

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository:<https://orca.cardiff.ac.uk/id/eprint/124247/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Carnegie, Rebecca, Zheng, Jie, Sallis, Hannah, Jones, Hannah, Wade, Kaitlin, Jonathan, Evans, Zammit, Stan, Munafò, Marcus and Richards, Martin 2020. Mendelian randomization for nutritional psychiatry. *Lancet Psychiatry* 7 (2), pp. 208-216. 10.1016/S2215-0366(19)30293-7

Publishers page: [https://doi.org/10.1016/S2215-0366\(19\)30293-7](https://doi.org/10.1016/S2215-0366(19)30293-7)

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



## **Mendelian Randomization for Nutritional Psychiatry**

Carnegie R, MRCPsych<sup>1,2,3</sup>; Zheng J, PhD<sup>2,3</sup>; Sallis HM, PhD<sup>1,2,3,4</sup>; Jones HJ, PhD<sup>1,2,3,5</sup>; Wade KH, PhD<sup>2,3</sup>; Evans J, PhD<sup>1</sup>; Prof. Zammit S, PhD<sup>1,3,5,6</sup>; Prof. Munafò MR, PhD<sup>2,4,5</sup> and Prof. Martin RM, PhD<sup>3</sup>

<sup>1</sup> Centre for Academic Mental Health, Population Health Sciences, Bristol Medical School, University of Bristol, UK

<sup>2</sup> Medical Research Centre (MRC) Integrative Epidemiology Unit (IEU), University of Bristol, UK

<sup>3</sup> Population Health Sciences, Bristol Medical School, University of Bristol, UK

<sup>4</sup> School of Psychological Science, University of Bristol, UK

<sup>5</sup> NIHR Biomedical Research Centre, University Hospitals Bristol NHS Foundation Trust, University of Bristol, Bristol, UK

<sup>6</sup> MRC Centre for Neuropsychiatric Genetics and Genomics, Division of Psychological Medicine and Clinical Neurosciences, Cardiff University School of Medicine, Cardiff, UK

Corresponding author: Rebecca Carnegie

Address: Centre for Academic Mental Health,  
Oakfield House,  
Oakfield Grove,  
Bristol BS8 2BN,  
United Kingdom

Email: [rebecca.carnegie@bristol.ac.uk](mailto:rebecca.carnegie@bristol.ac.uk)

Telephone: ++44(0)117 331 4007

2 **SUMMARY**

3 Nutritional psychiatry is a growing area of research, with several nutritional factors implicated in the  
4 aetiology of psychiatric ill health. However, nutritional research is highly complex, with multiple  
5 potential factors involved, highly confounded exposures and small individual effect sizes. This paper  
6 considers whether Mendelian randomization provides a solution to these difficulties, by  
7 investigating causality in a low risk and low-cost way. Current studies using MR in nutritional  
8 psychiatry are reviewed, along with the potential opportunities and challenges of using this  
9 approach for investigating the causal effects of nutritional exposures. Several studies have identified  
10 potentially causal nutritional exposures using Mendelian randomisation in psychiatry, offering  
11 opportunities for further mechanistic research, intervention development, and replication. Using  
12 Mendelian randomisation as a foundation for intervention development allows the best use of  
13 resources in an emerging discipline in which opportunities are rich, but resources are often poor.

14

15 **Key words**

16 Mendelian randomization, nutritional psychiatry, causality

17

18

19

20

21

22

23

24

25

26

27

## 28 INTRODUCTION

29           The founding of the International Society for Nutritional Psychiatry Research<sup>1</sup> reflects an  
30 increasing recognition of nutrition as a modifiable risk factor for mental ill-health, and the need for  
31 good quality research in this area. Whilst the adverse psychological effects of severe nutritional  
32 deficiency are well established,<sup>2</sup> the extent to which subtle nutritional factors might have on  
33 cognitive and affective processes, or on the increasing burden of psychological ill health at the  
34 population level remains unclear. As wholefood diets have been replaced by processed foods - high  
35 in sugar and low in essential fats, vitamins and minerals - many argue that subtle malnutrition may  
36 exist even in the presence of calorie-abundance,<sup>3,4</sup> with unclear repercussions for population mental  
37 health. **Several meta-analyses of prospective studies suggest that a high-quality diet can reduce the**  
38 **risk of mental illness,<sup>5,6</sup> warranting further investigation of specific nutritional factors and**  
39 **mechanisms.** Conventional epidemiological associations between nutritional intake or status and  
40 psychiatric outcomes are highly prone to confounding by lifestyle and correlated dietary factors.<sup>7</sup>  
41 Furthermore, as many aspects of nutrition are affected by mental ill-health,<sup>8</sup> it is likely that reverse  
42 causality, or at least a bi-directional relationship, explains some of these associations. Finally, as  
43 individual nutrients have small effect sizes, large sample sizes are required to explore such  
44 associations with adequate statistical power, in which accurate dietary measurement is difficult.  
45 Despite the best efforts of researchers to control for these limitations, nutritional epidemiology is  
46 limited by issues of residual confounding, biological complexities and limited power.

47           Interventional research in nutritional psychiatry is a potential solution to these limitations,  
48 as good quality randomized controlled trials (RCTs) eliminate issues of confounding and reverse  
49 causality. There are a growing number of RCTs in nutritional psychiatry. Although many studies have  
50 focused on individual nutritional supplements - probably reflecting the parallels with a  
51 pharmacological research model,<sup>9-11</sup> there are few supplements that have been robustly identified  
52 as beneficial in psychiatry.<sup>11</sup> Results are often inconsistent, and it is unclear which interventions are  
53 worth further investment. Given the complexities and inter-relatedness of dietary composition, a

54 more comprehensive nutritional approach may be preferable. Combination micronutrient  
55 supplement interventions<sup>12-14</sup> and interventions focused on making broader changes to dietary  
56 patterns might be advantageous.<sup>15, 16</sup> Dietary pattern interventions offer a potential solution to this  
57 complexity, with supporting meta-analytical evidence in both observational<sup>5</sup> and interventional  
58 research<sup>17</sup>. However, selecting the right intervention and participants, and accounting for behaviour  
59 change and attrition, make the planning and evaluation of such trials complex and costly. With a  
60 multitude of potential nutritional interventions, it can be difficult to prioritise the most likely to be  
61 effective. False negatives from underpowered designs or minor aberrations in a complex  
62 intervention, might hinder the development of potentially beneficial interventions. Conversely, false  
63 positives due to biased designs, compounded by publication bias, lead to wasted expenditure and  
64 potential harm in repeated trials. Further evidence to establish likely causality for specific nutritional  
65 factors to underpin nutritional interventions and identify the most likely beneficial components  
66 would prevent wasted time and expenditure.

67 This paper considers whether ‘Mendelian randomization’ is a viable method to inform  
68 intervention development in nutritional psychiatry, in a low-cost and low-risk way. We review  
69 existing Mendelian randomization studies in nutritional psychiatry, the challenges faced, and  
70 opportunities for further research.

71

## 72 **MENDELIAN RANDOMIZATION**

73 Mendelian randomization (MR) is a method that is increasingly used to infer causality in  
74 epidemiological research. MR uses genetic markers that are robustly associated with a particular  
75 potentially modifiable exposure as ‘instrumental variables’ in assessing the relationship between an  
76 exposure and an outcome.<sup>18, 19</sup>As genetic markers (or ‘alleles’) are randomly allocated at conception,  
77 many have compared MR to a natural RCT, in which variant alleles rather than

78 Interventions are randomized (figure 1a). The individual variations in genetic alleles are referred to  
79 as single nucleotide polymorphisms (SNPs). MR exploits this natural genetic variation to circumvent  
80 the problem of confounding and reverse causality (figure 1a).

81 The concept of MR relies on key assumptions for validity (figure 1b). Whilst a comprehensive  
82 review of MR is beyond the scope of this review, some key terms used to describe aspects of MR  
83 studies relevant to this review are explained in Table 1. For more detail, see Zheng et al 2017<sup>20</sup> and  
84 the MR Dictionary.<sup>21</sup>

85 There are potential benefits to applying MR methodology to nutritional psychiatry, as a  
86 cheap and powerful method for attributing causality to putative exposures, and it enables the  
87 exploration of multiple avenues for intervention development in a low-cost and low-risk way. This is  
88 particularly true with the development of two-sample MR, in which exposures and outcomes need  
89 not be measured in the same sample (figure 1c). Two-sample MR takes estimates of the SNP-  
90 exposure association from a one population (for example a genome-wide association study (GWAS)  
91 of a nutritional exposure,) and the SNP-outcome association from another (for example a GWAS of a  
92 given psychiatric outcome). This allows for the possibility of utilising the increasing sample sizes  
93 provided by large psychiatric genetic consortia, without the need to access individual-level data on  
94 specific nutritional measures. Given the relatively small effect sizes, and modest genetic contribution  
95 to nutritional exposures, a two-sample MR methodology using large outcome samples should  
96 provide adequate power to investigate them.

97 One advantage of MR is that, providing appropriate genetic instruments are available, it is  
98 theoretically possible to model the results of certain randomised trials, thereby reducing  
99 unnecessary potential harms and expenditure. One example in the context of nutritional  
100 epidemiology was given by a recent MR study to model the Selenium and Vitamin E Cancer  
101 Prevention Trial (SELECT) for prostate cancer, which was based on extensive epidemiological  
102 evidence at that time. The SELECT trial, randomised 35,533 men to use selenium supplementation,  
103 to investigate whether increasing selenium levels might prevent prostate cancer.<sup>22</sup> The \$114 million

104 trial ended prematurely as results showed that selenium supplementation not only failed to reduce  
105 prostate cancer risk, it was likely to increase the risk of advanced prostate cancer and type 2  
106 diabetes mellitus. These results were replicated by MR, using genetic instruments for circulating  
107 selenium in the PRACTICAL consortium.<sup>23</sup> Although retrospective, the MR study took a fraction of the  
108 financial and time burden of a trial, and more importantly avoided any potential harm to  
109 participants.<sup>23</sup>

110 A comparison between MR and a naturalised RCT, has its limitations. Firstly, as genetic  
111 variants reflect lifetime exposures rather than short durations of therapeutic intervention, MR may  
112 produce a stronger effect than in the best approximation of a time-limited intervention. Conversely,  
113 individual adaptation to genotype may reduce the effect of the SNP on the exposure and so may  
114 underestimate the effect (also known as canalization (see table 1)). Rather than a replacement for  
115 RCTs, MR might be viewed as a foundation from which interventions for further development can be  
116 identified, in combination with epidemiology and basic science, also referred to as triangulation<sup>24</sup>  
117 (figure 2).

118 MR may be particularly useful for a field such as nutritional psychiatry, in which many of the  
119 interventional trials have small to modest sample sizes. A well-powered MR study can be used to  
120 verify results in a potentially underpowered study, as well as to inform future studies. MR studies  
121 showing no evidence for a causal effect need careful consideration about whether it is possible to  
122 rule out a clinically significant effect based on the available parameters, and whether replication  
123 using updated background literature would be beneficial at a future date. This includes whether the  
124 methods and instruments are valid, power is adequate, and whether biological complexity might  
125 complicate results. This is particularly relevant in psychiatry, where diagnostic categorisation is yet  
126 to account for the diversity of symptoms and presentations categorized by a single 'disorder'.  
127 Studies showing strong evidence for an effect need equal consideration before intervention  
128 development is considered, - such as how to increase the nutritional exposure in the desired way,

129 whether participants are selected based on deficiency states, and whether supplementation might  
130 have potential adverse effects.

131

## 132 **MENDELIAN RANDOMIZATION STUDIES IN NUTRITIONAL PSYCHIATRY**

133 We identified 26 studies using MR to investigate causality in nutritional psychiatry (Table 2).

134 Many have investigated a single exposure or outcome, but some have investigated multiple  
135 exposures and outcomes within the same paper. The studies are broadly grouped into three main  
136 psychiatric outcomes - cognitive impairment and dementia, schizophrenia, and mood disorders.

137

### 138 *Dementia and Cognition*

139 We identified 17 studies using MR to investigate the causality of nutritional factors on  
140 dementia and cognitive outcomes. Evidence suggesting a protective effect of 25-hydroxyvitamin D  
141 (25(OH)D) in Alzheimer's disease has been shown in two studies in the International Genomics of  
142 Alzheimer's Project (IGAP) Cohort (OR 0.86 per SD increase in vitamin D, 95% CI 0.78 to 0.94),<sup>25, 26</sup>  
143 but not replicated in the Uppsala Longitudinal Study (Hazard ratio per allele 1.04, 95% CI 0.91 to  
144 1.19).<sup>27</sup> Studies investigating 25(OH)D as a causal factor in cognitive function have found no evidence  
145 for an association.<sup>27-29</sup> It may be that vitamin D is particularly relevant to Alzheimer's pathology, or  
146 that larger sample size or stronger genetic instruments are required to identify the effects in non-  
147 clinical population samples. Furthermore, a possible non-linear observational association between  
148 vitamin D and cognition, with both deficiency and excess associated with poor cognition, was noted  
149 by Maddock et al 2017.<sup>29</sup> This raises important considerations about the ability of traditional MR  
150 techniques to detect causality for cognitive outcomes,<sup>29</sup> as well as other associations in which a  
151 similar relationship has been noted.<sup>30</sup> Novel methods are being developed to manage non-linearity  
152 in MR,<sup>31</sup> but are not commonly employed.

153 Studies investigating the causal role for B vitamin pathways in dementia have had mixed  
154 results. A study looking at multiple exposures using the IGAP cohort did not provide evidence for



155 folate (OR 0.98 per SD, 95% CI 0.72 to 1.33), homocysteine (OR 0.99 per SD, 95% CI 0.88 to 1.11) or  
156 vitamin B12 (OR 1.11 per SD, 95% CI 0.95 to 1.30) in Alzheimer's disease.<sup>32</sup> However, previous  
157 studies looking at homocysteine using a single SNP in the methylenetetrahydrofolate reductase  
158 (*MTHFR*) gene have suggested strong evidence of causality.<sup>33</sup> The *MTHFR* gene produces an enzyme  
159 which activates folate to metabolise homocysteine, and SNPs in this gene have been identified in  
160 GWAS of both homocysteine and circulating folate levels. However, some have suggested caution in  
161 the use of the *MTHFR* gene for MR due to a complex interaction with folate intake, in which the  
162 same polymorphism leading to reduced enzymatic activity in low-intake states (and therefore low  
163 blood folate and high homocysteine), may not have any effect on blood folate or high homocysteine  
164 in high-intake states.<sup>34</sup> Several MR studies of homocysteine using a single SNP relating to *MTHFR*  
165 have failed to replicate using instruments containing more SNPs and explaining a greater variation in  
166 homocysteine levels, suggesting that this SNP may be acting via a different mechanistic pathway. A  
167 meta-analysis of the results for homocysteine in Alzheimer's disease using the different instruments  
168 suggests some causal evidence for homocysteine (pooled effect 1.34 per SD, 95% CI 1.03 to 1.66),  
169 but in light of the complex biology, this may be misleading. Another study investigating vascular  
170 dementia using the same single SNP in the *MTHFR* gene also showed strong causal evidence for  
171 homocysteine (OR 4.29 per SD log(homocysteine), 95% CI 1.11 to 16.57).<sup>35</sup> However, the same  
172 caveats apply.

173 A single identified study has investigated amino acids in psychiatric disease, suggesting a  
174 potential causal role for isoleucine in Alzheimer's disease (OR 1.35 per SD, 95% CI 1.08 to 1.69),  
175 though not for other branched chain amino acids such as valine and leucine.<sup>36</sup>

176 The established link between *APOE* genotype and Alzheimer's has been corroborated using  
177 MR studies (OR 1.41 per mg/dL of APOE, 95% CI 1.27 to 1.57).<sup>37</sup> Further exploration of the role of  
178 lipids in dementia have not shown evidence for a causal role for any specific lipid fraction when the  
179 *APOE* SNPs are excluded from analysis.<sup>38, 39</sup> MR studies investigating fasting glucose (OR 1.12 per SD,

180 95% CI 0.97 to 1.30),<sup>40</sup> and vitamin E levels (OR 0.96 per SD, 95% CI 0.47 to 1.94),<sup>41</sup> have not found  
181 any evidence for a causal association. A single study investigating minerals using several psychiatric  
182 outcomes including Alzheimer's disease found no causal evidence for magnesium (0.43 per SD, 95%  
183 CI 0.08 to 2.44), calcium (Ca 0.74 per SD, 95% CI 0.45 to 1.22), Iron (1.02 per SD, 95% CI 0.94 to 1.14)  
184 or zinc (0.99 per SD, 95% CI 0.85 to 1.14), with weak evidence for low copper (0.87 per SD, 95% CI  
185 0.75 to 1.00).<sup>42</sup>

186

### 187 *Schizophrenia*

188 We identified six studies that have investigated nutritional exposures in schizophrenia using  
189 MR. There was weak causal evidence for vitamin B6 (OR 0.99 per SD log(B6), 95% CI 0.65 to 1.51),<sup>43</sup>  
190 and for serum minerals (Calcium, Serum Magnesium, Copper, Iron and Zinc, see Table 2) in  
191 schizophrenia.<sup>42</sup> Two studies have identified an association between homocysteine and  
192 schizophrenia, in European (2.15 per SD, 95% CI 1.39 to 3.32)<sup>44</sup> and Japanese populations (1.14 per  
193 SD, 95% CI 1.03 to 1.27);<sup>45</sup> however, both used a single SNP related to the *MTHFR* gene, with the  
194 aforementioned limitations. A study looking at the causal role of glucose and insulin related traits  
195 found some evidence for fasting glucose (OR 0.84 per SD, 95% CI 0.71 to 0.99), but strong evidence  
196 for fasting insulin levels (OR 2.33 per SD, 95% CI 1.40 to 3.90).<sup>46</sup> Given the discrepancy with the  
197 strength of effect of fasting glucose in the same study, it is likely that insulin partially acts through an  
198 independent pathway to glucose, possibly related to a direct action as a 'neuropeptide', involved in  
199 neuroplasticity and modulation.

200 In contrast to findings in multiple sclerosis<sup>47</sup> and Alzheimer's Disease,<sup>26, 27</sup> no strong causal  
201 evidence has been found for vitamin D in schizophrenia (OR 0.99 per 10% increase in 25(OH)D, 95%  
202 CI 0.97 to 1.01).<sup>48</sup> This may suggest that the observational estimate is the result of confounding or  
203 reverse causality, but it is also possible that standard MR techniques have been unable to detect a  
204 true causal association due to limited power, population stratification, or biological complexities

205 (table 4). Although the power of the study appears more than adequate (example sample sizes  
206 based on MR power calculations are shown in table 3), diagnosis of schizophrenia is comparatively  
207 vague, and more subject to symptom interpretation than for an outcome such as multiple sclerosis  
208 or Alzheimer's Disease. This heterogeneity may require larger sample sizes to identify causal effects  
209 of a similar magnitude. A second limitation is MR results represent the causal impact of a lifetime  
210 exposure on an outcome, it is unable to account for exposures that are time-limited or during a  
211 sensitive period.

212 For example, if the sensitive period for vitamin D deficiency is intrauterine, as suggested by  
213 the higher prevalence among winter-born individuals,<sup>49</sup> an MR analysis would not reflect this. Finally,  
214 standard MR techniques assume a linear relationship between exposure and outcome, which in the  
215 case of vitamin D might be a fallacy, as both deficiency and excess states may be harmful.<sup>30</sup> Standard  
216 MR techniques assume a linear association between the exposure and outcome, and whilst novel  
217 methods are being developed to overcome this limitation, they are not yet standard practice.<sup>31</sup>

218

### 219 *Mood Disorders*

220 Several nutritional factors have been investigated using MR in major depression samples,  
221 with no strong evidence of effect. Nutritional factors include vitamins B12 and folate,<sup>50</sup> omega 3  
222 fatty acids,<sup>51</sup> and 25-hydroxyvitamin D.<sup>52</sup> The five minerals investigated in Cheng 2019 did not show  
223 evidence of causality, though the Psychiatric Genomics Consortium sample used as the outcome is  
224 small (N=10,640) in comparison to the latest PGC Major Depression sample (N=807,553).<sup>42</sup> An MR  
225 study using the Young Finns study<sup>53</sup> showed an inverse association between fasting glucose and  
226 depressive symptoms measured using the Beck Depression Inventory, (-0.43 BDI points per  
227 weighted effect allele, 95% CI -0.79 to 0.07), which the authors hypothesise to relate to the  
228 cognitive effects of hypoglycaemia. A study in UK Biobank suggested a potentially causal role for  
229 elevated triglycerides (but not LDL- or HDL-cholesterol) in the development of lifetime major  
230 depression (OR 1.18 per SD (1.09–1.27)).<sup>54</sup>

231 An MR study looking at multiple minerals identified a potentially causal role for low copper  
232 (OR 0.87 per SD, 95% CI 0.79 to 0.97) and for high serum magnesium (OR 8.78 per SD, 95% CI 1.16 to  
233 66.26) in bipolar disorder using the Bipolar Disorder Working Group sample of the Psychiatric  
234 Genomics Consortium. Both findings warrant replication and further investigation. Some  
235 observational literature has suggested higher serum magnesium (though lower intracellular  
236 magnesium) levels in bipolar disorder, and the pathophysiological mechanisms behind this could be  
237 further explored using two-step MR (figure 3a).

238

### 239 **OPPORTUNITIES FOR MR IN NUTRITIONAL PSYCHIATRY**

240 Although one of the biggest challenges for MR in nutritional psychiatry to date has been the  
241 lack of appropriate genetic instruments, nutritional genetics is evolving. Instruments for many  
242 nutritional exposures are being utilised in MR studies outside psychiatry or applied to only one of a  
243 multitude of psychiatric outcomes. In addition to biological nutritional markers, GWAS of dietary  
244 intake,<sup>55</sup> dietary patterns,<sup>56</sup> and even gut microbial diversity,<sup>57</sup> may provide useful potential  
245 instruments for future MR studies aiming to assess the impact of nutritional characteristics on  
246 psychiatric ill-health. For example, evidence suggests that gut microbial diversity and abundance is  
247 influenced by human genetics,<sup>57</sup> making MR studies of this exposure possible, with examples of  
248 causal relationships being identified using MR in other areas of medicine.<sup>58</sup> MR studies of the gut  
249 microbiome characterised in different ways may help to explain the association between reduced  
250 gut microbiome diversity and the presence of specific bacterial taxa in psychiatric disease,<sup>59</sup> and the  
251 apparent benefits of probiotics in psychiatry.<sup>60, 61</sup>

252 MR methods are continually evolving (see table 1), with several techniques relevant to  
253 research in nutritional psychiatry. An example is multivariable MR (see figure 3b), which can be  
254 employed in situations where genetic variants are related to several correlated exposures.  
255 Multivariable MR has been used successfully in untangling the association between high density  
256 lipoprotein cholesterol, low density lipoprotein cholesterol, and triglycerides with cardiovascular

257 disease<sup>62</sup> and depression.<sup>54</sup> Multivariable MR could similarly be used to unpick potentially complex  
258 associations, such as between omega 3 and 6 fatty acids, or B vitamin pathways, in psychiatry. For  
259 positive findings in nutritional psychiatry, potential off-target adverse effects of nutritional  
260 supplementation could be identified using MR phenome-wide association study (MR-PheWAS).<sup>63</sup> An  
261 MR-PheWAS uses a hypothesis-free approach to scan many outcomes for a given exposure, and  
262 could have potentially pre-empted the increased risk of diabetes with selenium supplementation  
263 seen in the SELECT trial.<sup>23</sup> As well as informing intervention development, MR can also be used to  
264 investigate biological mechanisms in psychiatry including metabolomic, microbiomic, proteomic and  
265 epigenomic intermediates, using two-step MR.<sup>64</sup> Two-step MR is a relatively new method for  
266 identifying and quantifying mediating mechanisms between an exposure and outcome using an MR  
267 framework (figure 3a). Novel MR methods to analyse gene-environment interactions are also under  
268 development, and may be particularly useful in the context of nutritional psychiatry. Finally, using  
269 MR of the human proteome in relation to psychiatric outcomes may identify novel drug targets.

270           Standard MR methods rely on a single exposure-outcome framework, which many consider  
271 to be oversimplified when in the context of complex nutritional biology. Many nutritional  
272 epidemiologists have moved beyond a single nutrient approach to consider whole dietary patterns,  
273 adiposity, and the inherently complex interaction between diet, hormones and physical activity.<sup>65</sup> It  
274 is possible that future MR methods could consider interactions between other nutritional exposures,  
275 as well as with gene-environment interactions considering nutritional intake or other lifestyle  
276 factors. Techniques such as machine learning and data mining using nutritional exposures, genetic  
277 data, dietary intake and psychiatric diagnoses and symptoms might be necessary for unpicking  
278 complex associations and gene-environment interactions further. Machine learning has already been  
279 suggested for augmenting MR, by predicting the most appropriate model to optimise power and  
280 detect pleiotropy, and could potentially enhance MR in the complex arena of nutritional  
281 psychiatry.<sup>66</sup>

282

## 283 CHALLENGES FOR MR IN NUTRITIONAL PSYCHIATRY

284 With increasing availability of genetic instruments, genetic samples, and platforms for MR  
285 analysis, false results can be obtained quickly. Results need careful consideration as to validity of the  
286 methods, samples, and instruments used, irrespective of their strength or direction. Subsequent  
287 replication in independent cohorts remains crucial.<sup>19</sup>

288 Several limitations of traditional MR methods may hinder the application to nutritional  
289 psychiatry (see table 4). The lack of valid, robust genetic instruments for many nutritional exposures  
290 is arguably one of the most fundamental limitations. GWAS studies identifying SNPs robustly  
291 associated with nutritional exposures depend on adequately sized genotyped samples of nutritional  
292 factors. Difficulties identifying robust and reliable nutritional biomarkers reflecting nutritional status  
293 may underlie this, along with the availability of such nutritional measures in adequately sized  
294 genotyped cohorts. Instruments that are only weakly associated with the exposure of interest (e.g.  
295 F-statistic <10, see table 2) will bias estimates in different directions depending on whether a one-  
296 sample or two-sample methodology is used (table 4).

297 Nutritional genetic epidemiology is a developing field and the expectation is that good  
298 quality, validated instruments for nutritional exposures should emerge and evolve. However, even  
299 where genetic instruments appear to exist, some consideration needs to be given to whether they  
300 are valid for the specific association being tested with MR analyses, checking as far as possible that  
301 the assumptions of MR hold, and by understanding their underlying biological function.

302 With the increasing development of large psychiatric genomics consortia samples, outcome  
303 sample sizes are rapidly increasing. At first glance these appear to provide ample power to detect  
304 nutritional exposures, even those with a very small effect (see table 3.)<sup>19</sup> However, as sample sizes  
305 increase, it is important to consider the extent to which the genetic heterogeneity of the population  
306 has increased, and the validity of the genetic instrument within this new population structure.  
307 Furthermore, the risk of overlapping exposure and outcome samples may invalidate some of the  
308 assumptions of two-sample MR. The relative benefits of using small samples with precisely

309 measured nutritional exposures and psychiatric symptomatology, compared to large samples with  
310 imprecise measures and heterogenous samples are not always clearly defined.

311

## 312 **FUTURE DIRECTIONS**

313 Genetic epidemiology is evolving. Sample sizes, genetic markers and MR techniques are  
314 continuing to increase in both number and complexity. Negative early findings need careful  
315 consideration, and positive findings warrant replication in independent cohorts. As sample sizes and  
316 genetic instruments develop, formal repetition of earlier studies and independent replication  
317 remains essential. Given the relative ease with which analyses can be conducted once an instrument  
318 is identified, a more systematic and thorough approach to evaluating nutritional factors in psychiatry  
319 would be beneficial, perhaps considering individual psychiatric presentations along with a 'cross-  
320 disorder' approach. Opportunities for undertaking GWAS of nutritional biomarkers should be sought  
321 and validated, to make future MR studies possible. Future MR studies should consider novel MR  
322 techniques such as multivariable MR where appropriate, techniques for accounting for non-linear  
323 associations, as well as two-step MR to identify causal mechanisms. Further understanding of gene-  
324 environment interactions using large biobanks with data on genetics as well as nutritional and  
325 lifestyle measures might be useful for triangulating with nutritional MR studies. Finally, as the  
326 research landscape evolves, replication of earlier studies using larger samples and improved genetic  
327 instruments, continues to be of value.

328 Beyond genetics, ongoing research from a broad range of disciplines including epidemiology,  
329 basic sciences, and clinical trials is needed, to identify novel biomarkers of nutritional intake and  
330 status, to develop new technologies for accurate dietary assessment, and to apply the results of MR  
331 studies to inform and conduct large-scale pragmatic trials.

332

## 333 **CONCLUSION**

334 Nutritional psychiatry, nutritional genetic epidemiology and psychiatric genetics are all at  
335 relatively early stages in their understanding. MR in nutritional psychiatry sits at the centre of these  
336 emerging disciplines, providing a unique way to investigate causality in nutritional psychiatry and  
337 understand its mechanisms. Despite some challenges in this area, emerging MR evidence for  
338 nutritional factors including vitamin D, folate, serum magnesium, copper, triglycerides, and glucose  
339 metabolic pathways on psychiatric outcomes highlight the potential utility of this technique for  
340 identifying causal factors in nutritional psychiatry and developing a firm evidence base for the  
341 causality of nutritional exposures from which successful interventions can develop.

342

#### 343 **SEARCH STRATEGY AND SELECTION CRITERIA**

344 References for this review were identified through systematic searches of OVID Medline  
345 (1946 to January 2019,) PsycINFO (1808 to January 2019) and EMBASE (1974-2019) database for  
346 articles published from by use of the terms “Mendelian randomization”, “Psychiatry OR Psychology”,  
347 and other diagnostic terms (see Appendix 1). All abstracts identified were screened to include any  
348 exposure related to nutrition. Exposures were included if they measured any factor that was directly  
349 related to nutritional components or nutritional status, including micronutrients (including vitamins  
350 and minerals), macronutrients (including glucose homeostatic markers, amino acids and peptides  
351 and lipids), and biological markers of nutritional status. These factors were not selected *a priori*, but  
352 identified *post-hoc* based on the MR exposures available. Studies using psychiatric diagnosis as an  
353 exposure rather than outcome, addressing broader lifestyle exposures such as body mass index,  
354 physical activity or alcohol, and considered inter-generational exposures (such as offspring outcomes  
355 of pregnancy exposures) were excluded. A full search strategy is given in Appendix 1, with a  
356 flowchart of included studies in Appendix 2. No exclusions were made on the basis of language.

357



358 **RESEARCH IN CONTEXT**

359 *Evidence before this study*

360 Nutritional psychiatry is an emerging area of research, but its complexities are numerous. Several  
361 nutritional factors have been implicated in psychiatric aetiology, but causal evidence remains scarce.  
362 Mendelian randomization (MR) is an epidemiological method that can help investigate causality.  
363 Outside of psychiatry, MR has identified likely causal associations between low vitamin D and  
364 multiple sclerosis, low serum iron and Parkinson’s disease, and low serum magnesium and  
365 cardiovascular disease. We searched the OVID Medline database for studies using “Mendelian  
366 randomization” with any outcome related to “Psychiatry OR Psychology”. We excluded studies in  
367 which psychiatric conditions were used as an exposure rather than outcome, which used broader  
368 lifestyle exposures such as body mass index, physical activity or alcohol, and for which the exposure  
369 and outcome was inter-generational (such as offspring outcomes of pregnancy exposures).

370

371 *Added value of this study*

372 Several studies have investigated potential causal nutritional factors in psychiatry using MR. This  
373 study summarizes the current evidence and explores the opportunities and challenges in using this  
374 method to underpin intervention development. This paper also summarises some of the novel  
375 methods in MR, and how they might overcome issues with correlated nutritional exposures, non-  
376 linear effects, and to identify potential harms of supplementation.

377

378 *Implications of all the available evidence*

379 Several MR studies have shown evidence for causal nutritional factors in psychiatry . A  
380 comprehensive approach to investigating nutritional exposures psychiatry would be beneficial for  
381 the current evidence base and would help to inform intervention development in a resource-  
382 constrained field. It is important to consider the validity of findings irrespective of the direction or

383 strength of evidence, and to replicate results as new samples, methods and biological insights  
384 become available.

385

386 **CONFLICT OF INTEREST**

387 RC was funded by a Wellcome Trust GW4 CAT Fellowship (grant number WT 203918/Z/16/Z), during  
388 the conduct of the study. The other authors declare no conflicts of interest.

389

390 **CONTRIBUTORS**

391 RC was primary author and undertook the literature search. JZ, KW, HS and HJ provided help and  
392 training in MR methodology. All other authors contributed through advice on content and editing  
393 drafts. All authors had approval of final draft for submission.

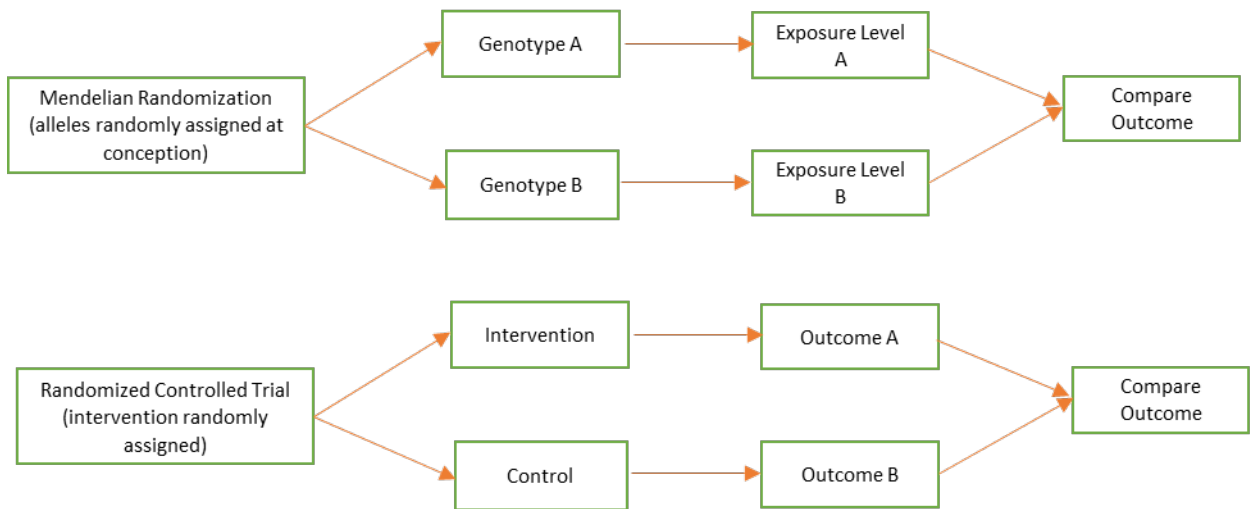
394

395

397 Figure 1: Mendelian Randomization: comparisons and assumption.

**1a Mendelian Randomization as a ‘natural’ Randomized Controlled Trial**

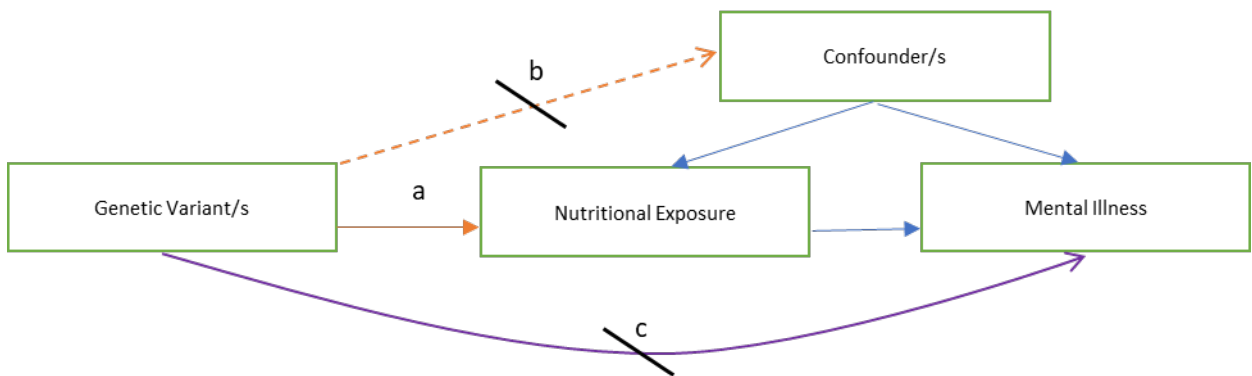
MR has been compared to a randomized controlled trial, with random allocation of genetic alleles at conception could be considered analogous to random allocation of interventions in a trial.



398  
399  
400

**1b Assumptions in Mendelian Randomization**

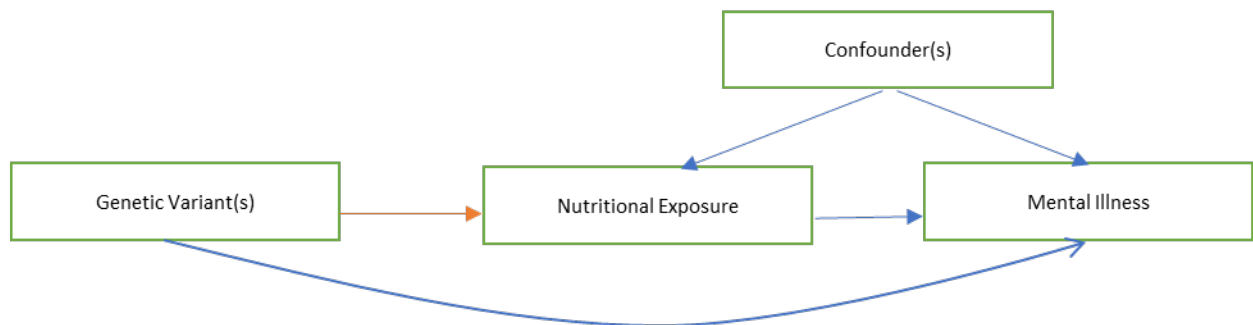
MR assumes that the genetic variants are: a. associated with the exposure of interest; b. not associated with confounders; and c. only associated with the outcome through the exposure



401  
402  
403

### 1c Two-sample Mendelian Randomization

Two-sample MR takes estimates of the SNP-exposure association from one population (e.g. a nutritional exposure GWAS) and the SNP-outcome association from a separate sample (e.g. a psychiatric outcome GWAS).



*First Sample is used to estimate the SNP-exposure association (i.e. genetic contribution to nutritional status)*



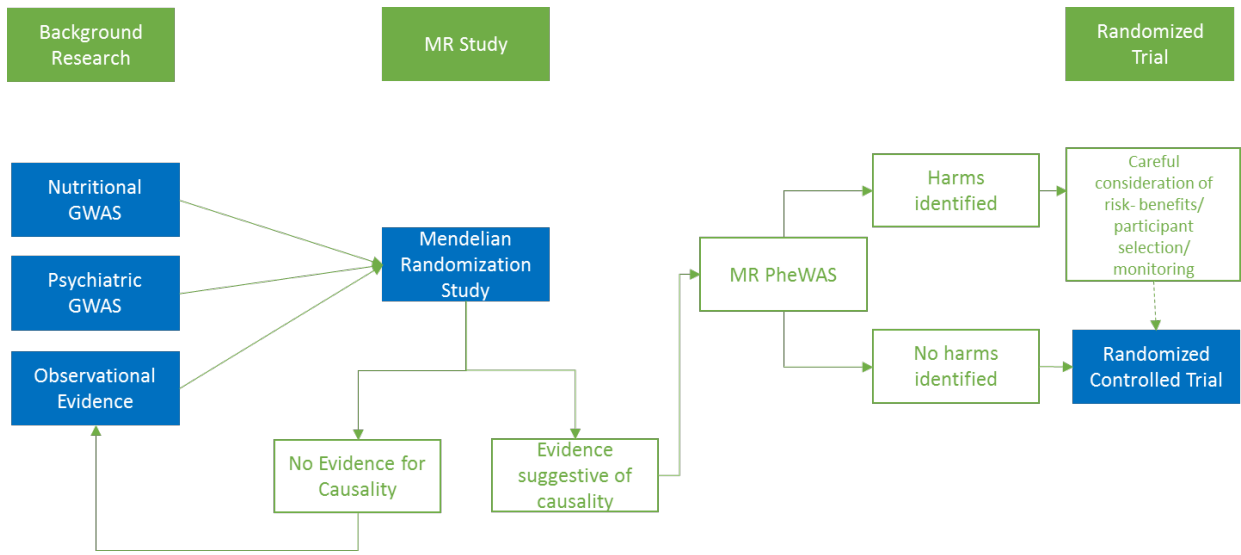
*Second Sample is used to estimate the SNP-outcome association (i.e. genetic contribution of the exposure SNP(s) on the outcome)*

404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414

**Figure 2: A theoretical pipeline for the use of MR studies in intervention development**

Whilst many have compared MR to 'nature's RCT', it may be more realistic to see MR studies as an interim step in intervention development.

415



416

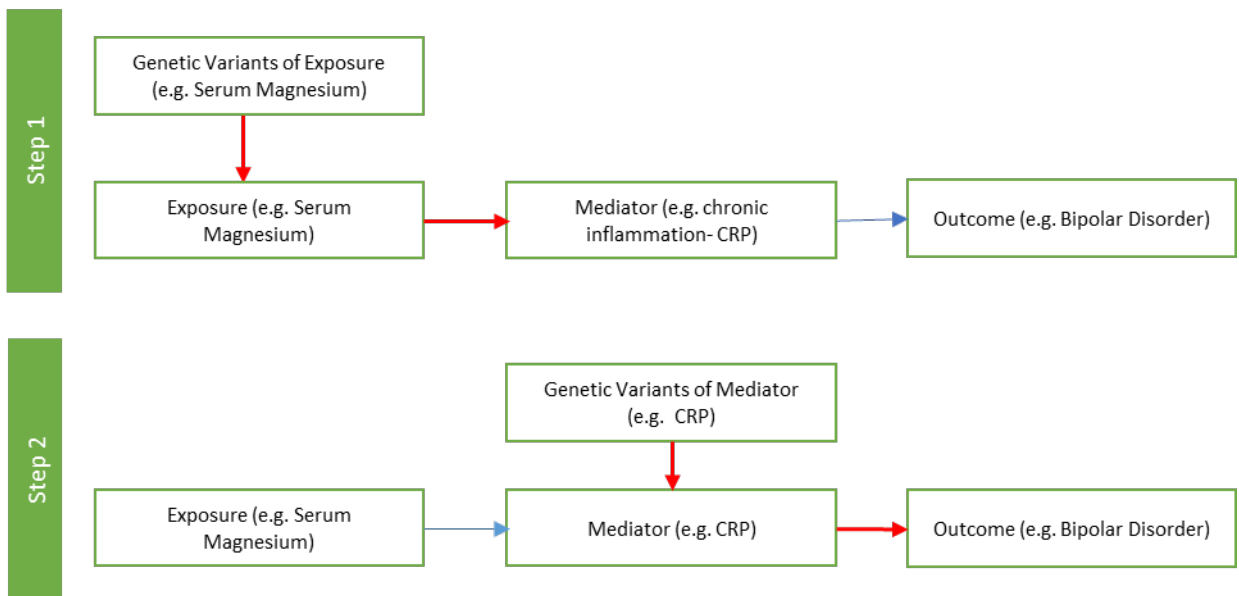
417

418

**Figure 3: Advanced MR methodologies**

**3a Two-step Mendelian Randomization**

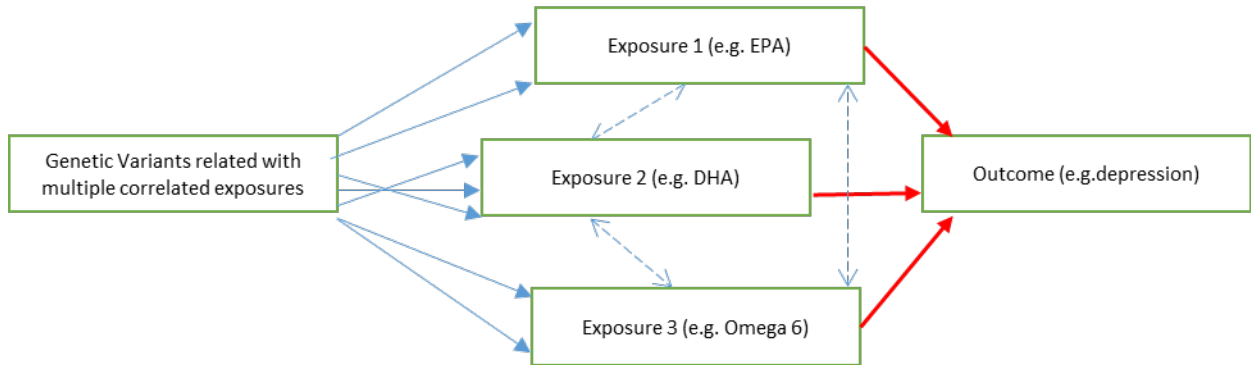
Two-step MR can be used to identify mediating mechanisms between an exposure and outcome using separate MR analyses which are combined in a traditional mediation analysis.



420

### 3b Multivariable Mendelian Randomization

Multivariable MR can be used where genetic variants are related to multiple correlated exposures. For example, a SNP for one lipid will often be correlated with others. This technique could be used to untangle potentially opposing associations between omega 3 (EPA/ DHA) and omega 6 fatty acids.



421

422 **Table 1: Glossary of MR Terms and potential uses in nutritional psychiatry** <sup>20</sup> For more information  
 423 **about other terms and the**

Term	Explanation
<b>F-Statistic</b>	The F-statistic measures the strength of genetic instruments. $F < 10$ is suggestive of weak instrument bias.
<b>Multivariable MR</b>	Multivariable MR is a technique to account for pleiotropy due to multiple correlated exposures.
<b>MR-PheWAS (MR Phenome Wide Association Study)</b>	MR PheWAS is a method using a hypothesis-free approach to scan many outcomes for a given exposure using MR methodology. Such approaches could be used to test for and identify any potential adverse off-target effects of dietary supplementation, providing genetic instruments exist.
<b>Pleiotropy</b>	Horizontal Pleiotropy is where the SNP or SNPs related to the exposure are associated with the outcome through a pathway independent of the exposure (i.e. a violation of assumption c in figure 1b).  Pleiotropy can be demonstrated by several methods, including Cochran's Q statistic testing heterogeneity in causal estimates from each SNP, MR-Egger intercept, and leave-one-out analysis to identify influential outliers
<b>Population Stratification</b>	Spurious associations may arise in MR where the genetic variant and the outcome are associated with ancestral background in a mixed or stratified sample. Using genetic associations from within homogenous populations, or checking that the GWAS has controlled for population substructure in the analysis is important.
<b>One-sample MR</b>	Conventional one-sample MR uses a single sample in which exposure, outcome and genetic instrument are measured within the same population. One-sample MR may have power issues due to inadequate

	sample sizes of studies that are required to have genotype, exposure and outcome data.
<b>Two-Sample MR</b>	The estimates of the SNP-exposure and SNP-outcome associations used in MR analyses are identified in independent studies (usually genome-wide association studies)
<b>Two-Step/ Mediation MR</b>	Two-step MR can be used to identify mediating mechanisms between an exposure and outcome using two steps- the first to assess the causal effect of the exposure on the potential mediator, and the second to assess the causal effect of the mediator on the outcome

424



**Table 2: Studies using Mendelian randomization in nutritional psychiatry**

Table summarizes current MR studies in nutritional psychiatry. Discrepancies exist between disorders, and the applicability of existing instruments to other outcomes, or to a combined 'cross disorder' cohort may be fruitful. Results are given as odds ratios per standard deviation change in the exposure unless otherwise specified. Abbreviations: IGAP (International Genomics of Alzheimer's), Psychiatric Genomics Consortium (PGC). For further details of instrument rsids, genes and beta coefficients please refer to the original publication.

Exposure	Study	Measure	Sample	N	MR Method	SNPs	Results Reported OR / beta/ Hazard ratio/ Risk difference (95% confidence intervals) p-value	
VITAMINS	Vitamin D	Maddock 2017 <sup>29</sup>	Global Cognitive tests	Cross cohort	172,349	Two-sample	2	$\beta$ 0.00 points per 25(OH)D decreasing allele (0.01, 0.01) p>0.99
			Memory tests					$\beta$ 0.00 points per 25(OH)D decreasing allele (-0.01, 0.01) p=0.6
	Jorde 2015 <sup>28</sup>	Cognitive Tests	Tromso Study	5,980	One-sample	4	No overall association	
	Mokry 2016 <sup>25</sup>	Alzheimer's Diagnosis	IGAP <sup>1</sup>	54,162	Two-sample	4	OR 0.8 per SD (0.97, 0.66) p=0.021	
	Olsson 2017 <sup>27</sup>	Dementia Diagnosis	Uppsala Longitudinal	1,087	One-sample	2	HR 1.04 points per effect allele (0.91, 1.19)	
		Cognitive Impairment (MMSE)	Study	408	One-sample	2	OR 1.03 per effect allele (0.80, 1.34)	
	Larsson 2018 <sup>26</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	7	OR 0.86 per SD (0.78, 0.94) p = 0.002	
	Taylor 2016 <sup>48</sup>	Schizophrenia Diagnosis	PGC <sup>2</sup>	79,845	Two-sample	4	OR 0.99 per 10% increase (0.97, 1.0)	
Michaelsson 2018 <sup>52</sup>	Major Depression Diagnosis	PGC	173,005	Two-sample	6	OR 1.02 per SD (0.97, 1.08) p = 0.44		

<sup>1</sup> IGAP International Genomics of Alzheimer's Project

<sup>2</sup> PGC Psychiatric Genomics Consortium

Vitamin E	Liu 2018 <sup>41</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	3	OR 0.96 per SD (0.47,1.94) p =0.936
Vitamin B6	Tomioka 2018 <sup>43</sup>	Schizophrenia Diagnosis	Tokushima University Hospital	10,689	One-sample	1	OR 0.99 per SD log(B6) (0.65, 1.51) p=0.96
Folate	Larsson 2017 <sup>32</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	2	OR 0.98 per SD (0.72, 1.33) p=0.89
	Mollehave 2017 <sup>50</sup>	Depression (SLCR90_r <sup>3</sup> )	Health 2006 & Inter 99	4,126	One-sample	2	OR 1.18 per effect allele (0.18, 7.66), P=0.86
Homocysteine	Hu 2016 <sup>33</sup>	Alzheimer's Diagnosis	34 studies	9,397	Two-Sample	1	OR 3.37 per SD ( 1.90, 5.95) p=2.9x10 <sup>-5</sup>
	Larsson 2017 <sup>32</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	18	OR 0.99 per SD (0.88, 1.11) 0.86
	Roostaei 2018 <sup>67</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	13	OR 1.01 per SD (0.89, 1.15), p=0.84
	Numata 2015 <sup>44</sup>	Schizophrenia Diagnosis	36 Studies	25,599	Two-sample	1	OR 2.15 per SD (1.39, 3.32) p=5.3x10 <sup>-4</sup>
	Kinoshita 2015 <sup>45</sup>	Schizophrenia Diagnosis	Meta-analysis	10,378	One-sample	1	OR 1.14 per SD (1.03-1.27), p=1.6x10 <sup>-2</sup>
	Wu 2017 <sup>35</sup>	Vascular Dementia Diagnosis	Meta-analysis	1,880	Two-sample	1	OR 4.29 per SD log (hcy) (1.11,16.57) P = 0.03

<sup>3</sup> SLCR90\_r diagnosis depression...

	B12	Mollehave 2017 <sup>50</sup>	Depression (SLCR90_r <sup>4</sup> )	Health 2006 & Inter 99	4,126	One-sample	12	0.96 (0.52,1.79), P=0.91
		Larsson 2018 <sup>32</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	7	OR 1.11 per SD (0.95, 1.30) p=0.18
MINERALS	Calcium	Cheng 2019 <sup>42</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	6	OR 0.74 per SD (0.45, 1.22) p=0.23
			Major Depression Diagnosis	PGC	10,640	Two-sample	6	OR 0.92 per SD (0.67, 1.28) p=0.63
			Bipolar Disorder Diagnosis	PGC	41,653	Two-sample	7	OR 1.85 per SD (0.74, 4.65) p=0.19
			Schizophrenia Diagnosis	PGC	65,967	Two-sample	7	OR 1.85 per SD (0.74, 4.65) p=0.19
	Copper	Cheng 2019 <sup>42</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	2	OR 0.87 per SD (0.75, 1.00) p=0.05
			Bipolar Disorder Diagnosis	PGC	41,653	Two-sample	2	OR 0.87 per SD (0.79, 0.97) p=0.01
			Schizophrenia Diagnosis	PGC	65,967	Two-sample	2	OR 0.96 per SD (0.85, 1.08) p=0.47

<sup>4</sup> SLCR90\_r diagnosis depression...

Magnesium	Cheng 2019 <sup>42</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	4	OR 0.43 per SD (0.08-2.44) p=0.34
		Major Depression Diagnosis	PGC	10,640	Two-sample	3	OR 1.19 per SD (0.22, 6.61) p=0.84
		Bipolar Disorder Diagnosis	PGC	41,653	Two-sample	4	OR 8.78 per SD (1.16, 66.26) p=0.04
		Schizophrenia Diagnosis	PGC	65,967	Two-sample	4	OR 0.87 per SD (0.24, 3.19) p=0.83
Iron	Cheng 2019 <sup>42</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	11	OR 1.02 per SD (0.94, 1.14) p=0.48
		Major Depression Diagnosis	PGC	10,640	Two-sample	9	OR 0.98 per SD (0.91, 1.05) p=0.60
		Bipolar Disorder Diagnosis	PGC	41,653	Two-sample	11	OR 1.17 per SD (0.89, 1.29) p=0.45
		Schizophrenia Diagnosis	PGC	65,967	Two-sample	10	OR 1.04 per SD (0.92, 1.18) p=0.55

	Zinc	Cheng 2019 <sup>42</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	2	OR 0.99 per SD (0.85, 1.14) p=0.85
			Major Depression Diagnosis	PGC	10,640	Two-sample	2	OR 0.99 per SD (0.95, 1.03) p=0.66
			Bipolar Disorder Diagnosis	PGC	41,653	Two-sample	2	OR 1.02 per SD (0.91, 1.14) p=0.70
			Schizophrenia Diagnosis	PGC	65,967	Two-sample	2	OR 0.94 per SD (0.86, 1.02) p=0.11
LIPID FAT AND GLUCOSE HOMEOSTASIS	Isoleucine	Larsson 2017 <sup>36</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	4	OR 1.35 per SD (1.08, 1.69) p=0.007
	Leucine						1	OR 1.16 per SD (95% CI, 0.78–1.72) p=0.46
	Valine						1	OR 1.13 per SD (95% CI, 0.82–1.57) p=0.46
	Fasting Glucose	Weslowska 2017 <sup>53</sup>	Depression (BDI)	Young Finns Study	1,217	One-Sample	35	-0.43 (-0.79, -0.07) p=0.02
		Li 2018 <sup>46</sup>	Schizophrenia Diagnosis	PGC	77,096	Two-sample	30	OR 0.84 per SD, (0.71, 0.99) p=0.038
				BIO-X	26,026		14	OR 1.04 per SD (0.84, 1.27) p=0.737
		Ostegaard 2015 <sup>40</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	36	OR 1.12 per SD (0.97, 1.30) p=0.112

Fasting insulin and insulin sensitivity	Ostegaard 2015 <sup>40</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	10	OR 1.32 per SD (0.88, 1.98) p=0.18	
	Walter 2016 <sup>68</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	9	OR 1.17 per unit (1.02,1.34) p=0.02	
	Li 2018 <sup>46</sup>	Schizophrenia Diagnosis	PGC	77,096	Two-sample	13	OR 2.33 per SD (1.40, 3.90) p=0.001	
DHA (Omega 3)	Sallis 2014 <sup>51</sup>	Perinatal Depression (EPDS)	ALSPAC mothers	2,378	One-sample	4	RD 0.08 (-0.05, 0.22) p=0.21	
Plasma APOE	Rasmussen <sup>37</sup>	Alzheimer's Diagnosis	Copenhagen	106,562	One-sample	5	OR 1.41 per mg/dL (1.27, 1.57)	
		All Dementia	General Population Study and Copenhagen City Heart Study				OR 1.33 per mg/dL (1.25, 1.43)	
Cholesterol & Triglycerides	Proitsi 2014 <sup>39</sup>	Alzheimer's Diagnosis	Cross Cohort	10,578	Two-Sample	70	OR 0.95 per unit (0.76,1.21) p=0.69 Total Cholesterol	
							40	OR 1.10 per unit (0.89,1.37) p=0.36 Triglycerides
							69	1.01 per unit (0.82,1.24) p=0.96 HDL-c
							55	0.90 per unit (0.65,1.25) p=0.53 LDL-c

Ostergaard 2015 <sup>40</sup>	Alzheimer's Diagnosis	IGAP	54,162	Two-sample	73	OR 1.94 per SD (1.79-2.10) p=3.1x10 <sup>-56</sup>
						Total Cholesterol
					39	OR 0.96 per SD (0.87,1.07) p=0.48
						Triglycerides
					71	OR 0.75 per SD (0.69, 0.82) p=1x10 <sup>-11</sup>
						HDL-c
					57	OR 2.31 per SD (2.12, 2.50) p=3x10 <sup>-87</sup>
						LDL-c
Benn 2017 <sup>38</sup>	Alzheimer's Diagnosis	Copenhagen	111,194	One-sample	380	OR 0.57 per mmolL <sup>-1</sup> (0.27, 1.17) LDL-c
	Vascular Dementia	General				OR 0.81 per mmolL <sup>-1</sup> (0.34, 1.89) LDL-c
	All Dementia	Population Study and Copenhagen City Heart Study				OR 0.66 per mmolL <sup>-1</sup> (0.34, 1.26) LDL-c
Khandaker 2019 <sup>54</sup>	Major Depression	UK Biobank	367,703	Two-sample	76	OR 1.02 per SD (0.91-1.14) LDL-c
					86	OR 0.97 per SD (0.91-1.03) HDL-c
					51	OR 1.18 per SD (1.09-1.27) Triglycerides





**Table 3: A rough guide to sample size requirements for MR studies**

An illustration of minimum sample sizes required for MR studies, taken from the online calculator available at <http://cnsgenomics.com/shiny/mRnd/><sup>69</sup> Results shown are for a binary outcome, assuming 25% cases in study, 0.8 power and alpha 0.05.

Variance explained	Estimated Effect Size (OR)	Minimum Sample size
1%	1.01	42,069,473
	1.1	439,015
	1.5	20,408
	2	5,756
5%	1.01	8,413,895
	1.1	87,803
	1.5	4,082
	2	1,152

**Table 4: Limitations of MR** <sup>20</sup>

Limitation	Description	Relevance to Nutritional Psychiatry	Potential Solution
<b>Lack of Available Instruments</b>	Genetic instruments are unavailable for certain exposures	Lack of GWAS for certain nutritional exposures. Also due to poor measurement of particular nutritional exposures (e.g. serum versus intracellular magnesium).	Choose a proxy exposure for which data is available. Continue to review instruments as nutritional GWAS are published.
<b>Weak instrument Bias</b>	Genetic variants that are weakly associated with an exposure (e.g. F-statistic <10) will bias estimates towards the observational estimate in one-	Weak instruments for nutritional exposures often result from limited sample sizes of pre-existing GWAS, as well as having	Increase sample sizes (e.g. through publicly available GWAS datasets and consortia). Explain more variation in the exposure using allele scores.

	sample MR, and to the null in Two-sample MR	a small proportion of variance explained by genetic variation.	
<b>Low Power</b>	May be caused by small sample size, low variance explained in the exposure by the SNP, confounding and type 1 error rate.	Inadequate power may result in null results and hinder important further research.	Increase sample size or instrument strength where possible  Power for one-sample MR can be calculated using free web application at <a href="http://cnsgenomics.com/shiny/mRnd/">http://cnsgenomics.com/shiny/mRnd/</a>
<b>Horizontal Pleiotropy</b>	The association between the genetic variant and the outcome of interest goes through an alternative pathway to the exposure.	Violates a core assumption of MR (figure 1c).	Understand underlying biological function of genetic variants.  Use variants directly coding for exposure of interest where possible.  Use MR-Egger estimation.
<b>Linkage Disequilibrium</b>	Non-random allocation of alleles in close	Confounding can be introduced by using an allele	Omit alleles in close genetic proximity to others.

	proximity during meiosis.	close to another allele, which affects the outcome of interest through another pathway.	Utilise genetic alleles on separate chromosomes  Use homogeneous populations where LD structures will be similar
<b>Developmental Compensation (Canalization)</b>	Individual adaptation to a genetic change, which reduces the phenotypic effect of the genetic change-	MR may produce causal estimates that are below the effect achieved by modifying the exposure.	The extent of the impact of canalization on MR is currently unclear.
<b>Population Stratification</b>	Spurious results may result from using mixed populations in which the genetic variant and outcome are associated with a particular genetic background.	Possible limitation of vitamin D in schizophrenia.	Use genetic associations derived from within homogenous populations only.  Use summary results statistics that have adequately controlled for population substructure through e.g. principal components analysis or linear mixed models.
<b>Biological Complexity</b>	MR may give misleading results	Several studies have suggested a	Improved understanding of biological pathways.

	<p>due to overly simplistic interpretation of complex biological pathways.</p>	<p>non-linear association between vitamin D and various outcomes, but standard MR techniques are not able to detect this. Likewise, MR is unable to account for time-limited exposures or sensitive periods, such as intrauterine exposures and psychiatric outcomes.</p>	<p>Use of novel methods to account for non-linear associations.</p>
--	--------------------------------------------------------------------------------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	---------------------------------------------------------------------

## Appendix 1: Search Strategy

Papers included in this review were identified using the following search strategy (adapted from the Cochrane Mental disorders search strategy at <https://cmd-cochrane-org/search-strategies-identification-studies>), executed on 5<sup>th</sup> May 2019. Modified MeSH terms were used for EMBASE/ PsychINFO databases-

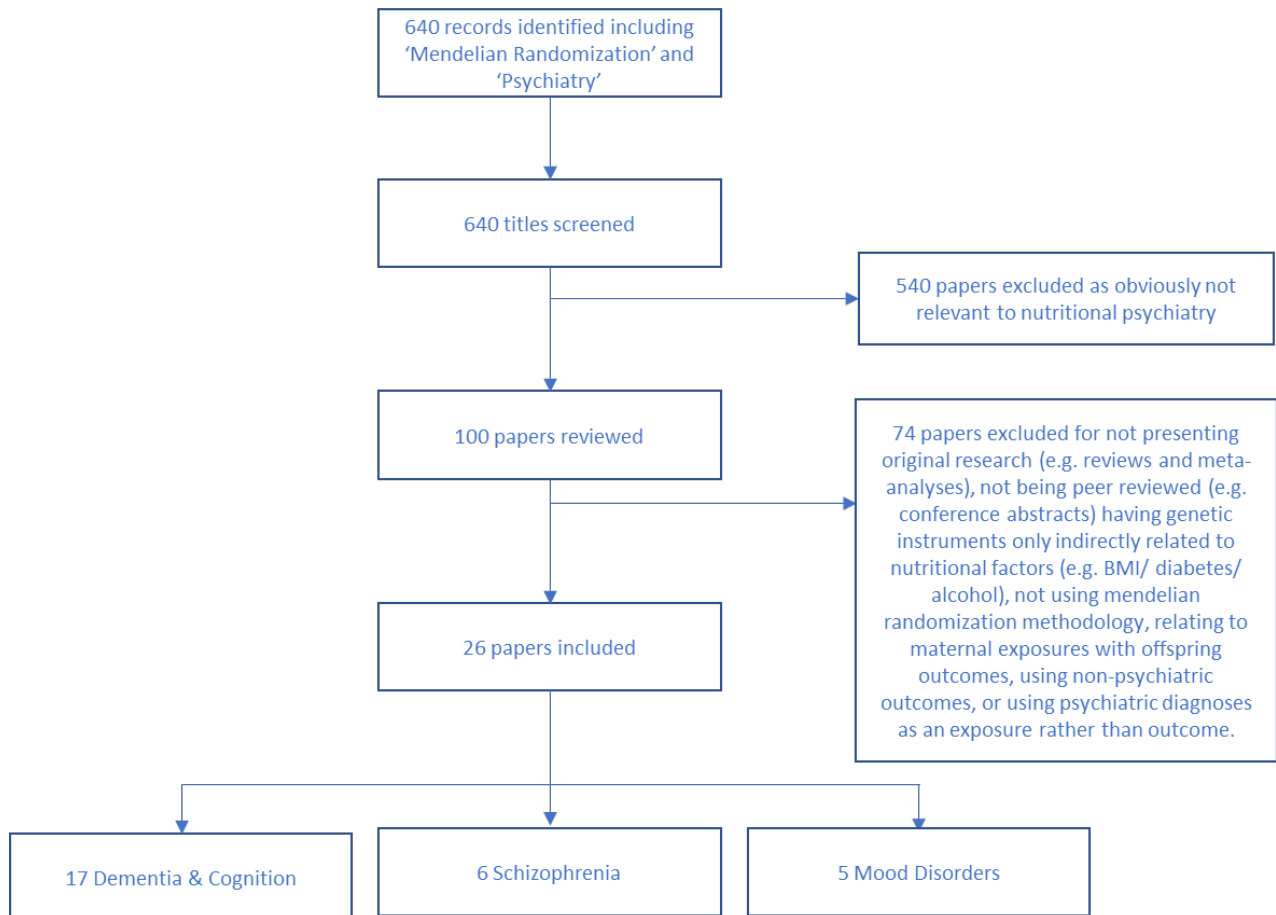
1. EATING DISORDERS/ or ANOREXIA NERVOSA/ or BINGE-EATING DISORDER/ or BULIMIA NERVOSA/ or FEMALE ATHLETE TRIAD SYNDROME/ or PICA/
2. HYPERPHAGIA/ or BULIMIA/
3. SELF-INJURIOUS BEHAVIOR/ or SELF MUTILATION/ or SUICIDE/ or SUICIDAL IDEATION/ or SUICIDE, ATTEMPTED/
4. MOOD DISORDERS/ or AFFECTIVE DISORDERS, PSYCHOTIC/ or BIPOLAR DISORDER/ or CYCLOTHYMIC DISORDER/ or DEPRESSIVE DISORDER/ or DEPRESSION, POSTPARTUM/ or DEPRESSIVE DISORDER, MAJOR/ or DEPRESSIVE DISORDER, TREATMENT-RESISTANT/ or DYSTHYMIC DISORDER/ or SEASONAL AFFECTIVE DISORDER/
5. NEUROTIC DISORDERS/
6. DEPRESSION/
7. ADJUSTMENT DISORDERS/
8. exp ANTIDEPRESSIVE AGENTS/
9. ANXIETY DISORDERS/ or AGORAPHOBIA/ or NEUROCIRCULATORY ASTHENIA/ or OBSESSIVE-COMPULSIVE DISORDER/ or OBSESSIVE HOARDING/ or PANIC DISORDER/ or PHOBIC DISORDERS/ or STRESS DISORDERS, TRAUMATIC/ or COMBAT DISORDERS/ or STRESS DISORDERS, POST-TRAUMATIC/ or STRESS DISORDERS, TRAUMATIC, ACUTE/
10. ANXIETY/ or ANXIETY, CASTRATION/ or KORO/
11. ANXIETY, SEPARATION/
12. PANIC/
13. exp ANTI-ANXIETY AGENTS/
14. SOMATOFORM DISORDERS/ or BODY DYSMORPHIC DISORDERS/ or CONVERSION DISORDER/ or HYPOCHONDRIASIS/ or NEURASTHENIA/
15. HYSTERIA/
16. MUNCHAUSEN SYNDROME BY PROXY/ or MUNCHAUSEN SYNDROME/
17. FATIGUE SYNDROME, CHRONIC/
18. OBSESSIVE BEHAVIOR/
19. COMPULSIVE BEHAVIOR/ or BEHAVIOR, ADDICTIVE/
20. IMPULSE CONTROL DISORDERS/ or FIRESETTING BEHAVIOR/ or GAMBLING/ or TRICHOTILLOMANIA/
21. STRESS, PSYCHOLOGICAL/ or BURNOUT, PROFESSIONAL/
22. SEXUAL DYSFUNCTIONS, PSYCHOLOGICAL/ or VAGINISMUS/
23. ANHEDONIA/
24. AFFECTIVE SYMPTOMS/
25. \*MENTAL DISORDERS/
26. (eating disorder\* or anorexia nervosa or bulimi\* or binge eat\* or (self adj (injur\* or mutilat\*)) or suicide\* or suicidal or parasuicid\* or mood disorder\* or affective disorder\* or bipolar i or bipolar ii or (bipolar and (affective or disorder\*)) or mania or manic or cyclothymic\* or depression or depressive or dysthymi\* or neurotic or neurosis or adjustment disorder\* or antidepress\* or anxiety disorder\* or agoraphobia or obsess\* or compulsi\* or panic or phobi\* or ptsd or posttrauma\* or post trauma\* or combat or

somatoform or somatization or medical\* unexplained or body dysmorphic\* or conversion disorder or hypochondria\* or neurasthenia\* or hysteria or munchausen or chronic fatigue\* or gambling or trichotillomania or vaginismus or anhedonia\* or affective symptoms or mental disorder\* or mental health)-ti-

27. Schizophrenia/ or schizophrenia.mp
28. depression.mp or Depression/
29. major depressive disorder.mp or Depressive Disorder, Major/
30. dementia.mp or Dementia/ or Frontotemporal Dementia/ or Dementia, Vascular/ or Dementia, Multi-Infarct/
31. autism.mp or Autistic Disorder/
32. eating disorder.mp or "Feeding and Eating Disorders"/
33. Borderline Personality Disorder/ or Mental Disorders/ or borderline personality.mp or Personality Disorders/
34. psychosis.mp or Psychotic Disorders/
35. exp "psychiatry and psychology (non mesh)"/ or psychiatry/
36. Attention Deficit Disorder with Hyperactivity/ or ADHD.mp./ or "attention deficit and disruptive behavior disorders"/ or child behavior disorders/
37. neurodevelopmental disorder.mp. or Neurodevelopmental Disorders/
38. communication disorders/ or language disorders/ or dyslexia/ or language development disorders/ or speech disorders/ or learning disorders/ or intellectual disability/
39. Developmental Disabilities/ or Motor Skills Disorders/ or motor delay.mp.
40. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39
41. mendelian adj2 random\*
42. Mendelian Randomization Analysis/ or mendelian randomization.mp
43. instrumental adj2 variable
44. 41 OR 42 OR 43
45. 40 AND 44



**Appendix 2: Flow chart for identification and inclusion of studies** (Numbers for inclusion do not add up to 26 as some studies investigated multiple outcomes)



**Appendix 3: Excluded papers**

The following papers were identified in the search strategy as potentially relevant. Reasons for exclusion from the current review is given where appropriate.

## References

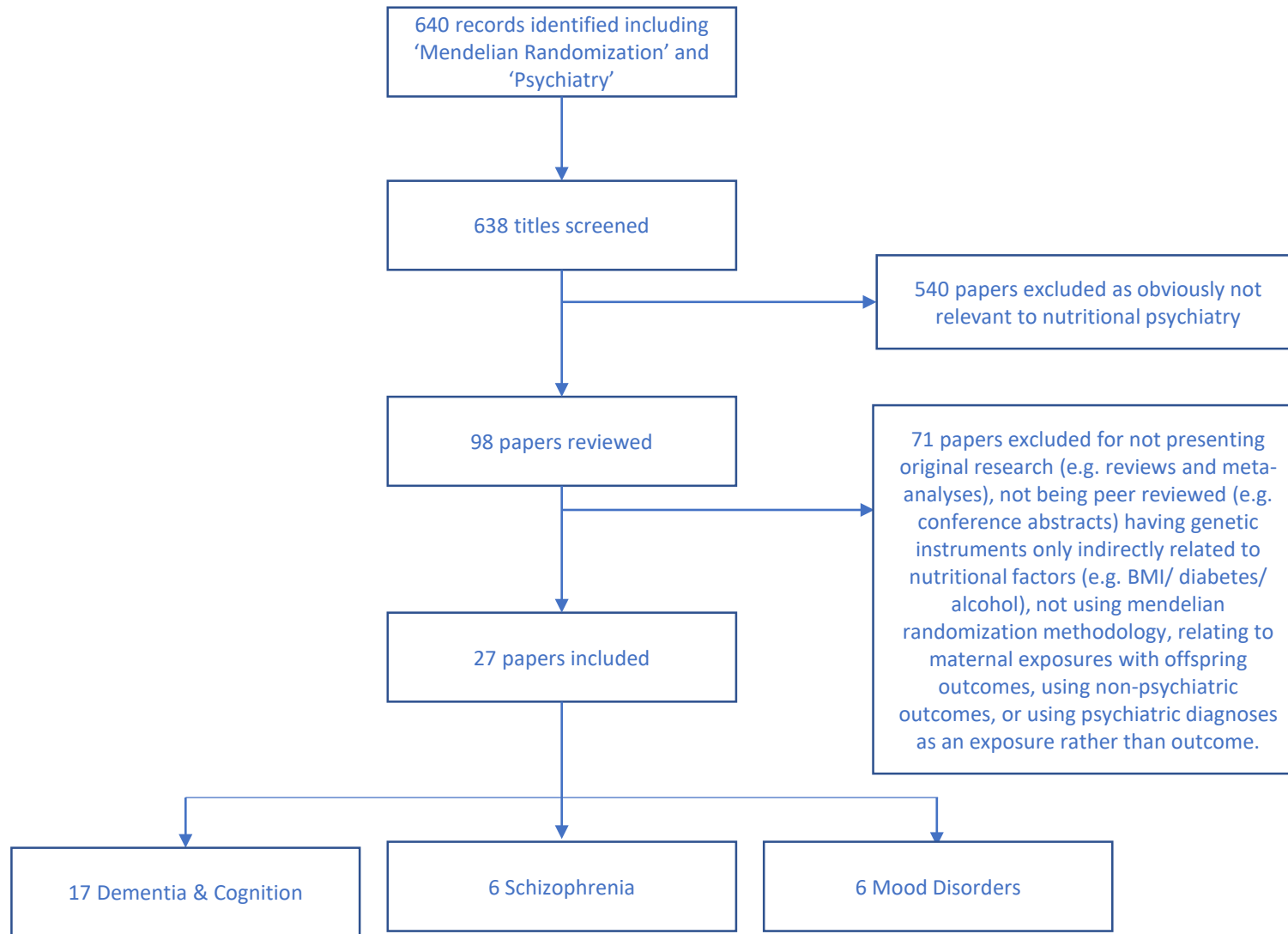
1. Sarris J, Logan AC, Akbaraly TN, Paul Amminger G, Balanza-Martinez V, Freeman MP, et al. International Society for Nutritional Psychiatry Research consensus position statement: nutritional medicine in modern psychiatry. *World Psychiatry*. 2015;14(3):370-1.
2. Tobey JA. The Biology of Human Starvation. *Am J Public Health N*. 1951;41(2):236-7.
3. Monteiro CA, Levy RB, Claro RM, de Castro IRR, Cannon G. Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. *Public Health Nutr*. 2011;14(1):5-13.
4. Sarris J. Nutritional Psychiatry: From Concept to the Clinic. *Drugs*. 2019;79(9):929-34.
5. Lassale C, Batty GD, Baghdadli A, Jacka F, Sanchez-Villegas A, Kivimaki M, et al. Healthy dietary indices and risk of depressive outcomes: a systematic review and meta-analysis of observational studies. *Mol Psychiatry*. 2019;24(7):965-86.
6. Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kostis R, Scarmeas N. Mediterranean Diet, Stroke, Cognitive Impairment, and Depression: A Meta-Analysis. *Ann Neurol*. 2013;74(4):580-91.
7. Ioannidis JPA. The Challenge of Reforming Nutritional Epidemiologic Research. *Jama-J Am Med Assoc*. 2018;320(10):969-70.
8. Dipasquale S, Pariante CM, Dazzan P, Aguglia E, McGuire P, Mondelli V. The dietary pattern of patients with schizophrenia: A systematic review. *J Psychiatr Res*. 2013;47(2):197-207.
9. Shaffer JA, Edmondson D, Wasson LT, Falzon L, Homma K, Ezeokoli N, et al. Vitamin D Supplementation for Depressive Symptoms: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Psychosomatic Medicine*. 2014;76(3):190-6.
10. Bloch MH, Hannestad J. Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis. *Mol Psychiatr*. 17(12):1272-82.
11. Sarris J, Murphy J, Mischoulon D, Papakostas GI, Fava M, Berk M, et al. Adjunctive Nutraceuticals for Depression: A Systematic Review and Meta-Analyses. *Am J Psychiatry*. 2016;173(6):575-87.
12. Rucklidge JJ, Eggleston MJF, Johnstone JM, Darling K, Frampton CM. Vitamin-mineral treatment improves aggression and emotional regulation in children with ADHD: a fully blinded, randomized, placebo-controlled trial. *J Child Psychol Psychiatry*. 2018;59(3):232-46.
13. Rucklidge JJ, Kaplan BJ. Broad-spectrum micronutrient formulas for the treatment of psychiatric symptoms: a systematic review. *Expert Rev Neurother*. 2013;13(1):49-73.
14. Kimball SM, Mirhosseini N, Rucklidge J. Database Analysis of Depression and Anxiety in a Community Sample-Response to a Micronutrient Intervention. *Nutrients*. 2018;10(2).

15. Jacka FN, O'Neil A, Opie R, Itsiopoulos C, Cotton S, Mohebbi M, et al. A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). *Bmc Med.* 2017;15.
16. Roca M, Kohls E, Gili M, Watkins E, Owens M, Hegerl U, et al. Prevention of depression through nutritional strategies in high-risk persons: rationale and design of the MoodFOOD prevention trial. *Bmc Psychiatry.* 2016;16.
17. Firth J, Marx W, Dash S, Carney R, Teasdale SB, Solmi M, et al. The Effects of Dietary Improvement on Symptoms of Depression and Anxiety: A Meta-Analysis of Randomized Controlled Trials. *Psychosomatic Medicine.* 2019;81(3):265-80.
18. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Human Molecular Genetics.* 2014;23:R89-R98.
19. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Smith GD. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr.* 2016;103(4):965-78.
20. Zheng J, Baird D, Borges MC, Bowden J, Hemani G, Haycock P, et al. Recent Developments in Mendelian Randomization Studies. *Curr Epidemiol Rep.* 2017;4(4):330-45.
21. A Lawlor D, Wade K, Borges M, Palmer T, Hartwig F, Hemani G, et al. A Mendelian Randomization dictionary: Useful definitions and descriptions for undertaking, understanding and interpreting Mendelian Randomization studies 2019.
22. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, et al. Effect of Selenium and Vitamin E on Risk of Prostate Cancer and Other Cancers The Selenium and Vitamin E Cancer Prevention Trial ( SELECT). *Jama-J Am Med Assoc.* 2009;301(1):39-51.
23. Yarmolinsky J, Bonilla C, Haycock PC, Langdon RJQ, Lotta LA, Langenberg C, et al. Circulating Selenium and Prostate Cancer Risk: A Mendelian Randomization Analysis. *J Natl Cancer Inst.* 2018;110(9):1035-8.
24. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *Int J Epidemiol.* 2016;45(6):1866-86.
25. Mokry LE, Ross S, Morris JA, Manousaki D, Forgetta V, Richards JB. Genetically decreased vitamin D and risk of Alzheimer disease. *Neurology.* 87(24):2567-74.
26. Larsson SC, Traylor M, Markus HS, Michaelsson K. Serum Parathyroid Hormone, 25-Hydroxyvitamin D, and Risk of Alzheimer's Disease: A Mendelian Randomization Study. *Nutrients.* 2018;10(9).
27. Olsson E, Byberg L, Karlstrom B, Cederholm T, Melhus H, Sjogren P, et al. Vitamin D is not associated with incident dementia or cognitive impairment: An 18-y follow-up study in community-living old men. *American Journal of Clinical Nutrition.* 2017;105(4):936-43.
28. Jorde R, Mathiesen EB, Rogne S, Wilsgaard T, Kjaergaard M, Grimnes G, et al. Vitamin D and cognitive function: The Tromso Study. *Journal of the Neurological Sciences.* 2015;355(1-2):155-61.

29. Maddock J, Zhou A, Cavadino A, Kuzma E, Bao Y, Smart MC, et al. Vitamin D and cognitive function: A Mendelian randomisation study. *Sci Rep.* 2017;7(1):13230.
30. McGrath JJ, Eyles DW, Pedersen CB, Anderson C, Ko P, Burne TH, et al. Neonatal Vitamin D Status and Risk of Schizophrenia: A Population-Based Case-Control Study. *Schizophrenia Research.* 2010;117(2-3):312-.
31. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. *Genet Epidemiol.* 2017;41(4):341-52.
32. Larsson SC, Traylor M, Malik R, Dichgans M, Burgess S, Markus HS, et al. Modifiable pathways in Alzheimer's disease: Mendelian randomisation analysis. *BMJ.* 2017;359:j5375.
33. Hu Q, Teng W, Li J, Hao F, Wang N. Homocysteine and Alzheimer's Disease: Evidence for a Causal Link from Mendelian Randomization. *Journal of Alzheimer's Disease.* 2016;52(2):747-56.
34. Schatzkin A, Abnet CC, Cross AJ, Gunter M, Pfeiffer R, Gail M, et al. Mendelian randomization: how it can--and cannot--help confirm causal relations between nutrition and cancer. *Cancer Prev Res (Phila).* 2009;2(2):104-13.
35. Wu SP, Ma JJ, Qi YW, Zhang JW. Plasma homocysteine levels and risk of vascular dementia: A Mendelian randomization study. *International Journal of Clinical and Experimental Medicine.* 2017;10(6):9142-51.
36. Larsson SC, Markus HS. Branched-chain amino acids and Alzheimer's disease: a Mendelian randomization analysis. *Sci Rep-Uk.* 2017;7.
37. Rasmussen KL, Tybjaerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma apolipoprotein E levels and risk of dementia: A Mendelian randomization study of 106,562 individuals. *Alzheimers Dement.* 2018;14(1):71-80.
38. Benn M, Nordestgaard BG, Frikke-Schmidt R, Tybjaerg-Hansen A. Low LDL cholesterol, PCSK9 and HMGCR genetic variation, and risk of Alzheimer's disease and Parkinson's disease: Mendelian randomisation study. *BMJ.* 2017;357:j1648.
39. Proitsi P, Lupton MK, Velayudhan L, Newhouse S, Fogh I, Tsolaki M, et al. Genetic predisposition to increased blood cholesterol and triglyceride lipid levels and risk of Alzheimer disease: a Mendelian randomization analysis. *PLoS Medicine / Public Library of Science.* 11(9):e1001713.
40. Ostergaard SD, Mukherjee S, Sharp SJ, Proitsi P, Lotta LA, Day F, et al. Associations between Potentially Modifiable Risk Factors and Alzheimer Disease: A Mendelian Randomization Study. *PLoS Medicine / Public Library of Science.* 12(6):e1001841; discussion e.
41. Liu G, Zhao Y, Jin S, Hu Y, Wang T, Tian R, et al. Circulating vitamin E levels and Alzheimer's disease: A Mendelian randomization study. *Neurobiology of Aging.* 2018;72:189.
42. Cheng WW, Zhu Q, Zhang HY. Mineral Nutrition and the Risk of Chronic Diseases: A Mendelian Randomization Study. *Nutrients.* 2019;11(2):12.

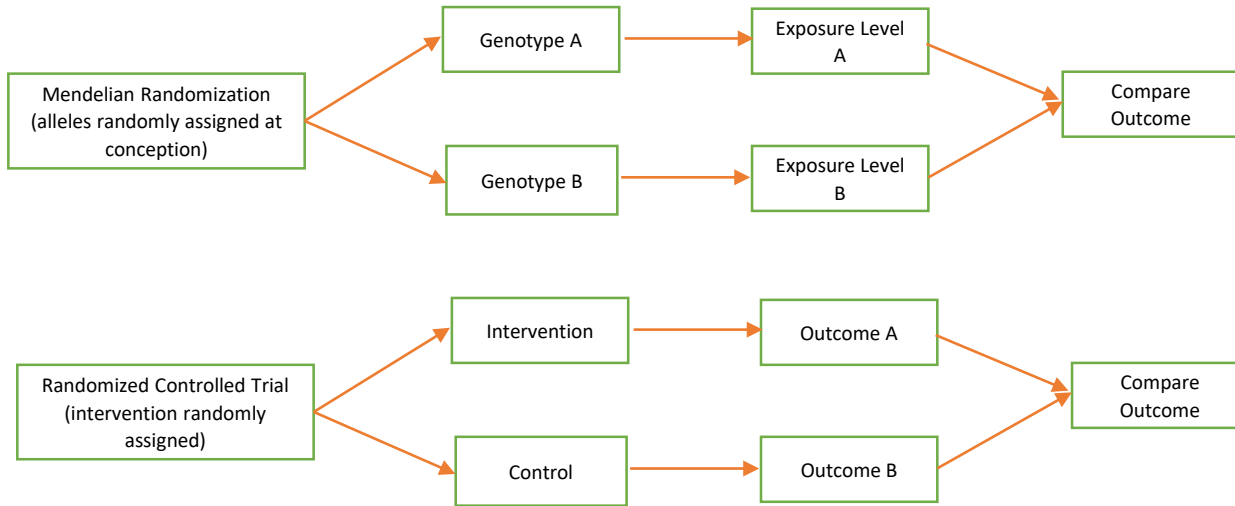
43. Tomioka Y, Numata S, Kinoshita M, Umehara H, Watanabe S, Nakataki M, et al. Decreased serum pyridoxal levels in schizophrenia: meta-analysis and Mendelian randomization analysis. *J Psychiatr Neurosci*. 2018;43(3):194-200.
44. Numata S, Kinoshita M, Tajima A, Nishi A, Imoto I, Ohmori T. Evaluation of an association between plasma total homocysteine and schizophrenia by a Mendelian randomization analysis. *BMC Medical Genetics*. 2015;16:54.
45. Kinoshita M. One-carbon Metabolism and Schizophrenia. [Japanese]. *Seishin shinkeigaku zasshi = Psychiatria et neurologia Japonica*. 2015;117(5):362-8.
46. Li Z, Chen P, Chen J, Xu Y, Wang Q, Li X, et al. Glucose and Insulin-Related Traits, Type 2 Diabetes and Risk of Schizophrenia: A Mendelian Randomization Study. *Ebiomedicine*. 2018;34:182-8.
47. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Leong A, et al. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. *Plos Med*. 2015;12(8).
48. Taylor AE, Burgess S, Ware JJ, Gage SH, Richards JB, Davey Smith G, et al. Investigating causality in the association between 25(OH)D and schizophrenia. *Sci Rep*. 2016;6:26496.
49. Dalen P. Month of Birth and Schizophrenia. *Acta Psychiatr Scand*. 1968;S:55-+.
50. Mollehave LT, Skaaby T, Simonsen KS, Thuesen BH, Mortensen EL, Sandholt CH, et al. Association studies of genetic scores of serum vitamin B12 and folate levels with symptoms of depression and anxiety in two danish population studies. *Eur J Clin Nutr*. 2017;71(9):1054-60.
51. Sallis H, Steer C, Paternoster L, Smith GD, Evans J. Perinatal depression and omega-3 fatty acids: A Mendelian randomisation study. *J Affect Disorders*. 2014;166:124-31.
52. Michaelsson K, Melhus H, Larsson SC. Serum 25-Hydroxyvitamin D Concentrations and Major Depression: A Mendelian Randomization Study. *Nutrients*. 2018;10(12).
53. Wesolowska K, Elovainio M, Hintsala T, Jokela M, Pulkki-Raback L, Pitkanen N, et al. Fasting Glucose and the Risk of Depressive Symptoms: Instrumental-Variable Regression in the Cardiovascular Risk in Young Finns Study. *International Journal of Behavioral Medicine*. 2017;24(6):901-7.
54. Khandaker GM, Zuber V, Rees JMB, Carvalho L, Mason AM, Foley CN, et al. Shared mechanisms between coronary heart disease and depression: findings from a large UK general population-based cohort. *Mol Psychiatr*. 2019;19:19.
55. Meddens SFW, Vlaming Rd, Bowers P, Burik CA, Linnér RK, Lee C, et al. Genomic analysis of diet composition finds novel loci and associations with health and lifestyle. *bioRxiv*. 2018.
56. Guenard F, Bouchard-Mercier A, Rudkowska I, Lemieux S, Couture P, Vohl MC. Genome-Wide Association Study of Dietary Pattern Scores. *Nutrients*. 2017;9(7).
57. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human Genetics Shape the Gut Microbiome. *Cell*. 2014;159(4):789-99.

58. Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vila AV, Vosa U, et al. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nature Genetics*. 2019;51(4):600-+.
59. Bastiaanssen TFS, Cowan CSM, Claesson MJ, Dinan TG, Cryan JF. Making Sense of ... the Microbiome in Psychiatry. *Int J Neuropsychoph*. 2019;22(1):37-52.
60. Kali A. Psychobiotics: An emerging probiotic in psychiatric practice. *Biomed J*. 2016;39(3):223-4.
61. Wallace CJK, Milev R. The effects of probiotics on depressive symptoms in humans: a systematic review (vol 16, 14, 2017). *Annals of General Psychiatry*. 2017;16.
62. Burgess S, Freitag DF, Khan H, Gorman DN, Thompson SG. Using Multivariable Mendelian Randomization to Disentangle the Causal Effects of Lipid Fractions. *Plos One*. 2014;9(10).
63. Millard LA, Davies NM, Timpson NJ, Tilling K, Flach PA, Davey Smith G. MR-PheWAS: hypothesis prioritization among potential causal effects of body mass index on many outcomes, using Mendelian randomization. *Sci Rep-Uk*.5:16645.
64. Relton CL, Davey Smith G. Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. *Int J Epidemiol*. 2012;41(1):161-76.
65. Giovannucci E. Nutritional epidemiology: forest, trees and leaves. *Eur J Epidemiol*. 2019;34(4):319-25.
66. Hemani G, Bowden J, Haycock P, Zheng J, Davis O, Flach P, et al. Automating Mendelian randomization through machine learning to construct a putative causal map of the human phenome. *bioRxiv*. 2017.
67. Roostaei T, Felsky D, Nazeri A, De Jager PL, Schneider JA, Bennett DA, et al. Genetic influence of plasma homocysteine on Alzheimer's disease. *Neurobiology of Aging*.62:243.e7-.e14.
68. Walter S, Marden JR, Kubzansky LD, Mayeda ER, Crane PK, Chang S-C, et al. Diabetic phenotypes and late-life dementia risk: A mechanism-specific Mendelian Randomization study. *Alzheimer Disease and Associated Disorders*. 2016;30(1):15-20.
69. Brion MJA, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol*. 2013;42(5):1497-501.



### 1a Mendelian Randomization as a 'natural' Randomized Controlled Trial

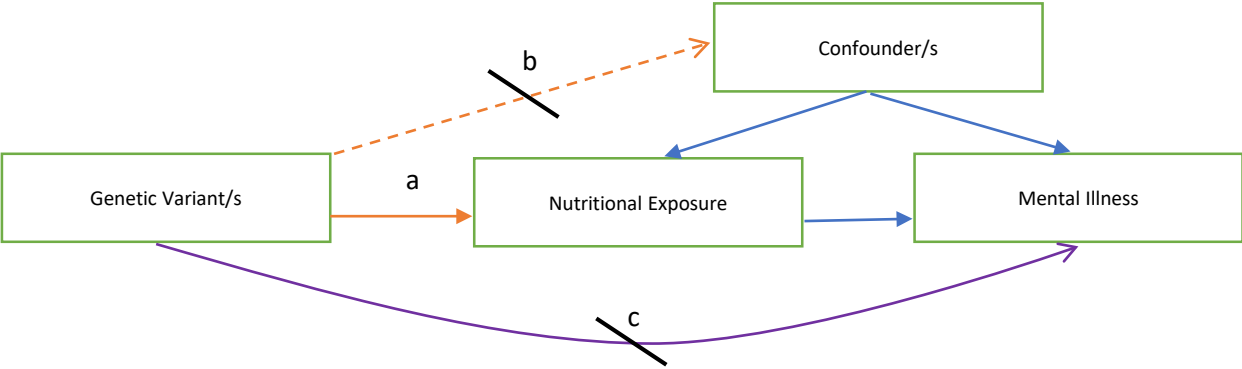
MR has been compared to a randomized controlled trial, with random allocation of genetic alleles at conception could be considered analogous to random allocation of interventions in a trial.





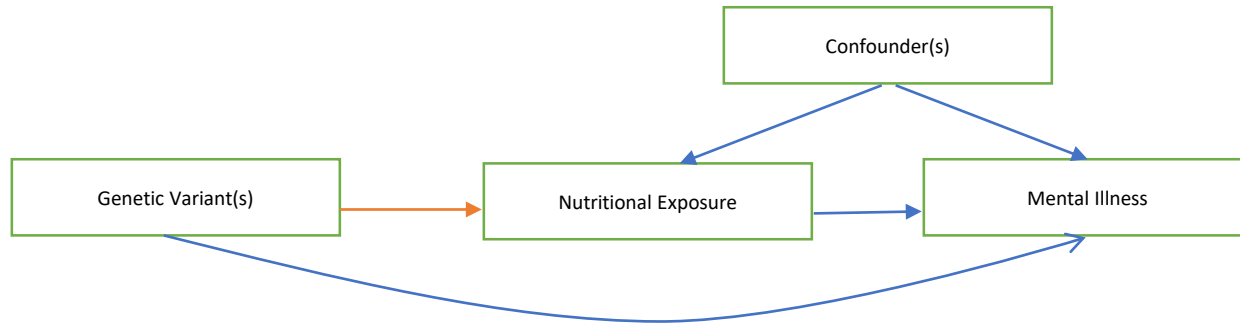
**1b Assumptions in Mendelian Randomization**

MR assumes that the genetic variants are: a. associated with the exposure of interest; b. not associated with confounders; and c. only associated with the outcome through the exposure



### 1c Two-sample Mendelian Randomization

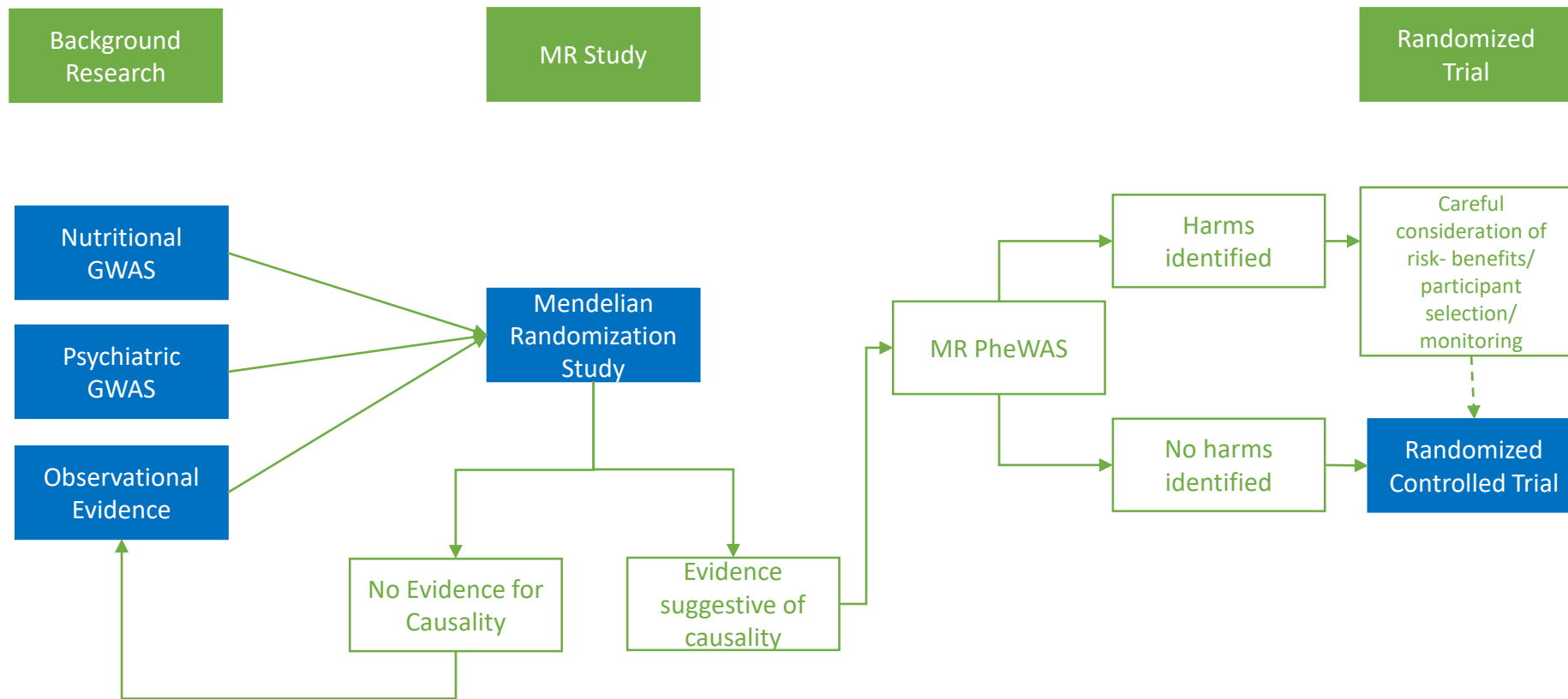
Two-sample MR takes estimates of the SNP-exposure association from one population (e.g. a nutritional exposure GWAS) and the SNP-outcome association from a separate sample (e.g. a psychiatric outcome GWAS).



*First Sample is used to estimate the SNP-exposure association (i.e. genetic contribution to nutritional status)*



*Second Sample is used to estimate the SNP-outcome association (i.e. genetic contribution of the exposure SNP(s) on the outcome)*

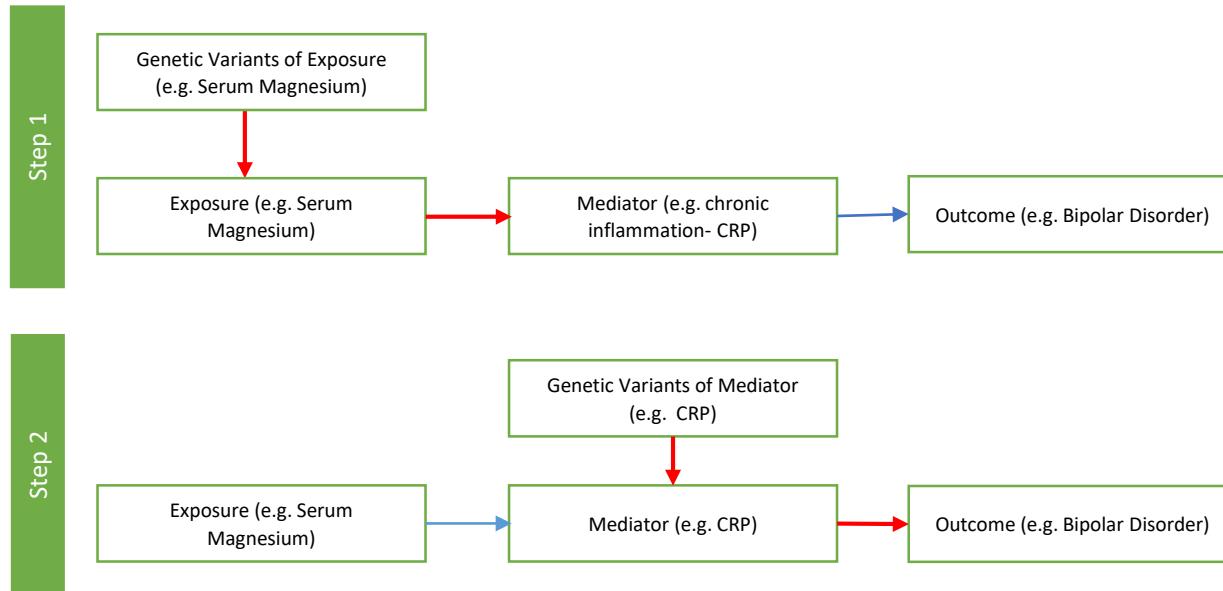


**Figure 2: A theoretical pipeline for the use of MR studies in intervention development**

Whilst many have compared MR to 'nature's RCT', it may be more realistic to see MR studies as an interim step in intervention development.

### 3a Two-step Mendelian Randomization

Two-step MR can be used to identify mediating mechanisms between an exposure and outcome using separate MR analyses which are combined in a traditional mediation analysis.



### 3b Multivariable Mendelian Randomization

Multivariable MR can be used where genetic variants are related to multiple correlated exposures. For example, a SNP for one lipid will often be correlated with others. This technique could be used to untangle potentially opposing associations between omega 3 (EPA/ DHA) and omega 6 fatty acids.

