac foci (EIF)' is indicative of the lack of association of these anomalies with ASD.

This is also not true. Figures 2 and 3 in the paper clearly demonstrate significant differences in the prevalence of several sonographic anomalies between the ASD children and their typically developing siblings. The fact that differences were smaller in magnitude relative to the differences between ASD children and typically developing children in the general population is expected because of their shared genetic and familial factors, as previously described.[3] The comparison between the ASD and TDS groups in our study allowed us to account for such genetic and familial factors in our analyses. The results clearly show that fetuses in the ASD group had significantly higher rates of foetal anomalies than their unaffected siblings and that these differences were consistent across different organs.

The fact that the rates of some of the study's sonographic anomalies were significantly higher in the ASD group compared to both the TDS and TDP groups while others did not, allowed us to highlight the sonographic anomalies that are more strongly related to ASD. This was precisely the point of performing the study with the two distinct control groups.

(iii) Sagi-Dain *et al.* further suggest that the differences deriving from the 'much lower rates of several sonographic anomalies' in the general population cohort, 'including EIF (0.4% versus ~5% in previous reports … as well as choroid plexus cysts (0.5% in the current analysis versus 1–2.5% according to formerly published studies)'[4,5] point to a selection bias of the TDP group.

This statement is misleading. There is heterogeneity in findings of sonographic anomalies in the general population. Sagi-Dain *et al.* highlighted specific studies that support their claims but unfortunately do not provide a fair description of this heterogeneity. For example, a study performed in the Israeli population (the most relevant population to our study), reported that EIF is present in only 0.17% of prenatal ultrasound scans[6] while another study reported a prevalence of 0.47%.[7] In addition, a study that examined choroid plexus cysts in multiple unselected populations reported rates of choroid plexus cysts between 0.18–3.5%.[8] Regardless, the heterogeneity in the general population is precisely why it was important to include the ASD siblings group and focus on differences that were apparent both in comparisons with the general population and with the ASD siblings.

Received June 6, 2022. Accepted July 3, 2022. Advance access publication July 8, 2022

Table 1 Ultrasonographic findings during prenatal anatomy survey

Variable	Group[a]	No. of foetuses (%)	Adjusted odds ratio	95% CI	P-value
Any foetal ultrasonography finding	ASD	67 (29.3)	Reference		
	TDS	32 (15.9)	2.23	1.32–3.78	**0.006**[b]
	TDP	22 (9.6)	3.50	2.07–5.91	**<0.001**[b]
Soft markers	ASD	52 (22.7)	Reference		
	TDS	29 (14.4)	1.98	1.12–3.49	**0.036**[b]
	TDP	17 (7.4)	3.06	1.77–5.29	**<0.001**[b]
Structural anomalies	ASD	23 (10.0)	Reference		
	TDS	7 (3.5)	2.99	1.19–7.51	**0.040**[b]
	TDP	5 (2.2)	5.50	1.90–15.96	**0.004**[b]

All P-values are Bonferroni corrected for multiple comparison ($n = 2$). Soft markers: pyelectasis, echogenic intracardiac focus, single umbilical artery, persistent right umbilical vein, echogenic bowel, choroid plexus cyst, short femur, enlarged foetal stomach. Structural anomalies: hydronephrosis (renal pelvis >10 mm), microcephaly (<3 standard deviations), ventricular-septal defect, ventriculomegaly, mega cisterna magna, clubfoot, single kidney, right aortic arch + vascular ring, abnormal cranial ossification.
[a]ASD = autism spectrum disorder ($n = 229$); TDS = typically developing siblings ($n = 201$); TDP = typically developing general population ($n = 229$).
[b]Conditional logistic regression, adjusted to foetal sex and mother's age.

(iv) Sagi-Dain *et al*. state that 'it is surprising that the authors did not expand the control population to at least 1:4 ratio'.

Our study involved a thorough exploration of the prenatal ultrasonography records of over 650 children and is the largest and most comprehensive study of this kind to date. It is of course always useful to enlarge samples—we implore Sagi-Dain *et al*. to perform a follow-up study at the extent that they describe.

(v) Sagi-Dain *et al*. write that 'Additional sources of bias include inadequate description of several sonographic anomalies. For instance, the degree of renal pelvic dilatation can be mild, constituting a soft marker with little risk for chromosomal anomalies, or hydronephrosis, defined as renal pelvis of over 1 cm and considered a major sonographic anomaly'.

We thank Sagi-Dain *et al*. for this important comment. It motivated us to revisit our data and to stratify all ultrasonography findings into 'structural anomalies' and 'soft markers', according to the guidelines issued by the Diagnostic Imaging and Genetic Committees of the Society of Obstetricians and Gynaecologists of Canada.[9] A comparison of these ultrasonography findings between the study groups is presented in Table 1 and shows that the rates of both soft markers and structural anomalies are significantly higher in ASD cases compared to the TDS and TDP groups [adjusted odds ratio (aOR) = 1.98, 95% confidence interval (CI) = 1.12–3.49, and aOR = 3.06, 95%CI = 1.77–5.29, respectively, for soft markers and aOR = 2.99, 95%CI = 1.19–7.51, and aOR = 5.50, 95%CI = 1.90–15.96, respectively, for structural anomalies], thus further strengthening the conclusions of our study.

(vi) 'Less crucial yet important drawback of the study is inadequate addressing of potential confounders. Notably, presence of chromosomal and microarray anomalies in cases could explain both the sonographic anomalies as well as the ASD itself. Was abnormal maternal serum screening present?'

We agree that genetic factors may underlie both ASD and the ultrasonography findings associated with it, as is clearly mentioned in our 'Discussion' section. Nevertheless, the goal of our study was to examine whether the ultrasoud findings in the foetal anatomy survey that are used as prenatal markers for a range of genetic disorders are also associated with the risk of ASD. Obviously, if genetic findings were available for these fetuses, ultrasound screening would not be required. We did, however, adjust for multiple other sociodemographic and familial factors in the matching of the ASD cases to the two control groups and in our multivariate logistic regression analyses. Furthermore, we acknowledge that despite all these adjustment efforts, 'the associations between foetal anomalies and ASD found in our study could still be confounded by other unmeasured variables', as was indeed mentioned in our 'Discussion' section.

(vii) The authors boldly state that sonographic anomalies associated with ASD "could form the basis of new prenatal screening approaches for ASD" which "will reveal foetuses at risk to develop ASD". However, performance of a case-controlled study does not allow calculation of positive predictive value for ASD'.

This statement is cited from the 'Discussion' section of our paper and suggests that the findings of our study 'could' (a word that is used to make suggestions and requests) form the basis of new prenatal screening approaches for ASD. Obviously, our findings should be confirmed in additional studies to further assess their clinical implications as ultrasonography markers for ASD risk.

(viii) 'Finally, with the publication of these unfounded allegations, health professionals may be charged with malpractice if they do not inform pregnant patients about these the alleged claims pointing to an increased risk for ASD with specific sonographic findings. This would have severe medico-legal implications.'

Our manuscript is a research article and is not written as guidelines for health professionals; thus it should not be treated as such. If such medico-legal concerns were guiding publication of scientific research, the majority of epidomiological studies would cease to exist.

(ix) 'It is of note that we have approached the authors, prior to publication, outlining our serious reservations and concerns and urging them to amend the manuscript according to the abovementioned points. Regretfully, we failed to convince them of the above.'

Indeed, Sagi-Dain *et al*. contacted us, but did it after the paper was published in its pre-printed form, when no major ammendments in the text can be made. Nevertheless, we offered them to revoke their letter and alternatively, to publish a correction to the paper that would address their medico-legal concerns as described above. Regretfully, they refused to this offer.

In summary, we welcome respectful scientific discussion and critiques of methodology and interpretation. We hope that our response clarifies issues that may have been misunderstood, and believe that it further supports the findings and conclusions of our original manuscript.

Data availability

Data availability is not applicable to this article as no new data were created or analysed in this study.

Competing interests

The authors report no competing interests.

References

1. Sagi-Dain L, Weisz B, Krajden KH, Singer A, Yaron Y, Maymon R. Methodological drawbacks in the alleged association between foetal sonographic anomalies and autism. *Brain*. 2022;145(10): e90–e91.

2. von Elm E, Altman DG, Egger M, *et al*. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Surg. (London, England)*. 2014;12:1495–1499.
3. Dawson S, Glasson EJ, Dixon G, Bower C. Birth defects in children with autism spectrum disorders: a population-based, nested case-control study. *Am J Epidemiol*. 2009; 169:1296–1303.
4. Sotiriadis A, Makrydimas G, Ioannidis JP. Diagnostic performance of intracardiac echogenic foci for Down syndrome: a meta-analysis. *Obstetrics Gynecol*. 2003;101(5 Pt 1):1009–1016.
5. Kurten C, Knippel A, Verde P, Kozlowski P. A Bayesian risk analysis for Trisomy 21 in isolated choroid plexus cyst: combining a prenatal database with a meta-analysis. *J Matern-Fetal Neonatal Med*. 2021;34:889–897.
6. Bronshtein M, Jakobi P, Ofir C. Multiple fetal intracardiac echogenic foci: not always a benign sonographic finding. *Prenat Diagn*. 1996;16:131–135.
7. How HY, Villafane J, Parihus RR, Spinnato JA 2nd. Small hyperechoic ventricle: a benign foci of the fetal cardiac sonographic finding? *Ultrasound Obstet Gynecol*. 1994;4:205–207.
8. Chitty LS, Chudleigh P, Wright E, Campbell S, Pembrey M. The significance of choroid plexus cysts in an unselected population: results of a multicenter study. *Ultrasound Obstet Gynecol*. 1998;12:391–397.
9. Van den Hof MC, Wilson RD, Diagnostic Imaging Committee, Society of Obstetricians and Gynaecologists of Canada; Genetics Committee, Society of Obstetricians and Gynaecologists of Canada. Fetal soft markers in obstetric ultrasound. *J Obstet Gynaecol Can*. 2005;27:592–636.