# Applicability of Mendelian Randomization in the Context of Life-Course Epidemiology

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## INTRODUCTION

One of the main goals of medical research is to determine whether an observed association between an exposure and an outcome reflects a true causal relationship. Major challenges in this context are the possibility of reverse causation or the presence of confounding variables, which can produce spurious associations. To address this, randomized controlled trials (RCTs) have traditionally been considered as the gold standard for establishing causal associations. However, RCTs face ethical, logistical, and generalizability limitations. They typically have limited follow-up periods, making them less suitable for studying long-term effects or life-course epidemiology.

In recent decades, Mendelian Randomization (MR) has emerged as a powerful tool for assessing causality in observational studies. Based on the instrumental variable approach, MR employs genetic variants that are randomly assigned at conception. This genetic randomization mimics the allocation process in RCTs, enabling researchers to make causal inference using observational data [1].

The growing availability of Genome-Wide Association Studies (GWAS) has further expanded the use of MR, enabling analyses without individual-level data and allowing broader investigation of diverse exposures [2]. Nevertheless, the scientific community continues to debate the applicability, strengths, limitations, and interpretation of MR findings [3].

## OBJECTIVES

The aim of our study aligns with the framework of the project "An Integrated Life-Course Approach for Person-Centred Solutions and Care for Ageing with Multi-morbidity in the European Regions – STAGE; Stay Healthy Through Ageing" [4]. In particular, it contributes to one of the project's tasks, which focuses on investigating the causal nature of associations between biological hallmarks and trajectories of ageing with multi-morbidity.

The specific objective of this study is to explore the feasibility of applying the MR approach to a selected research question. This application, beside obtaining specific MR estimates, should serve as a basis for critically reflecting on the strengths and limitations of the method, with particular attention to its relevance within the framework of life-course epidemiology.

## METHODS

We selected epigenetic age acceleration (EAA) as the exposure and attention deficit hyperactivity disorder (ADHD) as the outcome for our case study. EAA represents an example of a biological hallmark that may be identified within the STAGE project, and it has previously been linked to ADHD, specifically when measured using cord blood.

To conduct a two-sample MR analysis [5], we required summary statistics from GWAS for both the exposure and the outcome. Specifically, this involved identifying a GWAS on EAA to obtain the genetic instruments, and a separate GWAS on ADHD to extract the corresponding associations for those instruments.

For the exposure, we used the most recent and comprehensive GWAS on EAA including more than 40,000 individuals [6], which focused on the Horvath epigenetic clock [7]. For the outcome, we selected two of the latest GWAS on ADHD [8,9]. The first one [8] comprises 38,691 individuals with ADHD and 186,843 controls, while the second one [9], a genome-wide association meta-analysis still unpublished, includes 71,733 unique individuals from 28 population-based cohort.

We performed bidirectional MR analyses, i.e. we investigated the possible causal effect of EAA on ADHD as well as the possible causal effect of ADHD on EAA. The inverse-variance weighted (IVW) method was used as main method, but further sensitivity analyses were performed as well.

## RESULTS

Bidirectional MR analyses indicated no evidence of a causal effect of EAA on ADHD (ORIVW = 1.01, 95% CI: 0.98–1.03), nor of ADHD on EAA. This held true when considering both the first ADHD GWAS (βIVW = -0.06, 95% CI: -0.28 to 0.16) and the second, unpublished GWAS focused solely on childhood ADHD (βIVW = 0.23, 95% CI: -0.30 to

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0.77).

## CONCLUSIONS

Our results did not support a causal effect of EAA on ADHD, nor a causal effect of ADHD on EAA. However, conducting these analyses helped clarify several important methodological considerations.

Firstly, the feasibility of two-sample MR analyses is limited by the availability of appropriate GWAS data for both the exposure and the outcome. In our case, only one GWAS on EAA, based on the Horvath clock, was available. Genetic instruments derived from other epigenetic clocks, such as the Pediatric-Buccal-Epigenetic clock [10], may have been more appropriate for our research question, particularly given prior evidence suggesting that EAA in early life could be causally linked to ADHD onset.

Secondly, the time-varying nature of traits like EAA suggests that GWAS based on repeated measures may be more informative in contexts where exposures are not stable over time [11]. The genetic instruments associated with EAA in adulthood may differ from those influencing EAA at younger ages or over the course of life. The available GWAS on EAA was conducted on individuals with a mean age over 50 years, while the ADHD GWASs included both childhood and adult cases [8] and childhood-only cases [9]. This may suggest the possibility of reverse causation, which was not confirmed by our results.

Finally, within the framework of STAGE, where the outcomes of interest may involve aging with multimorbidity, MR may be less suitable. Constructing genetic instruments for composite phenotypes increases the likelihood of pleiotropy, potentially leading to biased estimates. In this context, more sophisticated methods including MR-Phenome Wide Association Studies (PheWAS) [12] may offer more appropriate frameworks for capturing causal relationships with complex outcomes.

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