A systematic review and meta-analysis of epigenetic clocks in schizophrenia

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ABSTRACT

Introduction: Evidence suggests that schizophrenia (SZ) is associated with accelerated biological aging. DNA methylation can be used as an indicator of biological aging by means of epigenetic clock estimates.
Objective: The aim of this systematic review and meta-analysis was to investigate the association between SZ and different epigenetic clocks.
Methods: Search terms were applied in different databases: Embase, MEDLINE (EBSCO), Cochrane Central Register of Controlled Trials, PubMed, PsychINFO and Web of Science. To assess for risk of bias we utilized an adapted version of the Newcastle-Ottawa Scale. Meta-analyses were conducted using the random effects model and meta-regressions were used to assess factors associated with heterogeneity.
Results: Eight studies were included (Controls, $n = 3394$; SZ subjects, $n = 3096$), which analyzed five different epigenetic clocks. Overall meta-analysis revealed no significant differences between SZ and controls on epigenetic aging (Standardized Mean Difference – SMD = -0.21; $p = 0.13$). However, epigenetic clock method was a significant moderator of heterogeneity ($p = 0.004$). Using Horvath's clock as reference, higher SMD's were found for PhenoAge and Intrinsic epigenetic age acceleration (IEAA) clocks. In a stratified meta-analysis restricted to the two clocks mentioned above, a significant accelerating effect was found in patients with SZ when compared to controls (SMD = 0.29; $p = 0.003$).
Conclusion: Our findings suggest that the method of epigenetic clocks is a critical factor associated with estimates of aging acceleration in SZ. However, more studies are needed to confirm these findings and in order to evaluate a possible minor effect in overall analysis.

A hypothesis of accelerated aging in schizophrenia (SZ) proposes that biological changes in the body occur earlier in individuals diagnosed with such condition. DNA methylation (DNAm) patterns have recognized value as biological aging markers. DNAm is an important epigenetic mechanism, which regulates genetic activity without changing the sequential structure of DNA. Thus, unique arrangements of DNAm proved to be important candidates in measuring aging processes, and so-called "epigenetic clocks" have been developed by measuring averages of methylation levels at specific CpG sites (Bell et al., 2021). A growing number of studies have investigated the effects of SZ on epigenetic clocks measured in peripheral and brain samples. However, there is no systematic review that has explored whether SZ is indeed associated with alterations on epigenetic clock estimates.

The search of this systematic review was conducted in the following databases: Embase, Medline, Cochrane, PsychINFO and Web of Science. The descriptors used for the search were: [schizoaffective or schizophr* or psychosis or psychoses] and [epigenetic clock or "epigenetic ag*" or "methylation ag*" or epigenetic acceleration or accelerated epigenetic aging or epigenetic drift]. Only studies involving humans were considered. Preprint studies were considered for this review. The mean and the standard deviation (SD) of epigenetic clocks were recorded of control and SZ groups. If other values were reported instead of mean and SD (median, standard error, or interquartile range), the mean and SD were estimated as follows: (1) median as mean; (2) standard error multiplied by the square root of sample size, as SD; and (3) interquartile range divided by 1.35, as SD. The data was extracted if only presented in

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graphs. A study could be included more than one time if it included different measures from different epigenetic clocks, or different tissue types. To assess the methodological quality and risk of bias in the included studies, an adapted version of the Newcastle-Ottawa Scale (NOS) (Stang, 2010). Meta-analysis was conducted using the random effects model (RE Model) and a multilevel approach to generate forest plots. The estimated effect size of the effects of SZ on epigenetic clock was determined using the standardized mean difference (SMD), calculated by use of Cohen's d. Sources of heterogeneity were explored by means of univariate meta-regression models with the inclusion of potential moderators: sample type (Blood or Brain tissue) and epigenetic clock method (Horvath age acceleration, Hannum age acceleration, PhenoAge age acceleration, Intrinsic epigenetic age acceleration calculated with Horvath's clock - IEAA, Extrinsic epigenetic age acceleration calculated with Hannum's clock - EEAA).

The initial search yielded 317 studies. After duplicates were removed ($n = 79$), we screened 238 studies through the review of title and abstract and 213 were excluded. The remaining studies ($n = 25$) were full text reviewed. A total of 8 studies were included in the review (Dada et al., 2021; Higgins-Chen et al., 2020; Kowalec et al., 2019; McKinney et al., 2017; McKinney et al., 2018; Okazaki et al., 2019; Ori et al., 2019; Voisey et al., 2017), with data from 3394 controls and 3096 SZ subjects. According to the NOS, most studies assessed had a score of 6 (87,5 %, $n = 7$), and were considered as "low risk of bias." Only one study was considered as "high risk of bias."

Influential case analysis detected three outliers, resulting in a total of 24 effect sizes derived from 8 studies. No significant difference was found between cases and controls on epigenetic clock (Fig. 1; SMD = -0.22; Standard error = 0.14; 95 % Confidence Interval: -0.50, 0.07; $p = 0.13$). Significant heterogeneity was detected (Q = 529.82; $p < 0.001$). Meta-regression analysis was performed to investigate potential effects of sample type and epigenetic clock method as moderators of heterogeneity. No significant effect was found for sample type ($p = 0.41$). A significant effect of epigenetic clock method was detected ($p = 0.004$). Using Horvath's clock as reference, higher SMD's were found for PhenoAge and IEAA. Therefore, a clock-specific meta-analysis was conducted using effect sizes obtained by the PhenoAge and IEAA epigenetic clocks (effect sizes = 6). A significant accelerating effect of these two epigenetic clocks was found in patients with SZ when compared to controls (SMD = 0.29; Standard error = 0.10; 95 % Confidence Interval: 0.09, 0.5; $p = 0.003$).

Therefore, although in the overall analysis there was no sufficient evidence to confirm the occurrence of accelerated epigenetic aging in the brain or blood of SZ individuals, a clock specific meta-analysis identified accelerated brain aging in SZ when stratifying the analysis for the PhenoAge and IEAA epigenetic clocks. This could be explained by the fact that these two clocks address mortality as a possible outcome and measure cellular aging processes, respectively. They substantially differentiate from the Horvath and Hannum clocks, which both estimate chronological age. Thus, alterations in epigenetic aging in SZ are clock dependent, and specific clock characteristics may indicate different results. These preliminary findings suggest that future studies are still needed.

CRediT authorship contribution statement

JHC, TWV, CHK designed this study. JHC and BPM performed literature search. JHC, BPM and TWV extracted the data. JHC and TWV analyzed data. JHC, RGO, RO and GRF wrote the first draft of the manuscript. All authors contributed to the final manuscript.

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There was no involvement of the funding source in the study design, data collection, data analysis, interpreting the results, preparation of the article or the decision to publish the article.
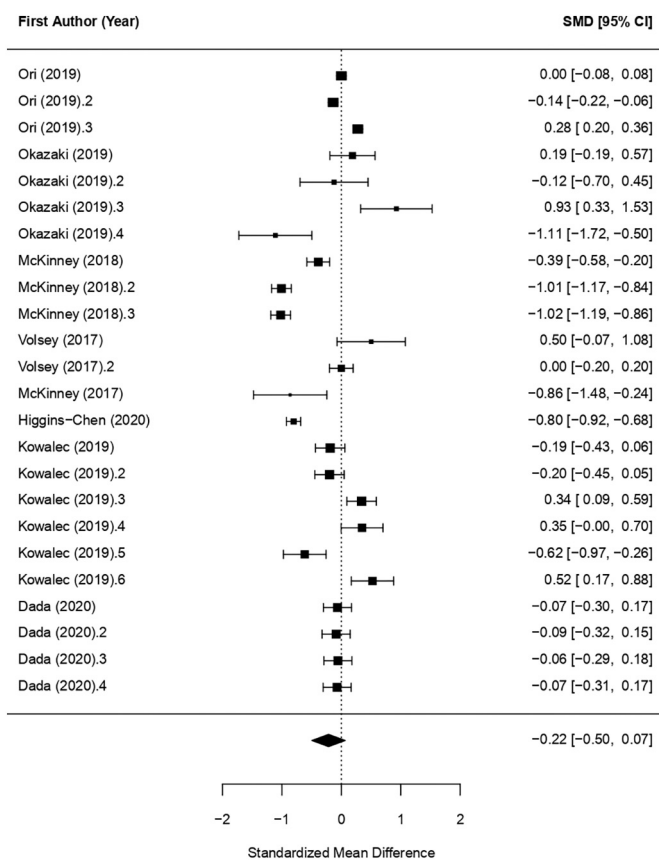

Fig. 1. Epigenetic age acceleration forest plot resulting in a total of 24 effect sizes derived from 8 studies. No significant differences between people with schizophrenia and healthy individuals. Positive effect sizes are associated with age acceleration, while negative effect sizes with the opposite. SMD, standardized mean difference.

Declaration of competing interest

None.

References

Bell, C., Lowe, R., Adams, P., Baccarelli, A., Beck, S., Bell, J., Christensen, B., Gladyshev, V., Heijmans, B., Horvath, S., Ideker, T., Issa, J.P., Kelsey, K., Marioni, R., Reik, W., Relton, C., Schalkwyk, L., Teschendorff, A., Wagner, W., Zhang, K., Rakyan, V., 2021. DNA methylation aging clocks: challenges and recommendations. Genome Biol. 20, 249. https://doi.org/10.1186/s13059-019-1824-y.

Dada, O., Adanty, C., Dai, N., Jeremian, R., Alli, S., Gerretsen, P., Graff, A., Strauss, J., De Luca, V., 2021. Biological aging in schizophrenia and psychosis severity: DNA methylation analysis. Psychiatry Res. 296, 113646 https://doi.org/10.1016/j.psychres.2020.113646.

Higgins-Chen, A.T., Boks, M.P., Vinkers, C.H., Kahn, R.S., Levine, M.E., 2020. Schizophrenia and epigenetic aging biomarkers: increased mortality, reduced cancer risk, and unique clozapine effects. Biol. Psychiatry 88 (3), 224–235. https://doi.org/10.1016/j.biopsych.2020.01.025.

Kowalec, K., Hannon, E., Mansell, G., Burrage, J., Ori, A.P.S., Ophoff, R.A., Mill, J., Sullivan, P.F., 2019. Methylation age acceleration does not predict mortality in schizophrenia. Transl. Psychiatry 9 (1), 157. https://doi.org/10.1038/s41398-019-0489-3.

McKinney, B.C., Lin, H., Ding, Y., Lewis, D.A., Sweet, R.A., 2017. DNA methylation evidence against the accelerated aging hypothesis of schizophrenia. NPJ Schizophr. 3, 13. https://doi.org/10.1038/s41537-017-0017-5.

McKinney, B.C., Lin, H., Ding, Y., Lewis, D.A., Sweet, R.A., 2018. DNA methylation age is not accelerated in brain or blood of subjects with schizophrenia. Schizophr. Res. 196, 39–44. https://doi.org/10.1016/j.schres.2017.09.025.

Okazaki, S., Otsuka, I., Numata, S., Horai, T., Mouri, K., Boku, S., Ohmori, T., Sora, I., Hishimoto, A., 2019. Epigenetic clock analysis of blood samples from Japanese schizophrenia patients. NPJ Schizophr. 5 (1), 4. https://doi.org/10.1038/s41537-019-0072-1.

Ori, A.P.S., Olde Loohuis, L.M., Guintivano, J., Hannon, E., Dempster, E., St. Clair, D., Bass, J., McQuillin, A., Mill, J., Sullivan, P.F., Kahn, R., Horvath, S., Ophoff, R.A., 2019. Schizophrenia is characterized by age- and sex-specific effects on epigenetic aging. bioRxiv. https://doi.org/10.1101/727859.

Stang, A., 2010. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur. J. Epidemiol. 25 (9), 603–605. https://doi.org/10.1007/s10654-010-9491-z.

Voisey, J., Lawford, B.R., Morris, C.P., Wockner, L.F., Noble, E.P., Young, R.M., Mehta, D., 2017. Epigenetic analysis confirms no accelerated brain aging in schizophrenia. NPJ Schizophr. 3 (1), 26. https://doi.org/10.1038/s41537-017-0026-4.