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Title: Epigenome-wide associations with ADHD in adults: the need for a longitudinal lifecourse approach in epigenetic psychiatry

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Considering that Attention-deficit/hyperactivity disorder (ADHD) is generally viewed a neurodevelopmental disorder, we have little understanding of the developmental aetiology of ADHD and the dynamic nature of the underlying biology across the lifecourse. Illustrative of this is the fact that ADHD is the most common psychiatric disorder in children with prevalence rates around 5% and higher (1, 2), but research of ADHD in adults has been relatively unexplored.

Although twin studies suggest a heritability of 74% (3), it is likely that ADHD symptoms are the result of a complex interplay between genetic and environmental influences with a strong developmental component. It is therefore not surprising to see a slow but steady rise in studies investigating epigenetic correlates of ADHD such as DNA methylation, assumed to represent developmentally dynamic interactions between genetic and environmental factors.

Most studies so far have focussed on candidate genes in cross-sectional, paediatric samples. Such designs however limit our ability to understand whether methylomic correlates of ADHD remain stable over time or are dynamic in nature, where different biological systems are implicated at distinct developmental periods (Fig 1A). In the latter case, only a fraction of all implicated genes or systems would be detectable at any single time. So, to fully understand the developmental processes linked to ADHD symptoms, we need epigenetic studies, in which methylation and ADHD symptoms are measured across development from birth to adulthood.

Although all three previous epigenome-wide studies on ADHD (5–7) have so far concentrated exclusively on children, two of these studies were prospective, predicting ADHD symptoms in children through DNA methylation, measured at birth. What seems to have emerged from this previous research was that DNA methylation patterns, measured at birth in cord blood, were more predictive of ADHD symptoms during childhood compared to more temporally proximal methylation measured at age 7. This suggests a methylomic system at birth, whose detectability in

blood fades away during childhood. So far, however, we could draw no conclusions as to whether these or other biological systems emerge again in adulthood.

In this issue of BP, van Dongen et al. (4) studied blood-based methylomic correlates of ADHD symptoms in adults using data from three different large cohorts with a combined sample size of 4,689. The authors investigated the association between 394,194 methylation probes and self-reported symptoms of ADHD, using data from the E-Risk Study (mean age at blood sampling of 18 years), Netherlands Twin Registry (mean age 37 years), the Dunedin Study (mean age 38 years). The study's hypotheses were that peripheral DNA methylation might provide insight into i) "epigenetic consequences of life conditions that correlate with ADHD symptoms", ii) "epigenetic mechanisms" of ADHD or iii) of those that correlate with "causal mechanisms in the brain". Study-specific results were meta-analysed across cohorts, followed by a range of sensitivity analyses related to e.g. ADHD subscale-specific effects, enrichment of epigenetic or genetic loci predictive of other psychiatric disorders, differentially methylated regions and gene expression.

Considering the sample size, it might be surprising that van Dongen et al. (4) reported mainly null findings. Although some suggestive CpG-specific or regional effects were identified, these showed some degree of heterogeneity across cohorts and did not replicate across datasets. A number of differentially methylated regions partially overlap with those linked to smoking initiation or exposure to maternal prenatal smoking, but less so with genetic or epigenetic markers for depression or autism. None of the methylation markers previously identified to be linked to ADHD symptoms in children replicated in the current study.

The sample size and meta-analytical approach are clear strengths of the current study, which also for the first time concentrated on adults with ADHD symptoms. In light of the study's results and including previous research findings, two patterns emerge. First, if there are *epigenetic consequences of ADHD-linked life conditions*, these might be linked to smoking exposure (although future studies should consider widening their search space beyond the five traits that were

investigated in the current study). The authors are cautious about a causal interpretation of this finding, which could be the result of residual confounding or shared biology. Indeed, the evidence of smoking as a causal risk factor for ADHD is limited. In fact, Mendelian Randomization studies rather suggest the reverse: childhood ADHD appears to be a risk factor for smoking initiation (8). This implies that the overlap between methylation signals for smoking and ADHD might be indicative of pleiotropic or confounding effects – or, indeed, of smoking as a downstream effect of ADHD, rather than a cause for disease.

Second, while we still do not fully understand the epigenetic mechanisms of ADHD, findings from the current study suggest that blood-based methylomic signals, measured at birth and predictive of ADHD in childhood, fade away within the first seven years of life and neither these nor other strong methylomic signals seem to re-emerge again in adulthood.

So, where should we go from here? First, we need to keep the momentum going regarding meta-analytical approaches and replication efforts (Figure 1B). Van Dongen et al. (4) meta-analysed results across three cohorts. Other initiatives such as the Pregnancy and Childhood Epigenetics (PACE) Consortium also pave the way towards large-scale collaborations. In PACE, similar efforts are currently underway to elucidate methylomic associations with mental health traits such as ADHD across development, using data of several thousand participants. However, such research endeavours are not easy. Large-scale meta-analyses rely on extensive collaborations, often take several years to complete and individual contributions are difficult to acknowledge within the current model of authorship listings. To maximize such efforts also calls for changes in research practise; e.g. establishing repositories and routines to publish complete (summary) data and re-evaluating the current standards of researcher contributions (9).

Second, we need to study the longitudinal trajectories of DNA methylation over longer periods of development (Figure 1C). A cross-sectional snapshot of methylation markers at a given time point might be less useful to shed light into the dynamic character of the methylome across the

lifecourse and its relation to ADHD. For example, it is possible that ADHD-linked methylation markers at birth set into motion a cascade of secondary processes that impact long-term methylation trajectories, which are not easily detected at any single time point during development.

Third, we have to remind ourselves of what we are trying to achieve (Figure 1D). Are we searching for predictors of ADHD risk or related health conditions; for causal, mechanistic methylation markers of ADHD; or for methylomic consequences of ADHD-linked life conditions? Each aim calls for slightly different study designs, populations and tissues. For example, while the observation of a methylation signal that fades away after birth might be true with respect to blood-based methylation signals, we do not know whether these also hold true for brain tissue. It is possible that (potentially mechanistic) methylation patterns remain stable in brain tissue across development or that new patterns emerge later in development as a result of earlier cascading processes. All we currently seem to know is that the predictive power or biomarker potential of these early peripheral signals, only detectable at birth, cease to be predictive of later ADHD symptoms after birth.

In conclusion, the findings by van Dongen et al. (4) provide an important contribution to the field of epigenetic psychiatry by highlighting the dynamic and transient nature of the human methylome. Future studies need to build on these findings to elucidate how longitudinal changes in the methylomic system link to psychiatric symptoms across the whole lifespan.

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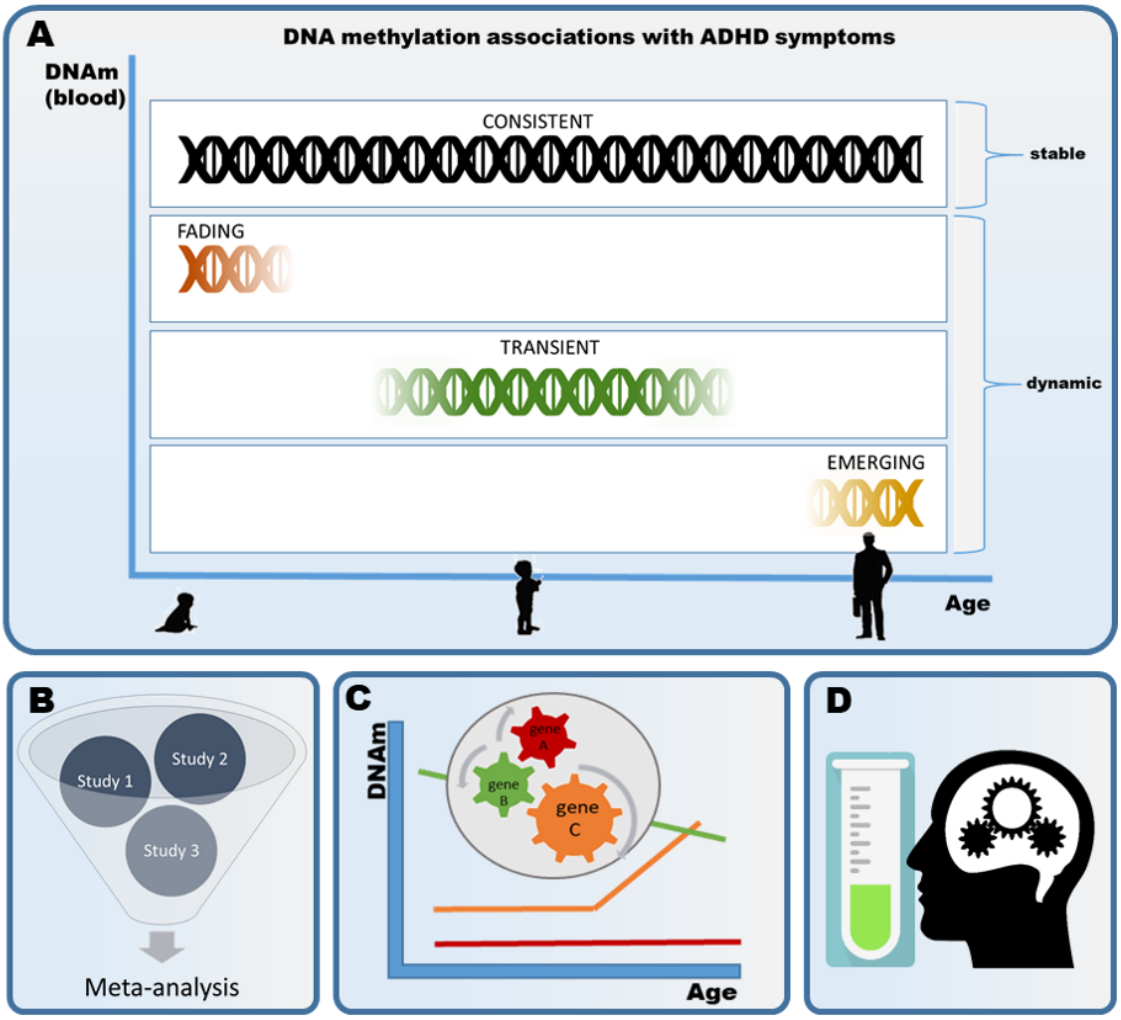

Figure 1. A) *Possible models describing the relationship between DNA methylation and psychiatric traits such as ADHD over the course of development. Methylomic correlates of ADHD could remain stable over time (top panel) or could be dynamic in nature (lower three panels), where different genes or biological systems (in red, green and yellow) are implicated at distinct developmental periods. The current results* (4) *provide little evidence for strong methylation signals in blood that are visible during adulthood.* ***B) – C)*** *To fully understand the relationship between DNA methylation and*

ADHD across the lifecourse, we need to B) continue meta-analytical efforts as demonstrated by van Dongen et al. (4)*, combining datasets across developmental stages; and C) study the changing methylome across the lifecourse to elucidate potential interacting or cascading biological systems;* ***D)*** *In combination, these efforts will help identifying biomarkers of risk or causal mechanisms.*