**Causal relationships between childhood-onset asthma and major mental disorders: a Mendelian randomization study**

**Abstract**

**Background:** Childhood-onset asthma is found to be associated with an increased risk of severe mental illnesses in later life. However, whether this association is causal remains to be determined.

**Methods:** A two-sample Mendelian randomization (MR) analysis was performed to investigate the causal effects of childhood-onset asthma (n=327,670) on six major mental illnesses, including major depressive disorders (n=143,265), bipolar disorder (n=353,899), schizophrenia (n=130,644), anxiety (n=10,240), autism (n=46,350), and attention deficit hyperreactivity disorder (ADHD) (n=225,534) using summary statistics from relevant genome-wide association studies (GWAS). The inverse variance weighted (IVW) method, weighted median, and MR-Egger test were used to obtain causal estimates. Multiple sensitivity analyses were conducted to examine the robustness of the estimates. Moreover, after adjusting for the effects of adult-onset asthma, the direct effects of childhood-onset asthma on mental disorders were determined through a multivariable MR (MVMR) analysis.

**Results:** Genetically predicted childhood-onset asthma was significantly associated with an increased risk of depression (IVW OR=1.059, 95%CI:1.025-1.095, p=5.72x10-04) and bipolar disorder (IVW OR=1,065, 95%CI:1.027-1.105, p=6.75x10-04), but not with the risk of other types of mental disorders. Further MVMR analysis confirmed that the causal relationships remained significant even after adjustment for adult-onset asthma. Furthermore, the causal effects of childhood- and adult-onset asthma were different on depression and bipolar disorder.

**Conclusions:** This MR analysis demonstrates a significant causal relationship between genetically predicted childhood-onset asthma and an increased risk of depression and bipolar disorder in later life. Moreover, the causal effects of childhood- and adult-onset asthma are different. Further studies are warranted to investigate the mechanisms underlying these causal relationships.

**Keywords:** childhood asthma, mental disorders, Mendelian randomization, causal relationships

**1. Introduction**

Asthma is the most prevalent chronic respiratory disorder, which manifests as airflow obstruction, wheezing, coughing, and shortness of breath [1]. More than 14% of children worldwide are diagnosed with asthma, which imposes a substantial economic burden and psychosocial challenges on the society [2]. Accumulating evidence has suggested that childhood-onset asthma is associated with an increased risk of severe mental illnesses in later life. Moreover, meta-analyses of observational studies have further revealed that children with asthma have a higher risk of developing major depressive disorder (MDD) [3, 4], bipolar disorder [4, 5], schizophrenia [5], anxiety [6], autism [7] and attention deficit hyperreactivity disorder (ADHD) [8, 9]. Although these observational studies adjusted for several covariates, some uncontrolled confounding factors and reverse causation bias still make it difficult to confirm causality [10]. Hence, exploring whether the reported association is causal is imperative, which can provide insights into the pathogenesis, management, and treatment of these diseases.

Mendelian randomization (MR) is a promising approach that uses genetic proxies for potential risk factors to investigate their causal effects on target diseases [11]. This approach minimizes the risk of bias arising from unmeasured confounding factors and reverse causation, because it leverages genetic variants that are randomly passed from the parents to the offspring and fixed at conception. Therefore, it is conceptually analogous to a randomized controlled trial (RCT) study, and is increasingly being utilized owing to its cost-effectiveness [12]. A recent MR study has reported that asthma (without considering the age of disease onset) does not play a causal role in the development of mental diseases [13]. However, it should be noted that asthma is a highly heterogeneous disease. Childhood-onset asthma and adult-onset asthma exhibited different etiological trajectories [14], severity [15], and genetic risk factors [16, 17]. Therefore, whether childhood-onset asthma exerts causal effects on the development of mental disorders remains unclear.

In this study, a two-sample MR analysis was performed to investigate whether childhood-onset asthma was causally associated with six major mental illnesses including depression, bipolar disorder, schizophrenia, anxiety, autism, and ADHD. Childhood-onset asthma (a binary phenotype, asthma in children under 12 years old) and age of onset (a contiguous phenotype) were used as the exposure variables. Multiple sensitivity analyses were performed to ensure the robustness of the results. In addition, a multivariable MR (MVMR) analysis was conducted to estimate the independent effect of childhood-onset asthma on mental disorders after adjusting for the effect of adult-onset asthma.

**2. Data and Methods**

***2.1 Study design***

A two-sample MR design was adopted, with childhood-onset asthma as the exposure and mental disorders as the outcomes. To ensure the validity of the causal estimates, three MR assumptions were applied: (i) the genetic instruments were significantly associated with the exposure variables; (ii) the genetic variants were not associated with confounding factors; (iii) the genetic variants affected the outcome only through the exposure [18]. The overview of the study design and the fundamental MR assumptions are presented in **Figure 1**.

***2.2 Summary GWAS data***

Summary association data from the latest GWAS study on childhood-onset and adult-onset asthma were used in the analysis [16]. This study comprised 37,846 asthma patients and 318,237 controls from the UK Biobank database. Asthma that occurred in children under 12 years old was identified as childhood-onset asthma (n=9,433), while asthma occurring in those aged between 26 and 65 years old was defined as adult-onset asthma (n=21,564). Individuals older than 38 years without a diagnosis of asthma were included as controls (n=318,237). In addition, we extracted the top GWAS loci associated with the age-of-onset of patients with asthma (n=37,846).

Similarly, summary statistics were derived from the largest-to-date GWAS for mental disorders performed by the Psychiatric Genomics Consortium (PGC). All data were extracted from participants of European ancestry, including MDD (45,591 cases and 97,674 controls) [19], bipolar disorder (40,463 cases and 313,436 controls) [20], schizophrenia (53,386 cases and 77,258 controls) [21], anxiety (2248 cases and 7992 controls) [22], autism (18381 cases and 27969 controls) [23], and ADHD (38,691 cases and 186,843 controls) [24].

Notably, all GWAS summary statistics used in this study are publicly available; therefore, no ethical approval was required for this investigation. The information on the phenotype, sample size, and PubMed ID of the original study is provided in **Supplementary Table 1**.

***2.3 Selection of genetic instruments***

Single nucleotide polymorphisms (SNPs) associated with childhood-onset asthma at the genome-wide significance threshold of p<5x10-8 but not associated with adult-onset asthma (p>0.05) were elected to estimate the specific causal effects of childhood-onset asthma and minimize potential horizontal pleiotropy bias. Then, independent SNPs were identified by clumping (r2 < 0.001 in a 10000-kb window) the SNP data based on the European reference data from the 1000 Genomes Project [25]. Subsequently, summary data for the associations of selected instruments with mental disorders were extracted from the GWAS studies mentioned previously. The results were harmonized between exposure and outcome data according to the same effect allele. For palindromic SNPs, the reported allele frequency was checked to avoid potential strand flipping. Palindromic SNPs with a minor allele frequency (＞ 0.42) were removed to avoid ambiguity in inferring strand. The strength of the instrument (F-statistic) was calculated as $\frac{(n−k−1)}{k}×\frac{R^{2}}{1−R^{2}}$, where n represents sample size, k represents the number of genetic variants, and R2 is the proportion of phenotypic variance explained by the genetic variants. The instruments with an F-statistic ＞10 were selected, ensuring that weak instruments were removed [26]. The details of genetic instruments used in this study are presented in **Supplementary Table 2.**

***2.4 Univariable MR analysis***

Causal effects of childhood-onset asthma on mental disorders were estimated using the inverse-variance weighted (IVW) method (multiplicative random-effects) to account for potential heterogeneity [26]. To adjust for the risk of horizontal pleiotropy associated with the IVW method, the causal effects were further analyzed using two alternative MR methods, the weighted median estimator and MR-Egger. The weighted median method could provide an unbiased estimate as long as at least 50% of the instruments are valid [27], while the MR-Egger method could provide an unbiased MR estimate, as it assumes that the instruments are independent of potential pleiotropy [28].

***2.5 Sensitivity analysis***
Multiple sensitivity analyses were performed to examine the robustness of the obtained estimates. Heterogeneity among SNPs was evaluated using the Cochran’s Q statistics. Leave-one-out analysis was also conducted to examine whether any specific genetic variant affected the MR estimates. Horizontal pleiotropy was evaluated using the MR-Egger intercept [28] and MR-PRESSO global test [29]. If horizontal pleiotropy was detected, the MR-PRESSO outlier test was performed to identify outliers. Identified outliers were removed, and the causal effects of the remaining SNPs were assessed. The causal effects were considered significant if the following three conditions were met: (i) the IVW P-value was below the Bonferroni-corrected threshold (p<0.05/6=0.008); (ii) the directions of the estimates obtained by the three methods (IVW, weighted median, and MR-Egger) were consistent; (iii) no significant horizontal pleiotropy was detected in the MR-Egger intercept test (p>0.05) and MR-PRESSO global test (p>0.05).

***2.6 Multivariable MR analysis***

Considering that childhood-onset asthma and adult-onset asthma are correlated and shared causative genetic risk factors [16, 17], MVMR analysis was performed to determine the direct effect of childhood-onset asthma on the mental disorders after the adjustment for the effect of adult-onset asthma. In addition, MVMR analysis was conducted for the mental disorders that exhibited significant causal relationships with childhood-onset asthma in the univariable MR analysis. The multivariable IVW method was employed to estimate the direct effects of childhood- and adult-onset asthma on mental disorders.

All the MR analyses were performed using the “TwoSampleMR” package (version 0.5.6)[30] in R (version 4.0.0).

**3. Results**

***3.1 Selection of genetic instruments***

In this MR analysis, two exposures were enrolled: childhood-onset asthma and the age of onset. Following robust instrumental variable selection procedures, the number of valid genetic variants associated with the exposures ranged from 9 to 19, depending on the outcomes assessed. All instruments exhibited F-statistics greater than 10 (ranging from 41.6 to 83.7), indicating a low likelihood of bias due to weak instruments **(Supplementary Table 2)**.

***3.2 Univariable MR******analysis of the causal effect of childhood-onset asthma on mental disorders***

Given the Bonferroni corrected threshold of p<0.05/6=0.008, genetic proxies for childhood asthma were significantly associated with elevated risks of depression (IVW OR=1.059, 95%CI:1.025-1.095, p=5.72x10-04) and bipolar disorder (IVW OR=1,065, 95%CI:1.027-1.105, p=6.75x10-04). The MR estimates remained unchanged even after testing by the other alternative MR methods, including weighted median and MR-Egger regression (**Figure 2, Table 1**). Similarly, we observed that each year decrease in the genetically predicted age of asthma onset was significantly associated with an increased risk of depression (IVW OR=0.993, 95% CI: 0.988–0.998, p=3.27×10⁻³). Although a negative trend was noted between the age of asthma onset and the risk of bipolar disorder, the MR estimate did not reach statistical significance. Notably, childhood asthma had no significant effect on other mental disorders including schizophrenia, anxiety, autism, and ADHD (all IVW p > 0.05) (**Supplementary Table 3)**.

Cochran’s Q statistics revealed no significant heterogeneity in most MR estimates (p > 0.05). Results of both MR-Egger intercept test and MR-PRESSO test suggested that the results were less likely to be affected by pleiotropy (all p > 0.05) **(Table 1)**. Furthermore, the leave-one-out analysis indicated that the MR estimates were not driven by one SNP (**Supplementary Figure 1)**. Collectively, the sensitivity analyses confirmed the robustness of the causal estimates.

***3.3 Distinct causal effects of childhood- and adult-onset asthma on mental disorders by multivariable MR analysis***

Given that childhood- and adult-onset asthma were correlated and shared genetic risk factors [16, 17], MVMR analysis was further performed to assess the direct effect of childhood-onset asthma on mental disorders (i.e., depression and bipolar disorder) after adjustment for the effect of adult-onset asthma **(Figure 3)**. Consistent with the results of univariable MR analysis, childhood-onset asthma remained significantly associated with an increased risk of depression (IVW OR=1.052, 95%CI: 1.020-1.084, p=1.25x10-03) after adjusting for adult-onset asthma. Interestingly, adult-onset asthma exerted a protective effect on depression (IVW OR=0.886, 95%CI: 0.817-0.962, p=3.68x10-03) after the adjustment for childhood-onset asthma. Similarly, childhood-onset asthma was significantly associated with an increased risk of bipolar disorder (IVW OR=1.072, 95%CI: 1.021-1.127, p=5.66x10-03), while adult-onset asthma was negatively associated with the risk of bipolar disorder (IVW OR=0.882, 95%CI: 0.772-1.006, p=0.062). Taken together, the MVMR analysis indicated that childhood- and adult-onset asthma had distinct causal effects on depression and bipolar disorder.

**4. Discussion**

This study investigated the causal relationship between childhood-onset asthma and six major mental disorders using a two-sample MR approach based on summary data from large-scale publicly available GWAS. Childhood-onset asthma had significant causal effects on elevated risks of depression and bipolar disorder. Consistently, earlier age of asthma onset was causally associated with an increased risk of depression. This causal relationship remained significant after adjusting for the effect of adult-onset asthma. Interestingly, adult-onset asthma was correlated with a decreased risk of mental illnesses (depression and bipolar disorder) compared to childhood-onset asthma. To the best of our knowledge, this is the first MR study to explore the causal effects of childhood- and adult-onset asthma on mental disorders. Our findings have several important clinical implications for understanding the disease pathogenesis and subtypes, enhancing the management of diseases, and developing effective therapeutics.

The association between asthma and mental disorders has been documented by several observational studies [3-8], but whether there is causality remains unclear. Previous studies using GWAS data demonstrated that asthma (without considering the age of disease onset) and depression [31] share a genetic basis, but MR analysis did not find a significant causal association [13, 32]. Given that childhood- and adult-onset asthma are increasingly being recognized as two different asthma subtypes, it is critical to delineate their causal effects by the age of disease onset. Our results showed that genetically predicted childhood-onset asthma was significantly associated with an increased risk of depression and bipolar disorder, while adult-onset asthma was correlated with a lower risk of mental illness. Recent studies suggested that disruption of the immune system by allergic diseases like asthma might contribute to the development of psychiatric disorders [33, 34]. Previous MR studies also found that genetically predicted inflammatory markers, e.g. IL-6 and C-reactive protein (CRP), were positively associated with the risk of depression [35] and bipolar disorder [35]. The distinct causal effects of childhood- and adult-onset asthma suggest that the immune profiles of the two subtypes of asthma might be different. This is consistent with the finding reported by a previous study, that is, adult-onset asthma tends to be more prevalent among non-Th2 high patients than childhood-onset asthma[36]. These findings suggest that different biological pathways might be involved in the pathogenesis of the two subtypes. Further studies are needed to clarify the molecular mechanisms by which childhood- and adult-onset asthma affect the development of depression and bipolar disorder.

Understanding the causal relationship between asthma and mental disorders has multiple clinical implications. For instance, this study demonstrates the potential benefits of early screening and/or intervention in children with asthma to reduce the risk of developing subsequent depression and bipolar disorders. Moreover, the causal associations identified in this study support the feasibility of reusing existing asthma drugs to treat depression and bipolar disorder. A meta-analysis of randomized clinical trials showed that nonsteroidal anti-inflammatory drugs (NSAIDs) could relieve depression symptoms [37]. Furthermore, no detrimental effects of NSAIDs on bipolar disorder were found [38]. Further clinical trials are needed to investigate the efficacy of anti-inflammation drugs as potential treatments for mental disorders.

This study has several limitations that should be discussed. First, the MR estimates were restricted to participants of European ancestry, and thus, the findings may not be extrapolated to other ethnic groups. Second, the diagnoses of asthma in the UK Biobank were predominantly self-reported. Hence, the information on the diagnosis and the age of onset may be misreported. However, the GWAS study replicated most of the previously reported asthma liability loci [38], suggesting that the results were robust. Further studies should enroll larger cohorts to clinically verify the phenotypes associated with the causal estimates.

In conclusion, this MR analysis found a significant causal relationship between genetically predicted childhood-onset asthma and increased risks of depression and bipolar disorder in later life. The causal effects of childhood-onset asthma were different from those of adult-onset asthma. Our findings have expanded the understanding of the disease pathogenesis and subtypes, providing novel ideas for developing effective therapeutics for the management of the disease.

**References**

1. Holgate, S.T., et al., *Asthma.* Nat Rev Dis Primers, 2015. **1**(1): p. 15025.

2. Hoch, H.E., P.R. Houin, and P.C. Stillwell, *Asthma in Children: A Brief Review for Primary Care Providers.* Pediatr Ann, 2019. **48**(3): p. e103-e109.

3. Easter, G., L. Sharpe, and C.J. Hunt, *Systematic Review and Meta-Analysis of Anxious and Depressive Symptoms in Caregivers of Children With Asthma.* J Pediatr Psychol, 2015. **40**(7): p. 623-32.

4. Chen, M.H., et al., *Higher risk of developing major depression and bipolar disorder in later life among adolescents with asthma: a nationwide prospective study.* J Psychiatr Res, 2014. **49**: p. 25-30.

5. Wu, Q., et al., *Childhood and Parental Asthma, Future Risk of Bipolar Disorder and Schizophrenia Spectrum Disorders: A Population-Based Cohort Study.* Schizophr Bull, 2019. **45**(2): p. 360-368.

6. Garcia-Sanchez, D., et al., *Asthma and anxiety development in Australian children and adolescents.* Pediatr Allergy Immunol, 2023. **34**(3): p. e13941.

7. Goodwin, R.D., et al., *Severity and persistence of asthma and mental health: a birth cohort study.* Psychol Med, 2013. **43**(6): p. 1313-22.

8. Kaas, T.H., et al., *Association between childhood asthma and attention deficit hyperactivity or autism spectrum disorders: A systematic review with meta-analysis.* Clin Exp Allergy, 2021. **51**(2): p. 228-252.

9. Blackman, J.A. and M.J. Gurka, *Developmental and behavioral comorbidities of asthma in children.* J Dev Behav Pediatr, 2007. **28**(2): p. 92-9.

10. Lawlor, D.A., et al., *Mendelian randomization: using genes as instruments for making causal inferences in epidemiology.* Stat Med, 2008. **27**(8): p. 1133-63.

11. Bowden, J. and M.V. Holmes, *Meta-analysis and Mendelian randomization: A review.* Res Synth Methods, 2019. **10**(4): p. 486-496.

12. Paternoster, L., K. Tilling, and G. Davey Smith, *Genetic epidemiology and Mendelian randomization for informing disease therapeutics: Conceptual and methodological challenges.* PLoS Genet, 2017. **13**(10): p. e1006944.

13. Budu-Aggrey, A., et al., *Investigating the causal relationship between allergic disease and mental health.* Clin Exp Allergy, 2021. **51**(11): p. 1449-1458.

14. Bush, A. and A. Menzies-Gow, *Phenotypic differences between pediatric and adult asthma.* Proc Am Thorac Soc, 2009. **6**(8): p. 712-9.

15. Busse, W., S.P. Banks-Schlegel, and G.L. Larsen, *Childhood- versus adult-onset asthma.* Am J Respir Crit Care Med, 1995. **151**(5): p. 1635-9.

16. Pividori, M., et al., *Shared and distinct genetic risk factors for childhood-onset and adult-onset asthma: genome-wide and transcriptome-wide studies.* Lancet Respir Med, 2019. **7**(6): p. 509-522.

17. Ferreira, M.A.R., et al., *Genetic Architectures of Childhood- and Adult-Onset Asthma Are Partly Distinct.* Am J Hum Genet, 2019. **104**(4): p. 665-684.

18. Sanderson, E., et al., *Mendelian randomization.* Nat Rev Methods Primers, 2022. **2**.

19. Wray, N.R., et al., *Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression.* Nat Genet, 2018. **50**(5): p. 668-681.

20. Mullins, N., et al., *Genome-wide association study of more than 40,000 bipolar disorder cases provides new insights into the underlying biology.* Nat Genet, 2021. **53**(6): p. 817-829.

21. Trubetskoy, V., et al., *Mapping genomic loci implicates genes and synaptic biology in schizophrenia.* Nature, 2022. **604**(7906): p. 502-508.

22. Forstner, A.J., et al., *Genome-wide association study of panic disorder reveals genetic overlap with neuroticism and depression.* Mol Psychiatry, 2021. **26**(8): p. 4179-4190.

23. Grove, J., et al., *Identification of common genetic risk variants for autism spectrum disorder.* Nat Genet, 2019. **51**(3): p. 431-444.

24. Demontis, D., et al., *Genome-wide analyses of ADHD identify 27 risk loci, refine the genetic architecture and implicate several cognitive domains.* Nat Genet, 2023. **55**(2): p. 198-208.

25. Auton, A., et al., *A global reference for human genetic variation.* Nature, 2015. **526**(7571): p. 68-74.

26. Burgess, S., A. Butterworth, and S.G. Thompson, *Mendelian randomization analysis with multiple genetic variants using summarized data.* Genet Epidemiol, 2013. **37**(7): p. 658-65.

27. Bowden, J., et al., *Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator.* Genet Epidemiol, 2016. **40**(4): p. 304-14.

28. Bowden, J., G. Davey Smith, and S. Burgess, *Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression.* Int J Epidemiol, 2015. **44**(2): p. 512-25.

29. Verbanck, M., et al., *Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases.* Nat Genet, 2018. **50**(5): p. 693-698.

30. Hemani, G., et al., *The MR-Base platform supports systematic causal inference across the human phenome.* Elife, 2018. **7**.

31. Tylee, D.S., et al., *An Atlas of Genetic Correlations and Genetically Informed Associations Linking Psychiatric and Immune-Related Phenotypes.* JAMA Psychiatry, 2022. **79**(7): p. 667-676.

32. Zhu, Z., et al., *Shared genetics of asthma and mental health disorders: a large-scale genome-wide cross-trait analysis.* Eur Respir J, 2019. **54**(6).

33. Yuan, N., et al., *Inflammation-related biomarkers in major psychiatric disorders: a cross-disorder assessment of reproducibility and specificity in 43 meta-analyses.* Transl Psychiatry, 2019. **9**(1): p. 233.

34. Dunn, A.J., A.H. Swiergiel, and R. de Beaurepaire, *Cytokines as mediators of depression: what can we learn from animal studies?* Neurosci Biobehav Rev, 2005. **29**(4-5): p. 891-909.

35. Prins, B.P., et al., *Investigating the Causal Relationship of C-Reactive Protein with 32 Complex Somatic and Psychiatric Outcomes: A Large-Scale Cross-Consortium Mendelian Randomization Study.* PLoS Med, 2016. **13**(6): p. e1001976.

36. Martin, P.E., et al., *Childhood eczema and rhinitis predict atopic but not nonatopic adult asthma: a prospective cohort study over 4 decades.* J Allergy Clin Immunol, 2011. **127**(6): p. 1473-9.e1.

37. Köhler, O., et al., *Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials.* JAMA Psychiatry, 2014. **71**(12): p. 1381-91.

38. Köhler-Forsberg, O., et al., *Nonsteroidal anti-inflammatory drugs (NSAIDs) and paracetamol do not affect 6-month mood-stabilizing treatment outcome among 482 patients with bipolar disorder.* Depress Anxiety, 2017. **34**(3): p. 281-290.

**Tables**

**Table 1.** The causal effect of childhood-onset asthma on mental disorders as determined by univariable MR analysis.

**Figure legends**

**Figure 1.** Overview of the study design and three MR assumptions.

MR1: the genetic instruments are significantly associated with the exposure variables; MR2: the genetic variants are not associated with confounding factors; MR3: the genetic variants affect the outcome only through the exposure and there are no alternative pathways. MDD: Major Depressive Disorder; ADHD: attention deficit hyperreactivity disorder.

**Figure 2.** Scatter plot of the selected causal estimates.

A. Scatter plot of the causal estimates between childhood-onset asthma and major depressive disorder; B. Scatter plot of the causal estimates between childhood-onset asthma and bipolar disorder; C. Scatter plot of the causal estimates between age-of-onset of asthma and major depressive disorder; D. Scatter plot of the causal estimates between age-of-onset of asthma and major depressive disorder.

**Figure 3.** Multivariable MR analysis of the causal effects of childhood- and adult-onset asthma on major depressive disorder (MDD) and bipolar disorder.

**Supplementary materials**

Supplementary Figure 1. Leave-one-out analysis.

Supplementary Table 1. Characteristics of the GWAS summary data.

Supplementary Table 2. The strengths of instrumental variables used in this study.

Supplementary Table 3. Causal effects of childhood asthma on mental disorders with three MR approaches.