Value of twin and family study designs for epigenetic research

Shuai Li[a,b,c], John L. Hopper[a]

[a]*Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia,*
[b]*Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom,*
[c]*Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, VIC, Australia*

1 Introduction

First proposed by C.H. Waddington in 1942, epigenetics was defined as a field of research aiming to "discover the processes involved in the mechanism by which the genes of the genotype bring about phenotypic effects."[1] Now epigenetics is commonly defined as studying the modifications that change gene expression without changing DNA sequence. Epigenetics, therefore, challenges the concept of "genetic determinism," which has implicitly underpinned many interpretations of the findings of twin and family studies of the genetic and environmental causes of variation of human traits.

Epigenetic modification has been proposed to unify genetic and environmental effects and play a critical role in the etiology of human diseases and traits.[2] Epigenetic modification could be a cause of a disease, a mediator of the effects of exposures on disease risk, act as a biomarker of an exposure or a disease, and/or play a role in the effectiveness of treatment for a disease.[3]

Large-scale population-based epigenetic research is emerging. Many studies have found that DNA methylation, the type of epigenetic modification most often considered, is associated with human traits, diseases and health-related exposures, such as body mass index (BMI),[4] type 2 diabetes[5] and smoking.[6] These studies have provided novel knowledge in understanding human health.

Twin studies have several unique values that have been exploited in epigenetic research.[7–10] As a more general study design, twin family and other family studies can have even greater value, especially in testing if the conclusions and assumptions of studies of twins alone apply more generally. This chapter introduces and summarizes the value of twin and family study designs for epigenetic research.

2 Quantifying genetic and environmental influences on variation in epigenetic modification, heritability and the equal environment assumption

The classic twin model has been used to estimate the extents to which (unmeasured) genetic and environmental factors influence the variation in a vast number of human traits, following the seminal work of R.A. Fisher (himself a twin) a century ago who introduced the concepts of genetic and environmental components of variation.[11, 12] This has come to be called heritability analysis, even though Fisher himself abhorred the concept of heritability. A thorough summary of the theory and caveats of this design and relevant analyses can be found in a review paper by Hopper.[12]

Monozygotic (MZ) twin pairs are genetically identical, and dizygotic (DZ) twin pairs share, on average, 50% of their genetic variation. Under the null hypothesis that genetic factors do not influence variation in a trait, MZ twin pairs will have the same resemblance in that trait, i.e., phenotypic covariance, as DZ twin pairs. Any excess in the resemblance for MZ twin pairs compared with DZ twin pairs will reject the null hypothesis in favor of the hypothesis that genetic factors influence variation in the trait of interest. Under this hypothesis, the resemblance in MZ twin pairs is an indication of *maximum* genetic variance—a useful parameter in its own right.

Note, however, that rejection of the null hypothesis is not enough to prove that genetic factors cause that variation, let alone are the only familial cause of variation. And on the other hand, failure to detect any statistically significant difference in resemblance by zygosity still gives an indication of the upper limit of genetic variation, based on the power of the study to detect a given difference.

The classic twin model has been applied to epigenetic research by many studies, most of which aim to estimate the heritability of DNA methylation.[13–25]

The term "heritability of DNA methylation" can have several distinct meanings and this can confuse researchers from different backgrounds. At the cellular level, heritability refers to DNA methylation patterns that are maintained during cellular differentiation, mitosis and meiosis. This is often called heritable DNA methylation. At the family level, heritability refers to DNA methylation patterns that are passed to younger generations who inherit DNA methylation patterns from their parents or even older generations. This is often called transgenerational inheritance. At the population level, heritability refers to the influence of genetic factors on variation in DNA methylation across the population, defined as the ratio of genetic variance to the total variance in DNA methylation. The classic twin studies mentioned above use the expression "heritability of DNA methylation" in the latter sense, rather than heritable DNA methylation *per se*.

The classic twin model makes a very important and controversial assumption—that the variability caused by non-genetic factors shared by twins is exactly the same for MZ pairs as it is for DZ twin pairs. This is called as the Equal Environment Assumption (EEA) and if true, means that *all* of any excess in the resemblance for MZ twin pairs, compared with DZ twin pairs, is attributed to genetic factors. The estimates of genetic variance (heritability) that arise from applying the classic twin model are therefore *upper* estimates of the role of genetic factors, and need to be always interpreted with this in mind.

Whether the EEA holds for any, let alone all, epigenetic modifications is questionable. From the time of conception, there could be zygosity-dependent prenatal environmental effects because MZ

twin pairs originate from the same zygote while DZ twin pairs originate from two epigenomically different zygotes. DZ twin pairs, therefore, are potentially more epigenetically different from MZ twin pairs from the very starting point.[2] Some studies of variation in DNA methylation levels have found evidence that, at many sites, MZ twin pairs are extremely similar while DZ twin pairs are not similar at all.[26, 27] This observation is highly inconsistent with what would be expected under the EEA if the difference is to be accounted for by genetic factors only. It is, however, consistent with the existence of non-genetic factors specific to MZ twin pairs that make them more similar than DZ twin pairs, contrary to the EAA.

There is also a suggestion that the EEA might not hold due to postnatal environmental effects. A twin and family study found that the familial aggregation in epigenetic aging increased with time spent living together, but at different rates for different types of relatives. This increase in correlation was most rapid for MZ pairs, less rapid for DZ and sibling pairs, and least rapid for parent-offspring pairs. The pattern whereby the rate of increase in correlation differs between the latter two groups of first-degree relatives might be better explained by different types of relatives sharing the cohabitation environmental effects to different degrees depending on their familial relationships, than by genetic factors.[28]

In summary, there are both theoretical and empirical evidence suggesting that the EEA might not hold at least for some epigenetic measures, and the classic twin model based on the EEA could overestimate the influences of genetic factors on the variation in those and other epigenetic measures.

A limitation of previous twin studies for investigating the causes of DNA methylation variation is that most of these studies focus on limited age ranges only, so they cannot provide an overview of the causes of DNA methylation variation over the lifespan. By pooling data from multiple twin and family studies for which the age range of participants covers the whole lifespan, studies have found evidence that environmental factors shared by relatives when they are cohabitating play an important role in causing the variation in global DNA methylation and epigenetic aging across the lifespan.[28, 29] These insights would have been unlikely to be made from studying twins of limited age ranges only.

More importantly, studying twins of limited age ranges could lead to different interpretations of the causes of variation in epigenetic measures. For example, the lifespan approach suggests that variation in epigenetic aging is likely to be determined by cohabitation-dependent environmental effects,[28] but studies focusing on limited age ranges would interpret the same data as evidence for genetic effects with heritability estimates that depend on the age ranges.[30–38]

The classic twin model can be extended. A first extension is to study families of twins. This enables simultaneous estimation of additive and dominant genetic effects and shared environmental effects and also allows investigation of the role of twin-specific environmental factors. For example, a twin family study found that both MZ and DZ twin pairs were similarly and highly correlated in global DNA methylation at birth (the correlation coefficients were both approximately 0.8) while the other types of relative pairs (sibling pairs, including a sibling paired with an individual twin, and parent-offspring pairs) were not correlated. These correlations suggest that sharing the same environment in the uterus at the same time has a very large influence on global DNA methylation variation.[29] This finding could not have been made without including the extended family members, because the intrauterine and postnatal environmental effects cannot be distinguished by studying twins alone.

A second extension is to include replicate samples from the epigenetic experiment. This allows investigation of the extent to which measurement error explains the observed epigenetic variation. In the classic twin design, measurement error is encapsulated within the individual-specific environmen-

tal variance component and cannot be assessed independently without replicate samples. With respect to DNA methylation, there is evidence that the Infinium HumanMethylation450 BeadChip array has low-to-moderate reliability in site-specific DNA methylation measurement,[39] and a twin and family study has provided evidence that this measurement error explains a substantial proportion of methylation variation.[25]

A third extension is to include single nucleotide polymorphism (SNP) data to simultaneously estimate the heritability based on identity-by-descent and the heritability based on measured SNPs. The study of van Dongen et al. is an example applied to DNA methylation.[24]

3 Controlling for the effects of genetic and non-genetic factors shared within the family

Another value of twin and family study designs for epigenetic research is to control for the effects of genetic and non-genetic factors shared between relatives when investigating the relationships between epigenetic modifications and traits of interest, i.e., the within-family design.

Case-control designs that include only unrelated individuals compare cases with controls who are dissimilar in many (and mostly unmeasurable) genetic and environmental factors, and these might confound the observed associations between epigenetic modifications and traits of interest. Furthermore, studies have found sites differentially methylated across races or ethnicities,[40–43] suggesting that population stratification might also be an issue which needs to be considered in epigenetic research.

Relatives from the same family share to varying extents the same genetic background, with the proportion of genetic factors shared depending on their biological relationship, as well as the same environmental background, depending on biological and social factors. While some of these genetic and environmental factors might be known, and even measured, they are for the most part unknown and even unknowable.

Therefore, a comparison between relatives can control for the potentially confounding effects of at least some familial factors, genetic and environmental, manifested in population stratification. Under the within-family design, an association between the discordance (or difference) in the exposure and the discordance (or difference) in the outcome between relatives from the same family is a stronger level of evidence for a causal relationship between the exposure and the outcome because it has controlled for—by design—a much greater amount of (known and unknown) familial confounding than could ever be achieved by measuring, and adjusting for, known familial risk factors.

A classic within-family design is the discordant MZ twin pair design, which uses the co-twin as a control and utilizes the advantages that MZ twins are perfectly matched with one another for their age, sex, genetic background and various environmental factors. There is evidence that the discordant MZ twin pair design has more statistical power (per subject) than the ordinary case-control study in detecting epigenetic associations.[44] This is because the within-pair MZ twin design naturally controls for major sources of variation (noise), and at the same time protects against some potential biases.

There are two major forms of the discordant MZ twin pair design. The twins can be discordant for the outcome, resembling an ordinary case-control study, or they can be discordant for the exposure, resembling an intervention or cohort study. In terms of epigenetic research, the discordant MZ twin

pair design has been applied to investigate the associations of DNA methylation with several human traits such as psychiatric symptoms,[45–47] birth weight,[18, 48, 49] breast cancer[50] and type 1 diabetes.[51, 52]

The within-family design can control for the effects of the factors shared within the family by design; however, a within-family association itself does not support the existence of family-shared factors. One way to detect the existence of family-shared factors is to fit within- and between-family models and compare the within- and between-family results.[53–55] The two results are expected to be different if family-shared factors exist. This approach has been applied to epigenetic research for the association between BMI and DNA methylation of CpG cg2763521 in *SOCS3* gene—while there was a between-family association (change in percentage methylation per unit change in BMI $= -7.4$, 95% confidence interval [CI]: -12.0, -2.8), there was no evidence for a within-family association (-0.8, 95% CI: -5.1, 3.5) and the two associations were different ($P = 0.03$); this observation is consistent with there being family-shared factors contributing to the association between BMI and CpG cg2763521.[56]

Note that the family-shared factors do not necessarily include only familial confounders; familial mediators, i.e., mediators between the exposure and outcome that are shared between relatives, might also be included. Familial mediators are likely to be environmental factors only, as genetic factors are unlikely to be caused by the exposure. A simple comparison between the within- and between-family results is not able to detect what type the family-shared factors are. For example, the between-family association between BMI and CpG cg2763521 above might be due to both of them being affected by the same factors that are shared within the family, i.e., familial confounding, or to factors shared within the family that fully mediate the association between them, or to a mixture of the two scenarios. Under all the scenarios, the within-family association is expected to be null and different from the between-family association. Therefore, these scenarios are not able to be distinguished from one another using a within-family design. All that can be said is that there are family-shared factors underlying the association between BMI and CpG cg2763521. An understanding of the causal pathways requires sophisticated approaches, such as causal mediation analysis, in order to try to distinguish whether the family-shared factors are confounders and/or mediators.

4 Facilitating causation assessment

While the within-family design controls to some extent for the effects of familial confounding, and a within-family epigenetic association is therefore a stronger level of evidence for causation, it is still only an association that could be a consequence of other uncontrolled confounding, or even reverse causation. Causation cannot be "proved" by the within-family design, let alone the direction of causation, although information on the later could come from using a cohort design in which there a temporal relationship between the exposure and outcome. There are several twin and family designs, however, that can be used to better assess causation.

(i) Randomized Controlled Trial

A gold standard for addressing causation is the Randomized Controlled Trial (RCT). A special and powerful version is a RCT involving MZ twin pairs, in which twins within the same pair are randomly assigned to different interventions (or both at different times if a cross-over design is used). As for the discordant MZ twin pair design, this RCT design takes advantage of teh fact that the twin re-

ceiving one intervention and their co-twin receiving the other intervention are perfectly matched for age, sex and genetic background. This both reduces noise and guards against various sources of potential (unmeasured) confounding.

This MZ twin pair RCT design is therefore expected to be more powerful per subject, and more accurate, than the conventional RCT design that involves unrelated individuals. An example of this design is a RCT including 42 female twin pairs (22 MZ, 20 DZ) aged 10–17 years which found that calcium supplementation in adolescence has little effect on female bone density.[57] To the best of our knowledge, there has not yet been a RCT involving twins applied to epigenetic research.

(ii) Mendelian randomization

Observational data can also be used to assess evidence for causation. Mendelian randomization (MR) uses genetic variant(s) associated with the exposure as a presumed instrumental variable to make inference about causation.[58] The presumed instrumental variable by definition needs to satisfy perfectly the three assumptions in the next paragraph. As genetic variants are randomly assigned during conception, MR mimics a RCT with each genotype resembling one arm of the RCT.

MR makes three absolutely critical assumptions, and for the latter two their validity can be very hard to thoroughly test:

(1) Relevance: the genetic variant(s) is(are) strongly associated with the exposure (and the strength is not only in terms of statistical significance);
(2) Independence: the genetic variant(s) is(are) not associated with any confounder between the exposure and the outcome; and
(3) Exclusion: the only way the genetic variant(s) is(are) associated with the outcome is through pathways involving the exposure.

If these three assumptions are perfectly true, then any real association between the genetic variant(s) and the outcome can only occur if there is causation between the exposure and the outcome.

In application, to meet the relevance assumption researchers choose genetic variant(s) they consider are reliably associated with the exposure, typically genetic variant(s) found by genome-wide association studies to be associated with the exposure at a genome-wide level of significance. With respect to the exclusion assumption, there are methods to try to minimize the bias due to this assumption being violated.[59–63] The independence assumption is hard to test, and rarely tested. Some new designs can be used to detect and adjust for the violation of this assumption; see the within-family MR design below.

MR results, therefore, can be highly biased and contentious without good assessment on the validity of the three assumptions, as is well-recognized by those who have championed its use in epidemiology.[64]

MR has been applied to epigenetic research and provided evidence that BMI and breast cancer have a causal effect on their associated DNA methylation changes found from genome-wide DNA methylation association analyses.[4, 65]

MR analysis does not necessarily need twin and family data, but twin and family data have a unique value for MR analysis. Environmental and social factors, such as assortative mating, dynastic effects and population structure, can affect both outcomes and the distribution of the presumed "instrumental" genetic variant(s) for the exposure. That is, there could be confounders that are at least in

part familial. As a result, the independence assumption is violated, and the conclusions of the MR analysis could be wrong.

The issue of familial confounders can be at least in part addressed by incorporating the within-family design into MR analyses and estimating the association between the presumed instrumental genetic variant(s) and the outcome. The Within-family Consortium[66] has used this approach and found that, compared with the causal effect estimates using unrelated individuals, within-family MR gave slightly-to-moderately reduced but still evident causal effect estimates of BMI on type 2 diabetes risk and blood pressure. This suggests that the associations between BMI and these conditions are not greatly confounded by familial factors.

On the other hand, when they considered height and BMI as causes of educational attainment, the supposedly causal associations seen when studying unrelated individuals were greatly reduced when the within-family approach was used, and there was no longer evidence for causal effects. This implies that there is uncontrolled assortative mating, dynastic effects and/or population structure effects that bias the MR results for this socio-economic trait.[66]

Such biases might also apply to using MR of unrelated individuals to assess causation between epigenetic modifications and traits of interest, especially for socio-economic traits. To date, no within-family MR has yet been applied to epigenetic research, partly due to this approach requiring genetic and epigenetic data to be generated for families on a sufficiently large scale.

(iii) Inference about causation from examination of familial confounding

Inference about Causation from Examination of FAmiliaL CONfounding (ICE FALCON) is a family-based causation assessment method using observational paired data.[67] Given data from relatives, such as pairs of twins, siblings, etc., ICE FALCON fits three regression models:

(1) Model 1, $E\left(Y_{self}\right) = \alpha + \beta_{self} \cdot X_{self}$
(2) Model 2, $E\left(Y_{self}\right) = \alpha + \beta_{relative} \cdot X_{relative}$
(3) Model 3, $E\left(Y_{self}\right) = \alpha + \beta'_{self} \cdot X_{self} + \beta'_{relative} \cdot X_{relative}$

where X_{self} and $X_{relative}$ are the exposures of a person and their relative, and Y_{self} and $Y_{relative}$ are the outcomes of that person and their relative.

Under different causal scenarios (X causes Y; Y causes X; X and Y are associated due to familial confounding; or mixtures of these three beforementioned causal scenarios), different patterns of *changes* in regression coefficients are expected from comparing the conditional estimates with the marginal estimates (β'_{self} vs β_{self}, $\beta'_{relative}$ vs $\beta_{relative}$).

By assessing the consistency between the observed and expected *changes* in regression coefficients, the causal scenario(s) with which the associations (1), (2) and (3) between X and Y are most consistent can be inferred. ICE FALCON has been empirically applied to try to understand the causes of several traits including mammographic density,[68, 69] allergic conditions,[70] psychological behaviors,[71] bone architecture,[72, 73] longitudinal BMI measures[67] and epigenetic modifications.[67, 74, 75]

(iv) Direction of causation (DoC) model and MR-DoC model

Another family-based causation assessment method using observational data is the Direction of Causation (DoC) model.[76] The DoC model assumes that the covariances of two correlated traits measured for pairs of relatives are influenced by latent genetic factors and environmental factors, and that there is causation between the two traits. Using structural equation modeling of the *marginal* associa-

tions only (i.e., not considering conditional associations, let alone change in association arising from conditioning, as in ICE FALCON), the DoC model assesses the goodness of fit of presumed models to identify the single most parsimonious model consistent with the data, and estimates relevant parameters. However, in order to achieve model identification, the DoC model needs to restrict the within-individual and cross-pair cross-trait genetic and environmental correlations to be zero, i.e., it assumes there is no familial confounding. This is a major shortcoming; the results of fitting the DoC model need to be interpreted as being conditional on there actually being no familial confounding. It cannot be used to assess if causation provides a better fit than familial confounding.

The multivariable classic twin model, instead assumes there is no causation, but only familial confounding, between two correlated traits. Therefore, both the DoC and multivariable classic twin models fit marginal associations, and neither can differentiate between causation and familial confounding.

The DoC model has recently been incorporated into MR by including genetic variant(s) associated with the exposure in the pathway analysis, and is called MR-DoC.[77] But being based on the DoC model, the MR-DoC model shares the same shortcomings of the DoC model. The MR-Doc model essentially adopts the MR idea of using genetic variant(s) as a presumed instrumental variable to estimate a causal effect by making the same assumptions of MR. Under the assumption of the DoC model that there is no familial confounding, the MR-DoC model can test the pathways between the genetic variant(s) and the outcome that do not go through the exposure, i.e., pleiotropic effects, and therefore provides an estimate of the causal effect even if pleiotropic effects exist—but this is only valid if there is no familial causation. The MR-DoC model has not yet been applied to epigenetic research; there is currently only one published empirical application of this method, which is to height and educational attainment.[78]

(v) Comparison between MR, ICE FALCON, DoC and MR-DoC models

ICE FALCON shares similarities with MR, as it essentially uses an unmeasured instrumental variable to assess the evidence for causation between X_{self} and Y_{self}. The familial causes of the trait X are the presumed instrumental variable; these causes are not measured but proxied by $X_{relative}$.

ICE FALCON differs from MR in four aspects: (1) it does not need genetic data, nor *a priori* knowledge on a genetic association, for trait X, (2) the instrumental variable that causes both X_{self} and $X_{relative}$ captures all the familial causes of the trait X, and is therefore stronger than a finite number of genetic variants used by MR which typically only explain a small proportion of variance in X, (3) ICE FALCON analysis conditions X_{self} and $X_{relative}$ on each other (model 3), essentially controlling for the effects of assortative mating, dynastic effects and/or population structure that would bias a MR analysis using unrelated individuals, as mentioned above, and (4) ICE FALCON still works even if the instrumental variable has directional pleiotropy, as suggested by the empirical analysis of the longitudinal tracking of BMI.[67]

The advantages of ICE FALCON over MR are supported by applications of ICE FALCON to epigenetic research. ICE FALCON has provided evidence that BMI and smoking have a causal effect on their associated DNA methylation changes; this is the same causal conclusion as that from MR analyses, but ICE FALCON has teh same power using smaller sample sizes than MR.[67, 74, 75] For example, in the application to BMI and DNA methylation, ICE FALCON analysis included 66 MZ pairs while MR analyses included >4000 individuals, and ICE FALCON appeared to be extracting about 2.5 times more information on causality per subject than MR.[4, 67, 74]

The DoC and MR-DoC models assume there is no familial confounding between the exposure and outcome. This is different from ICE FALCON, which can still make inference about causation even when there is familial confounding. ICE FALCON quantifies the effects of causation and familial confounding simultaneously.[67]

Another advantage of ICE FALCON over the DoC and MR-DoC models is that ICE FALCON uses the familial similarity of the traits of interest but does not necessarily need knowledge about the actual causes of the familial similarity, let alone decompose the variance into genetic and/or non-genetic components. ICE FALCON, therefore, does not need to assume latent causal models for X or Y traits or use complex structural equation modeling.

The key difference between ICE FALCON and the other causation assessment models is that it makes inference from comparing *changes* between conditional and unconditional regression coefficients when other variables are included in the models, not just on the unconditional regression coefficients themselves. It can also use a variety of ways to assess the statistical properties of the variation in the change in regression coefficients. Several different causal scenarios can be simulated using the observed correlation matrix, and ICE FALCON can be applied to each simulated causal scenario. The consistency between the simulated ICE FALCON regression results and the observed ICE FALCON regression results can be assessed by graphical plots and formal statistical tests to infer which simulated causal scenario(s) is(are) most consistent with the observed data; see Li et al.[67] for more details.

Under certain simulated scenarios, it has been suggested that the MR-DoC model has a greater statistical power than MR.[77] From an empirical analysis of height and educational attainment, however, the MR-DoC model and MR appeared to have similar statistical powers; the χ^2 on 1 degree of freedom statistics for the estimated causal effects were 6.9 and 6.7, respectively, for the two methods using the same sample[78] (see Li et al.[67] for a discussion on using the test statistic as a measure of the amount of information provided by a method on causality assessment). It will be of interest to compare empirically the statistical powers of ICE FALCON and the MR-DoC model.

5 Addressing issues of specific relevance to twins

MZ twins share almost identical genome sequence, so it is not possible to reliably discriminate MZ twins from one another based on genetic sequence using, e.g., the short tandem repeat (STR) markers that are used for forensic DNA profiling; the STR marker comparison is indeed a method for confirming if twins are monozygotic.

Epigenetic modifications could be used to discriminate MZ twins from one another. Studies have found that MZ twins can be different from one another in some DNA methylation measures,[27, 79] and several studies have suggested that DNA methylation measures could be used to discriminate MZ twins in forensic settings.[80–85] However, most of these studies included only a few MZ twin pairs (some studies even included only one MZ twin pair) and assessed the within-pair methylation difference without any statistical test, i.e., the difference could be simply due to chance. Studies with greater sample sizes and that use appropriate statistical methods to assess within-pair differences are needed to find reliable methylation measures that could be used for discriminating MZ twins in forensic settings.

Another application of twin and family study designs is to investigate the epigenetic signature related to a twin birth. An Australian twin and family study found that pairs of siblings born after a

twin birth were correlated in both global and site-specific DNA methylation, while no such correlation was found for pairs of siblings both born before a twin birth, or pairs of siblings born either side of a twin birth. The findings were replicated using a Korean twin and family study.[86] These observations are consistent with a twin birth changing the intrauterine environment sufficient for siblings born after a twin birth to be correlated in DNA methylation.

6 Summary

Twin and family study designs have unique value in the rapidly emerging research field of epigenetics. These designs with appropriate statistical analysis can give important insights into what it is to be human, and facilitate the translation of a higher level of scientific knowledge into practice so as to improve health and wellbeing.

References

1. C.H. Waddington The epigenotype. 1942. Int J Epidemiol. 2012;41(1):10–13. doi:10.1093/ije/dyr184.
2. A. Petronis Epigenetics as a unifying principle in the aetiology of complex traits and diseases. Nature. 2010;465(7299):721–727. doi:10.1038/nature09230.
3. S. Li, P.-A. Dugué, R.L. Milne, J.L. Hopper Epigenetic prospects in epidemiology and public health. In: T.O. Tollefsbol, ed. Epigenetics in Human Disease. Academic Press; 2018:995–1017.
4. S. Wahl, A. Drong, B. Lehne, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. Nature. 2017;541(7635):81–86. doi:10.1038/nature20784.
5. J.C. Chambers, M. Loh, B. Lehne, et al. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: a nested case-control study. Lancet Diabetes Endocrinol. 2015;3(7):526–534. doi:10.1016/S2213-8587(15)00127-8.
6. L.P. Breitling, R. Yang, B. Korn, B. Burwinkel, H. Brenner Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. Am J Hum Genet. 2011;88(4):450–457. doi:10.1016/j.ajhg.2011.03.003.
7. J.T. Bell, R. Saffery The value of twins in epigenetic epidemiology. Int J Epidemiol. 2012;41(1):140–150. doi:10.1093/ije/dyr179.
8. J.T. Bell, T.D. Spector A twin approach to unraveling epigenetics. Trends Genet. 2011;27(3):116–125. doi:10.1016/j.tig.2010.12.005.
9. J. van Dongen, P.E. Slagboom, H.H. Draisma, N.G. Martin, D.I. Boomsma The continuing value of twin studies in the omics era. Nat Rev Genet. 2012;13(9):640–653. doi:10.1038/nrg3243.
10. J.T. Bell, T.D. Spector DNA methylation studies using twins: what are they telling us? Genome Biol. 2012;13(10):172. doi:10.1186/gb-2012-13-10-172.
11. R. Fisher The correlation between relatives on the supposition of Mendelian inheritance. Trans Roy Soc Edinb. 1918;52:399–433.
12. J.L. Hopper Variance components for statistical genetics: applications in medical research to characteristics related to human diseases and health. Stat Methods Med Res. 1993;2(3):199–223. doi:10.1177/096228029300200302.
13. B.T. Heijmans, D. Kremer, E.W. Tobi, D.I. Boomsma, P.E. Slagboom Heritable rather than age-related environmental and stochastic factors dominate variation in DNA methylation of the human IGF2/H19 locus. Hum Mol Genet. 2007;16(5):547–554. doi:10.1093/hmg/ddm010.
14. H.T. Bjornsson, M.I. Sigurdsson, M.D. Fallin, et al. Intra-individual change over time in DNA methylation with familial clustering. JAMA. 2008;299(24):2877–2883. doi:10.1001/jama.299.24.2877.
15. M.P. Boks, E.M. Derks, D.J. Weisenberger, et al. The relationship of DNA methylation with age, gender and

genotype in twins and healthy controls. PLoS One. 2009;4(8):e6767. doi:10.1371/journal.pone.0006767.

16. K. Gervin, M. Hammero, H.E. Akselsen, et al. Extensive variation and low heritability of DNA methylation identified in a twin study. Genome Res. 2011;21(11):1813–1821. doi:10.1101/gr.119685.110.

17. J.T. Bell, P.C. Tsai, T.P. Yang, et al. Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. PLoS Genet. 2012;8(4):e1002629. doi:10.1371/journal.pgen.1002629.

18. L. Gordon, J.E. Joo, J.E. Powell, et al. Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. Genome Res. 2012;22(8):1395–1406. doi:10.1101/gr.136598.111.

19. C. Pirazzini, C. Giuliani, M.G. Bacalini, et al. Space/population and time/age in DNA methylation variability in humans: a study on IGF2/H19 locus in different Italian populations and in mono- and di-zygotic twins of different age. Aging (Albany NY). 2012;4(7):509–520. doi:10.18632/aging.100476.

20. E. Grundberg, E. Meduri, J.K. Sandling, et al. Global analysis of DNA methylation variation in adipose tissue from twins reveals links to disease-associated variants in distal regulatory elements. Am J Hum Genet. 2013;93(5):876–890. doi:10.1016/j.ajhg.2013.10.004.

21. A.F. McRae, J.E. Powell, A.K. Henders, et al. Contribution of genetic variation to transgenerational inheritance of DNA methylation. Genome Biol. 2014;15(5):R73. doi:10.1186/gb-2014-15-5-r73.

22. S. Busche, X. Shao, M. Caron, et al. Population whole-genome bisulfite sequencing across two tissues highlights the environment as the principal source of human methylome variation. Genome Biol. 2015;16:290. doi:10.1186/s13059-015-0856-1.

23. B.L. Tremblay, F. Guenard, B. Lamarche, L. Perusse, M.C. Vohl Familial resemblances in blood leukocyte DNA methylation levels. Epigenetics. 2016;11(11):831–838. doi:10.1080/15592294.2016.1232234.

24. J. van Dongen, M.G. Nivard, G. Willemsen, et al. Genetic and environmental influences interact with age and sex in shaping the human methylome. Nat Commun. 2016;7:11115. doi:10.1038/ncomms11115.

25. S. Li, E.M. Wong, T.L. Nguyen, et al. Causes of blood methylomic variation for middle-aged women measured by the HumanMethylation450 array. Epigenetics. 2017;12(11):973–981. doi:10.1080/15592294.2017.1384891.

26. T.E. Van Baak, C. Coarfa, P.A. Dugue, et al. Epigenetic supersimilarity of monozygotic twin pairs. Genome Biol. 2018;19(1):2. doi:10.1186/s13059-017-1374-0.

27. Z.A. Kaminsky, T. Tang, S.C. Wang, et al. DNA methylation profiles in monozygotic and dizygotic twins. Nat Genet. 2009;41(2):240–245. doi:10.1038/ng.286.

28. S. Li, T.L. Nguyen, E.M. Wong, et al. Genetic and environmental causes of variation in epigenetic aging across the lifespan. Clin Epigenetics. 2020;12(1):158. doi:10.1186/s13148-020-00950-1.

29. S. Li, E.M. Wong, P.A. Dugue, et al. Genome-wide average DNA methylation is determined in utero. Int J Epidemiol. 2018;47(3):908–916. doi:10.1093/ije/dyy028.

30. M.E. Levine, A.T. Lu, D.A. Bennett, S. Horvath Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. Aging (Albany NY). 2015;7(12):1198–1211. doi:10.18632/aging.100864.

31. A.T. Lu, E. Hannon, M.E. Levine, et al. Genetic variants near MLST8 and DHX57 affect the epigenetic age of the cerebellum. Nat Commun. 2016;7:10561. doi:10.1038/ncomms10561.

32. A.T. Lu, L. Xue, E.L. Salfati, et al. GWAS of epigenetic aging rates in blood reveals a critical role for TERT. Nat Commun. 2018;9(1):387. doi:10.1038/s41467-017-02697-5.

33. R.E. Marioni, S. Shah, A.F. McRae, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 2015;16(1):25. doi:10.1186/s13059-015-0584-6.

34. A.J. Simpkin, G. Hemani, M. Suderman, et al. Prenatal and early life influences on epigenetic age in children: a study of mother-offspring pairs from two cohort studies. Hum Mol Genet. 2016;25(1):191–201. doi:10.1093/hmg/ddv456.

35. S. Horvath DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115.

doi:10.1186/gb-2013-14-10-r115.

36. J. Gibson, T.C. Russ, T.K. Clarke, et al. A meta-analysis of genome-wide association studies of epigenetic age acceleration. PLoS Genet. 2019;15(11):e1008104. doi:10.1371/journal.pgen.1008104.

37. S. Li, E.M. Wong, J.E. Joo, et al. Genetic and environmental causes of variation in the difference between biological age based on DNA methylation and chronological age for middle-aged women. Twin Res Hum Genet. 2015;18(6):720–726. doi:10.1017/thg.2015.75.

38. P.-A. Dugué, S. Li, J.L. Hopper, R.L. Milne DNA methylation–based measures of biological aging. In: T.O. Tollefsbol, ed. Epigenetics in Human Disease. Academic Press; 2018:39–64.

39. P.A. Dugue, D.R. English, R.J. MacInnis, et al. Reliability of DNA methylation measures from dried blood spots and mononuclear cells using the HumanMethylation450k BeadArray. Sci Rep. 2016;6:30317. doi:10.1038/srep30317.

40. M.B. Terry, J.S. Ferris, R. Pilsner, et al. Genomic DNA methylation among women in a multiethnic New York City birth cohort. Cancer Epidemiol Biomarkers Prev. 2008;17(9):2306–2310. doi:10.1158/1055-9965.EPI-08-0312.

41. H. Heyn, S. Moran, I. Hernando-Herraez, et al. DNA methylation contributes to natural human variation. Genome Res. 2013;23(9):1363–1372. doi:10.1101/gr.154187.112.

42. B. Kwabi-Addo, S. Wang, W. Chung, et al. Identification of differentially methylated genes in normal prostate tissues from African American and Caucasian men. Clin Cancer Res. 2010;16(14):3539–3547. doi:10.1158/1078-0432.CCR-09-3342.

43. J. Liu, K. Hutchison, N. Perrone-Bizzozero, M. Morgan, J. Sui, V. Calhoun Identification of genetic and epigenetic marks involved in population structure. PLoS One. 2010;5(10):e13209. doi:10.1371/journal.pone.0013209.

44. P.-C. Tsai, J.T. Bell Power and sample size estimation for epigenome-wide association scans to detect differential DNA methylation. Int J Epidemiol. 2015. doi:10.1093/ije/dyv041.

45. C.C. Wong, E.L. Meaburn, A. Ronald, et al. Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. Mol Psychiatry. 2014;19(4):495–503. doi:10.1038/mp.2013.41.

46. E.M. Byrne, T. Carrillo-Roa, A.K. Henders, et al. Monozygotic twins affected with major depressive disorder have greater variance in methylation than their unaffected co-twin. Transl Psychiatry. 2013;3:e269. doi:10.1038/tp.2013.45.

47. E.L. Dempster, R. Pidsley, L.C. Schalkwyk, et al. Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. Hum Mol Genet. 2011;20(24):4786–4796. doi:10.1093/hmg/ddr416.

48. Q. Tan, M. Frost, B.T. Heijmans, et al. Epigenetic signature of birth weight discordance in adult twins. BMC Genomics. 2014;15:1062. doi:10.1186/1471-2164-15-1062.

49. N.Y. Souren, P. Lutsik, G. Gasparoni, et al. Adult monozygotic twins discordant for intra-uterine growth have indistinguishable genome-wide DNA methylation profiles. Genome Biol. 2013;14(5):R44. doi:10.1186/gb-2013-14-5-r44.

50. H. Heyn, F.J. Carmona, A. Gomez, et al. DNA methylation profiling in breast cancer discordant identical twins identifies DOK7 as novel epigenetic biomarker. Carcinogenesis. 2013;34(1):102–108. doi:10.1093/carcin/bgs321.

51. V.K. Rakyan, H. Beyan, T.A. Down, et al. Identification of type 1 diabetes-associated DNA methylation variable positions that precede disease diagnosis. PLoS Genet. 2011;7(9):e1002300. doi:10.1371/journal.pgen.1002300.

52. M. Stefan, W. Zhang, E. Concepcion, Z. Yi, Y. Tomer DNA methylation profiles in type 1 diabetes twins point to strong epigenetic effects on etiology. J Autoimmun. 2014;50:33–37. doi:10.1016/j.jaut.2013.10.001.

53. J.B. Carlin, L.C. Gurrin, J.A. Sterne, R. Morley, T. Dwyer Regression models for twin studies: a critical review. Int J Epidemiol. 2005;34(5):1089–1099. doi:10.1093/ije/dyi153.

54. L.C. Gurrin, J.B. Carlin, J.A. Sterne, G.S. Dite, J.L. Hopper Using bivariate models to understand between- and within-cluster regression coefficients, with application to twin data. Biometrics. 2006;62(3):745–751. doi:10.1111/j.1541-0420.2006.00561.x.

55. J. Stone, L.C. Gurrin, V.M. Hayes, M.C. Southey, J.L. Hopper, G.B. Byrnes Sibship analysis of associations between SNP haplotypes and a continuous trait with application to mammographic density. Genet Epidemiol. 2010;34(4):309–318. doi:10.1002/gepi.20462.

56. S. Li, E.M. Wong, M.C. Southey, J.L. Hopper Association between DNA methylation at SOCS3 gene and body mass index might be due to familial confounding. Int J Obes (Lond). 2017;41(6):995–996. doi:10.1038/ijo.2017.56.

57. C.A. Nowson, R.M. Green, J.L. Hopper, et al. A co-twin study of the effect of calcium supplementation on bone density during adolescence. Osteoporos Int. 1997;7(3):219–225. doi:10.1007/bf01622292.

58. G.D. Smith, S. Ebrahim "Mendelian randomization": can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003;32(1):1–22.

59. F. Windmeijer, H. Farbmacher, N. Davies, S.G. Davey On the use of the lasso for instrumental variables estimation with some invalid instruments. J Am Stat Assoc. 2019;114(527):1339–1350. doi:10.1080/01621459.2018.1498346.

60. F.P. Hartwig, G. Davey Smith, J. Bowden Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol. 2017;46(6):1985–1998. doi:10.1093/ije/dyx102.

61. J. Bowden, G. Davey Smith, S. Burgess Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–525. doi:10.1093/ije/dyv080.

62. J. Bowden, G. Davey Smith, P.C. Haycock, S. Burgess Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol. 2016;40(4):304–314. doi:10.1002/gepi.21965.

63. S. Burgess, S.G. Thompson Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. Am J Epidemiol. 2015;181(4):251–260. doi:10.1093/aje/kwu283.

64. D. Adam The gene-based hack that is revolutionizing epidemiology. Nature. 2019;576(7786):196–199. doi:10.1038/d41586-019-03754-3.

65. Z. Xu, D.P. Sandler, J.A. Taylor Blood DNA methylation and breast cancer: a prospective case-cohort analysis in the sister study. J Natl Cancer Inst. 2019. doi:10.1093/jnci/djz065.

66. B. Brumpton, E. Sanderson, K. Heilbron, et al. Avoiding dynastic, assortative mating, and population stratification biases in Mendelian randomization through within-family analyses. Nat Commun. 2020;11(1):3519. doi:10.1038/s41467-020-17117-4.

67. S. Li, M. Bui, J.L. Hopper Inference about causation from examination of familial confounding (ICE FALCON): a model for assessing causation analogous to Mendelian randomization. Int J Epidemiol. 2020;49(4):1259–1269. doi:10.1093/ije/dyaa065.

68. G.S. Dite, L.C. Gurrin, G.B. Byrnes, et al. Predictors of mammographic density: insights gained from a novel regression analysis of a twin study. Cancer Epidemiol Biomarkers Prev. 2008;17(12):3474–3481. doi:10.1158/1055-9965.EPI-07-2636.

69. J. Stone, G.S. Dite, G.G. Giles, J. Cawson, D.R. English, J.L. Hopper Inference about causation from examination of familial confounding: application to longitudinal twin data on mammographic density measures that predict breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2012;21(7):1149–1155. doi:10.1158/1055-9965.EPI-12-0051.

70. J.L. Hopper, Q.M. Bui, B. Erbas, et al. Does eczema in infancy cause hay fever, asthma, or both in childhood? Insights from a novel regression model of sibling data. J Allergy Clin Immunol. 2012;130(5):1117–1122. e1 https://doi.org/10.1016/j.jaci.2012.08.003.

71. C.G. Davey, C. Lopez-Sola, M. Bui, et al. The effects of stress-tension on depression and anxiety symptoms: evidence from a novel twin modelling analysis. Psychol Med. 2016;46(15):3213–3218.

doi:10.1017/S0033291716001884.

72. M. Bui, A. Bjornerem, A. Ghasem-Zadeh, G.S. Dite, J.L. Hopper, E. Seeman Architecture of cortical bone determines in part its remodelling and structural decay. Bone. 2013;55(2):353–358. doi:10.1016/j.bone.2013.04.020.
73. M. Bui, R. Zebaze, S. Li, J.L. Hopper, A. Bjornerem Are the relationships of lean mass and fat mass with bone microarchitecture causal or due to familial confounders? A novel study of adult female twin pairs. JBMR Plus. 2020;4(9):e10386. doi:10.1002/jbm4.10386.
74. S. Li, E.M. Wong, M. Bui, et al. Inference about causation between body mass index and DNA methylation in blood from a twin family study. Int J Obes (Lond). 2019;43(2):243–252. doi:10.1038/s41366-018-0103-4.
75. S. Li, E.M. Wong, M. Bui, et al. Causal effect of smoking on DNA methylation in peripheral blood: a twin and family study. Clin Epigenetics. 2018;10(1):18. doi:10.1186/s13148-018-0452-9.
76. A.C. Heath, R.C. Kessler, M.C. Neale, J.K. Hewitt, L.J. Eaves, K.S. Kendler Testing hypotheses about direction of causation using cross-sectional family data. Behav Genet. 1993;23(1):29–50. doi:10.1007/bf01067552.
77. C.C. Minică, C.V. Dolan, D.I. Boomsma, E. de Geus, M.C. Neale Extending causality tests with genetic instruments: an integration of Mendelian randomization with the classical twin design. Behav Genet. 2018;48(4):337–349. doi:10.1007/s10519-018-9904-4.
78. C.C. Minică, D.I. Boomsma, C.V. Dolan, E. de Geus, M.C. Neale Empirical comparisons of multiple Mendelian randomization approaches in the presence of assortative mating. Int J Epidemiol. 2020;49(4):1185–1193. doi:10.1093/ije/dyaa013.
79. M.F. Fraga, E. Ballestar, M.F. Paz, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A. 2005;102(30):10604–10609. doi:10.1073/pnas.0500398102.
80. A. Vidaki, V. Kalamara, E. Carnero-Montoro, T.D. Spector, J.T. Bell, M. Kayser Investigating the epigenetic discrimination of identical twins using buccal swabs, saliva, and cigarette butts in the forensic setting. Genes (Basel). 2018;9(5). doi:10.3390/genes9050252.
81. A. Vidaki, C. Díez López, E. Carnero-Montoro, et al. Epigenetic discrimination of identical twins from blood under the forensic scenario. Forensic Sci Int Genet. 2017;31:67–80. doi:10.1016/j.fsigen.2017.07.014.
82. C. Li, S. Zhao, N. Zhang, S. Zhang, Y. Hou Differences of DNA methylation profiles between monozygotic twins' blood samples. Mol Biol Rep. 2013;40(9):5275–5280. doi:10.1007/s11033-013-2627-y.
83. Q. Du, G. Zhu, G. Fu, et al. A genome-wide scan of DNA methylation markers for distinguishing monozygotic twins. Twin Res Hum Genet. 2015;18(6):670–679. doi:10.1017/thg.2015.73.
84. J. Xu, G. Fu, L. Yan, et al. LINE-1 DNA methylation: a potential forensic marker for discriminating monozygotic twins. Forensic Sci Int Genet. 2015;19:136–145. doi:10.1016/j.fsigen.2015.07.014.
85. C. Li, S. Zhang, T. Que, L. Li, S. Zhao Identical but not the same: the value of DNA methylation profiling in forensic discrimination within monozygotic twins. Forensic Sci Int Genet Suppl Ser. 2011;3(1):e337–e338. doi:10.1016/j.fsigss.2011.09.031.
86. S. Li, E. Kim, E.M. Wong, et al. Twin birth changes DNA methylation of subsequent siblings. Sci Rep. 2017;7(1):8463. doi:10.1038/s41598-017-08595-6.