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Response to Dr Fried & Dr Kievit, and Dr Malhi *et al.*

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We thank Dr. Fried & Dr. Kievit, and Dr. Malhi and colleagues for their insightful comments. Here we further clarify the design and outcome of our meta-analysis of subcortical volume differences between patients with major depressive disorder (MDD) and controls.

Fried and Kievit¹ and Malhi *et al.*² commend the collaborative achievement of the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) MDD consortium in analyzing sample sizes unprecedented in neuroimaging of depression. They also raise some concerns and provide additional interpretations of our results. Malhi *et al.* suggest that we should better exploit this vast data set to study the heterogeneity of MDD. MDD is a heterogeneous disorder, and the scope and extent of brain alterations depends on specific clinical characteristics of the sample studied. That is why we reported meta-analytic results of subcortical volume differences in depressed patients stratified by stage of illness (first episode versus recurrent) and age of onset (early versus late).³ Compared to controls, hippocampal volume was lower in recurrent and early-onset patients, on an average, but not in first-episode patients or patients diagnosed after age 21. These findings indeed raise interesting questions: for instance, whether hippocampal volume reductions are detectable in early-onset first-episode patients. Malhi *et al.* suggest partitioning our sample into finer subgroups to study these interactions. We agree that it is important to analyze between-subject differences across these complex interactions, but our study design did not allow for delineating these more complex interactions (for example, diagnosis x recurrence x age of onset). Our meta-analysis comprised standardized processing, statistics and quality control to harmonize methods in a sample of unprecedented size. In a mega-analysis, one can access all participant-level data. By contrast, our initial meta-analysis distributed work efficiently across many sites, and we meta-analyzed summary statistics at a central site. Coordinated analyses at this scale must consider what is feasible and achievable, to motivate more fine-grained analysis. So far, partitioning patients into fine-grained subgroups has not been feasible in most individual samples, as the numbers per cell quickly become too small to assess more complex clinical interactions. As ENIGMA grows, several analyses are showing

consistency worldwide. A mega-analysis may be feasible with a subsample of participating sites who meet legal and ethical requirements for the sharing of individual subject data to a central site.

Our study is the largest meta-analysis to date and reveals the profile of subcortical volume alterations in MDD, and some factors that affect it. Brain structure was consistently altered across: (1) MDD patients residing in the community or primary care, and (2) patients recruited from specialized mental health services, many of whom had more severe and recurrent MDD. Unlike smaller studies, we found no consistent subcortical brain alterations beyond the hippocampus, and even this was observed only in specific patient subgroups: this is, unquestionably, new information from a worldwide sample offering very high power. As Fried and Kievit note, subcortical abnormalities in MDD are moderate, but consistent. Past claims reporting smaller volumes of (for example) the amygdala in MDD patients were not robust across the cohorts we analyzed. As with any other study, ours has limitations. Not all individual studies had detailed information on duration, number of episodes and treatment history. When combining already collected data across worldwide samples, data collection protocols are not prospectively harmonized. Clinical assessments therefore differed across studies, which limits the analysis of sources of heterogeneity. For instance, different instruments were used to assess depression symptom severity across the studies included in the meta-analysis. New subprojects were recently initiated within our ENIGMA MDD consortium specifically focused on how severity impacts neural changes in MDD, which intend to (1) establish a common metric for depressive symptoms for various questionnaires, and (2) explore different ways of defining symptom and disease severity and their association with brain measures. Regarding the effects of antidepressants (cf., the letter to the editor by Malhi *et al.*), a cross-sectional study design such as ours cannot determine how antidepressant medication affects brain structure. Interventional studies comparing patients pre- and post-treatment are required to establish how antidepressants affect brain structure.

Fried and Kievit rightly note that hippocampal volume reduction in the MDD group and its subgroups is known to be small (Cohen's *d* between -0.14 and -0.21) and not specific to MDD. As our colleagues found in the ENIGMA Schizophrenia Working Group,⁴ larger effects are observed in schizophrenia, motivating cross-disorder comparisons across ENIGMA eventually. However, our finding is robust: the hippocampus was consistently smaller, on average, across a large number of samples encompassing the broad heterogeneity of MDD (I^2 scores showed low heterogeneity of findings across studies). Smaller hippocampal volume has been associated with executive function impairments,⁵ learning and memory deficits⁶ and poorer treatment response⁷ in MDD; so the hippocampal volume reduction is important despite its small effect size. Establishing the degree of hippocampal volume difference in MDD, and its modulators, with this precision is crucial, as the disorder affects billions of people worldwide.

Indeed we did not estimate any form of classification accuracy. Researchers in the field of neuroimaging already realize that no single univariate data point differentiates MDD patients from controls. If classification were the goal, one could include other subcortical regions whose effects do not reach the significance threshold, but within a multivariate analysis could boost classification accuracy. Moreover, cortical regions or other imaging measures could be included. Ultimately, consortia such as ENIGMA may discover multivariate patterns predictive of diagnosis, but progress is unlikely without first publishing studies of measures that are easier to harmonize. It is widely known that findings based on group-level (univariate and mass-univariate) approaches may not offer sufficient predictive value for individual patients within a multivariate classification approach. Measures from future ENIGMA MDD projects studying cortical thickness, surface area,

shape, hippocampal subfields, diffusion tensor imaging or functional measures may help multivariate prediction methods, eventually.⁸ Moreover, the harmonization of processing, statistics and quality control protocols across ENIGMA disease working groups will eventually allow classification across different psychiatric disorders.⁹

Fried and Kievit discuss some alternative mechanisms that may drive hippocampal volume reduction in MDD. In our original paper, we did not claim 'that depression causes structural changes' (cf. letter to the editor by Fried and Kievit). In fact, not only patients with recurrent episodes, but also the group with early age of onset (consisting of almost 50% of first-episode patients) showed smaller hippocampal volumes; so structural changes are not merely a consequence of depression. We speculated that hippocampal volume reductions may be promoted by a chronic hyperactivity of the hypothalamic–pituitary–adrenal axis via remodelling and downregulation of growth factors including brain-derived neurotrophic factor, associated with (chronic) stress.¹⁰ Stressors include multiple episodes of depression, early-life stress and a family history of depression, which are all linked to early-onset depression,^{11–13} higher risk for recurrent depression,^{14–16} an overactive hypothalamic–pituitary–adrenal axis^{17,18} and smaller hippocampal volume.¹⁹ As we stated in our article, smaller hippocampal volume may even be a risk factor for depression: 'morphological hippocampal alterations may represent risk markers for depression, recurrence and chronicity' and 'Clearly, there is a continued need for longitudinal studies tracking hippocampal volume changes over the disease course, to further elucidate whether hippocampal abnormalities result from prolonged duration of chronic stress (i.e. 'scarring'), represent a vulnerability factor for MDD, or both', which agrees with Fried and Kievit.

CONSORTIUM MEMBERS

The members of the ENIGMA-Major Depressive Disorder Working Group consortium are listed at <http://enigma.ini.usc.edu/ongoing/enigma-mdd-working-group/>.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Parental age, birth order and neurodevelopmental disorders

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Advanced paternal age is a well-established risk factor for the development of schizophrenia (SZ),^{1–3} and an increased rate of *de novo* mutations with increasing paternal age has been proposed as the chief explanation for this association.⁴ However, the paternal age effect could also be as a result of other potential explanations. For example, analyses of Danish registry data revealed that the paternal age effect was attributable to paternal age at birth of the first child in the sibship, rather than to age at birth of the child with SZ,⁵ which suggests that some explanation other than *de novo* mutations may explain the reported paternal age association with SZ. Furthermore, advanced maternal age, that has also been implicated in the risk of neurodevelopmental disorders (NDDs) via unknown mechanisms (that is, not *de novo* mutation), should also be incorporated in this conceptualization.^{6,7} Therefore, findings regarding *de novo* mutations as the explanation for the association between advanced paternal age and SZ are inconclusive because covariates, such as maternal age⁸ and family size,⁹ which may index other potential mechanisms than paternally derived *de novo* mutations, have not been simultaneously considered in most prior analyses.

Studies of birth order effects in SZ in both population-based samples^{10,11} and clinical samples^{12,13} have yielded conflicting findings. Nevertheless, Jaffe *et al.*¹⁴ consider affected proband birth order as a proxy for *de novo* mutations. Their analyses did not support an association between paternal age and birth order as an index of *de novo* mutations in a SZ data set after controlling for maternal age and family size. In order to determine if this was a robust finding, we attempted to replicate this work in our local SZ data using similar methods to those described by Jaffe *et al.*¹⁴ Furthermore, because both advanced paternal age and increased *de novo* mutations have also been reported among cases of autism spectrum disorder (ASD)¹⁵ and other NDDs,² we examined whether the paternal age/proband birth order association is specific to SZ or is more broadly related to NDDs by extending the analyses to an ASD sample.

The study samples included in these analyses were cases with SZ from an Irish collection ($N=264$, 69% male)¹⁶ and cases with ASD from the Simons Simplex Collection (SSC, version 14, $N=2539$, 87% male).¹⁷ Cases were limited to those with available data on birth order of the proband, and either maternal or paternal age at the proband's birth (see Supplementary Information for further details on the Irish SZ collection, and see Figure 1 for proband birth order and parental age distributions). We hypothesised that the results of