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Article

Differences in DNA Methylation-Based Age Prediction Within Twin Pairs Discordant for Cancer

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Abstract

DNA methylation-based age acceleration (DNAmAA) is associated with cancer, with both cancer tissue and blood showing increased DNAmAA. We aimed to investigate whether DNAmAA is associated with cancer risk within twin pairs discordant for cancer, and whether DNAmAA has the potential to serve as a biomarker for such. The study included 47 monozygotic and 48 same-sex-dizygotic cancer-discordant twin pairs from the Finnish Twin Cohort study with blood samples available between 17 and 31 years after the cancer diagnosis. We studied all cancers (95 pairs), then separately breast cancer (24 pairs) and all sites other than breast cancer (71 pairs). DNAmAA was calculated for seven models: Horvath, Horvath intrinsic epigenetic age acceleration, Hannum, Hannum intrinsic epigenetic age acceleration, Hannum extrinsic epigenetic age acceleration, PhenoAge and GrimAge. Within-pair differences in DNAmAA were analyzed by paired *t* tests and linear regression. Twin pairs sampled before cancer diagnosis did not differ significantly in DNAmAA. However, the within-pair differences in DNAmAA before cancer diagnosis increased significantly the closer the cancer diagnosis was, and this acceleration extended for years after the diagnosis. Pairs sampled after the diagnosis differed for DNAmAA with the Horvath models capturing cancer diagnosis-associated DNAmAA across all three cancer groupings. The results suggest that DNAmAA in blood is associated with cancer diagnosis. This may be due to epigenetic alterations in relation to cancer, its treatment or associated lifestyle changes. Based on the current study, the biomarker potential of DNAmAA in blood appears to be limited.

Keywords: Epigenetic age acceleration; cancer-discordant twin pairs; breast cancer

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Age and aging are two of the biggest risk factors for many diseases, including cancer (Aunan et al., 2017; López-Otín et al., 2013). However, aging can be approached in two ways: chronological aging and biological aging. Chronological age is defined as the calendar time between birth and the time of observation. Biological age can be defined as the physiological state of a person in terms of cellular and organ functions (Horvath & Raj, 2018; López-Otín et al., 2013). Biological aging rates may differ from chronological aging rates and are often associated with health-related factors (Ahadi et al., 2020; Horvath & Raj, 2018). From this perspective, the difference between chronological and biological age may serve as a better biomarker for individual cancer risk than chronological age alone.

For the study of biological aging, DNA methylation (DNAm, addition of a methyl group to a cytosine base next to guanine) provides a valuable source of information. DNAm-based age prediction measures age-related changes in DNAm and can serve as a surrogate for biological age (Horvath & Raj, 2018). Such prediction tools enable a straightforward study of biological aging of each individual in large cohorts (Horvath & Raj, 2018). Increased DNAm-based age has been shown to be associated, for example, with breast cancer in the cancer tissue (Horvath, 2013) and in the adjacent healthy tissue (Hofstatter et al., 2018) and in the blood of breast cancer patients (Kresovich et al., 2019). Further, biological age captured by DNAm is associated with different risk factors for cancer (M. Chen et al., 2019; Xiao, Miller et al., 2021), suggesting that DNAm-based age could summarize exposure to such risk factors. Higher exposure would lead to higher cancer risk and increased DNAm-based age; thus, DNAm-based age could serve as a biomarker for cancer risk.

To calculate DNAm-based age, the DNAm is measured in each sample using Illumina BeadChips (Illumina, San Diego, CA, USA). Using mathematical models, individual age estimates (termed DNAm age) are predicted based on the methylation status of a predefined subset of DNAm sites (CpG sites) whose methylation correlate with age (Horvath & Raj, 2018; Yu et al., 2020). A variant of this is DNAm-based age acceleration (DNAmAA), which is calculated by regressing the DNAm age on chronological age. The DNAmAA values are the residuals of this regression. Consequently, the DNAmAA is independent of chronological age and indicates a difference between the chronological age and the measured DNAm age (i.e., biological aging of an individual). Using DNAmAA instead of DNAm age allows for comparison of individuals in a cohort sampled at different chronological ages (B. H. Chen et al., 2016; Horvath & Raj, 2018).

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Two of the first and frequently used DNAmAA models are the Horvath model (Horvath, 2013), also referred to as the 'epigenetic clock' or the 'multi-tissue DNAm age estimator', and the Hannum model (Hannum et al., 2013). Both of these models were developed by selecting CpG sites that correlate with chronological age (Hannum et al., 2013; Horvath, 2013). DNAmAA measured by both of these tools are associated with cancer (Horvath, 2013; Kresovich et al., 2019). Later, to enable better prediction of morbidity and mortality, the second-generation DNAmAA models were developed, such as the PhenoAge models (Levine et al., 2018) and GrimAge (Lu et al., 2019). In addition to chronological age, these two models were built by incorporating blood-derived biomarkers of aging (PhenoAge and GrimAge) and time to death (GrimAge) as a reference. Association between DNAmAA and cancer has been shown by the PhenoAge (Levine et al., 2018) and the GrimAge model (Lu et al., 2019).

Twin pairs discordant for a disease (here, pairs with a cancer diagnosis in one twin and no cancer diagnosis in the other twin during the study period) provide a powerful study design, since the twins are matched for many known factors (such as age, sex, genetic relatedness and family background) and potentially for many unknown factors. These factors shared by the co-twins of each pair may affect DNAm and DNAmAA (Czyz et al., 2012). Hence, for cancer-discordant twin pairs, within-pair differences in DNAmAA can be considered cancer diagnosis-related, and confounding factors that affect DNAmAA but not cancer risk are largely mitigated by matching the twins. It is known that DNAmAA is correlated between twins, with a considerable amount of heritability (Jylhävä et al., 2019; Reynolds et al., 2020). However, this correlation decreases over time, and the twins diverge from each other in their DNAmAA (Li et al., 2020). To date, it is not known whether twins in a pair discordant for cancer differ by their DNAmAA, and whether these differences are associated with cancer diagnosis. Knowing this could be important in understanding whether DNAmAA serves as an appropriate biomarker for individual cancer risk. In addition, this would allow us to determine the extent to which a cancer diagnosis affects biological aging post diagnosis, reflected by DNAmAA.

To this end, we identified twin pairs discordant for incident cancer from the Finnish Twin Cohort. After blood-based DNAm measurement, DNAmAA was calculated. Within-pair differences of DNAmAA were first computed to determine whether cancer diagnosis is associated with increased DNAmAA. These analyses were performed separately for pairs where DNA was sampled prior to cancer diagnosis, and for those where DNA was sampled after a diagnosis. This division allowed for investigation of DNAmAA, both independent of and associated with diagnosis and treatment as possible factors affecting DNAmAA. Furthermore, these within-pair differences in DNAmAA were analyzed for associations between the time interval from DNA sampling to cancer diagnosis. All the analyses were performed for all cancers together and then separately for breast cancer and nonbreast cancer.

Materials and Methods

Twin Cohort

The data used in this study originate from the older Finnish Twin Cohort, including same-sex twin pairs born before 1958, and followed up with lifestyle, health and behavioral surveys in four waves (years 1975, 1981, 1990 and 1999–2017). Cancer incidence and cause of death were updated from the Cancer Registry and Statistics Finland, with the latest update in 2018 (Kaprio et al., 2019). The extracted cancer diagnoses of the cohort were provided in the form of 'International Classification of Diseases 10th revision' (ICD10) codes (Harris et al., 2019; Skytthe et al., 2019). The ICD10 codes were studied using the ICD10 code browser provided by the WHO (World Health Organization, 2019) to identify the different sites of diagnosis (Supplementary Table S1 and Table 1). Epigenetic analyses of DNA samples in the older cohort have been undertaken for a number of specific projects, which provided the DNAm data (Kaprio et al., 2019). A total of 95 twin pairs discordant for any cancer with available blood DNAm data were included in the current study. The methylation data of the co-twins in each pair is from the same time point, and all twin pairs are same-sex pairs, thereby age and sex are accounted for in the within-pair analyses. For all individuals, smoking status at the time of DNA collection was coded categorically: 77.9% ($n = 148$) were nonsmokers, 14.7% ($n = 28$) were former smokers and 7.4% ($n = 14$) were current smokers, with 21 pairs discordant for smoking behavior (Table 2).

Table 1. Number of the Finnish Twin Cohort twin pairs discordant for cancer shown for the different cancer groups

	Total number of pairs (% females)	Pairs sampled before the diagnosis (% females)	Pairs sampled after the diagnosis (% females)
All cancers pooled (pan-cancer)	95 (92)	46 (100)	49 (83)
Breast cancer	24 (100)	10 (100)	14 (100)
Nonbreast cancer[a]	71 (89)	36 (100)	35 (77)

Note: [a]other cancers than breast cancer.

DNAm Data

High-molecular-weight blood DNA was bisulphite-converted using the EZ-96 DNA Methylation-Gold Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions and hybridized on the Infinium HumanMethylation450 ('450k') or EPIC BeadChip ('EPIC') (Illumina, San Diego, CA, USA; 21 and 74 twin pairs, respectively) for DNAm profiling following the standard protocols. Samples of both twins in a pair were always processed at the same time.

Sample quality control (QC) was performed using the R package *MethylAid* (van Iterson et al., 2014) and *minfi* (Aryee et al., 2014) to assess quality based on five control probe metrics with default thresholds, as follows. A sample passed the QC when the log2 intensity of converted red/green channel bisulphite Type I control was higher than 12.75, median methylated versus unmethylated log2 intensity was higher than 10.5, log2 intensity of sample-dependent control probes was higher than 11.75, log2 intensity of sample-independent control probes was higher than 13.25 and when less than 5% of the control probes in a sample showed only background signal. All samples passed the sample QC. Finally, data generated on the 450K and EPIC platforms were combined using *minfi*'s *combineArrays* function.

Probe QC was performed using the R packages *minfi* (Fortin et al., 2017) and *wateRmelon* (Pidsley et al., 2013). Probes with a bead count < 3, detection p value > 0.01 or intensity value of exactly zero were removed, and probes with a call rate of $\geq 95\%$ across all samples were retained. In total, 1058 probes were set to missing. Afterward, ambiguously mapping and poor-quality

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Table 2. Description of the cancer-discordant Finnish Twin Cohort twin pairs

	All ($N = 95$ pairs)	Pairs sampled before the diagnosis ($n = 46$ pairs)	Pairs sampled after the diagnosis ($n = 49$ pairs)
Age at diagnosis[a]			
Mean (*SD*)	65.8 (13.0)	76.4 (6.0)	55.7 (9.5)
Range	32.5–87.1	63.5–87.1	32.5–71.0
Age at DNA sampling[a]			
Mean (*SD*)	67.4 (4.6)	68.0 (4.1)	66.9 (5.0)
Range	57–75	58–75	57–75
Time to diagnosis[a]			
Mean (*SD*)	1.7 before (12.0)	8.4 before (5.0)	11.1 after (8.3)
Range	16.8 before–31.0 after	0.5 before–16.8 before	0.8 after–31.0 after
MZ pairs (% females)	47 (87)	26 (100)	21 (71)
ssDZ pairs (% females)	48 (96)	20 (100)	28 (92)
Smoking status, *n* (%)			
Nonsmoker[b]	148 (77.9)	74 (80.4)	74 (75.5)
Current smoker[b]	14 (7.4)	7 (7.6)	7 (7.1)
Former smoker[b]	28 (14.7)	11 (12.0)	17 (17.3)
Pairs discordant for smoking	21 (22)	10 (22)	11 (22)
DNA methylation platform, pairs			
450K	21	4	17
EPIC	74	42	32

Note: [a]ages and time to diagnosis are in years; [b]per individual, at the time point of sampling.

probes according to Zhou and colleagues (negative selection criteria: single-nucleotide polymorphisms with minor allele frequency > 1% near target site, color-channel-switching single-nucleotide polymorphisms, off-target hybridization events and incorrect mapping on the *GRCh38* genome; Zhou et al., 2017) were removed. Additionally, probes binding to sex chromosomes were set to missing.

Functional normalization was applied using *ssnoob* from the R package *minfi* (Fortin et al., 2017) with default settings to remove unwanted between-array variability (Zhou et al., 2017). Afterward, beta mixture quantile normalization was applied to the normalized data using the R package *wateRmelon* (Pidsley et al., 2013) to further correct the data for probe design bias.

DNAm-Based Age Prediction

The DNAm-based age was calculated on the preprocessed DNAm data using the online calculator (http://dnamage.genetics.ucla.edu/). DNAm-based age prediction was obtained for seven models: Horvath (Horvath, 2013) and Horvath intrinsic epigenetic age acceleration (Horvath IEAA; B. H. Chen et al., 2016), Hannum (Hannum et al., 2013), Hannum IEAA (B. H. Chen et al., 2016), Hannum extrinsic epigenetic age acceleration (Hannum EEAA; B. H. Chen et al., 2016), PhenoAge (Levine et al., 2018) and GrimAge (Lu et al., 2019). DNAmAA was calculated by linearly regressing DNAm age over chronological age, with the residuals serving as the DNAmAA measure that was used in further analyses. Detailed information on the epigenetic age acceleration models is presented in the supplements (Supplementary materials and methods on DNAm-based age prediction and Supplementary Table S2 available at the Cambridge Core website (https://www.cambridge.org/core/).

Statistical Analysis

A paired *t* test was used to examine within-pair differences in DNAmAA. This was done separately for the pairs sampled before and after diagnosis. The association between the time to diagnosis (the time interval between the blood sampling for the DNA and diagnosis) and the within-pair difference in DNAmAA (dDNAmAA) was examined using linear regression (1). A negative time to diagnosis indicates that the sample was collected prior to cancer diagnosis, and a positive time to diagnosis that the sample was collected after diagnosis. A negative dDNAmAA indicates that the cancer-diagnosed twin in a pair is predicted to be younger, and a positive dDNAmAA that the cancer-diagnosed twin in a pair is predicted to be older than the healthy co-twin. A categorical correction term was included in the regression model to correct for confounding by differences in smoking behavior between twins in a pair. This correction term describes the smoking status of the pair by combining the smoking status of each twin in a pair into a single term ('nonsmoker/nonsmoker', 'former smoker/nonsmoker', 'current smoker/nonsmoker', etc.). F-statistics' p values and adjusted R^2 (adR^2) values were reported for the regression models.

(1) $\text{dDNAmAA (Pair}_X) \sim \text{Intercept}_{\text{Time Point of Diagnosis}} + \text{Slope} * \text{Time to Diagnosis (Pair}_X) + \text{Smoking (Pair}_X)$

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Table 3. Within-pair differences in DNAmAA in cancer-discordant twin pairs ($N = 95$). The presented units are in years

	Before the diagnosis ($n = 46$)				After the diagnosis ($n = 49$)				Time to diagnosis ($n = 95$)			
	Mean difference	95% CI	p value[a]	Power	Mean difference	95% CI	p value[a]	Power	Estimate	p value[b]	Power	adR^{2c}
Horvath	0.14	−1.25, 1.53	.84	0.16	2.48	1.09, 3.87	.0008	1	0.16	.0005	1	0.21
Horvath IEAA	0.37	−0.89, 1.64	.56	0.70	2.18	0.87, 3.49	.001	1	0.14	.0002	1	0.23
Hannum	−0.24	−1.93, 1.45	.78	0.36	1.98	0.65, 3.31	.004	1	0.11	.11	1	0.06
Hannum IEAA	0.40	− 1.06, 1.86	.59	0.75	1.12	0.08, 2.15	.03	1	0.05	.40	1	0.01
Hannum EEAA	−0.66	−2.80, 1.48	.54	0.99	2.89	1.01, 4.76	.003	1	0.17	.06	1	0.08
PhenoAge	−0.42	− 2.64, 1.80	.70	0.81	1.66	−0.69, 4.01	.16	1	0.15	.38	1	0.01
GrimAge	−0.04	− 1.02, 0.93	.93	0.06	1.23	0.47, 2.00	.002	1	0.05	.08	1	0.07

Note: [a]t-test p value; [b]F-statistics p value; [c]adjusted coefficient of determination (R^2), proportion of variance in the data explained by the linear model.

A *post hoc* power analysis was performed for the paired t test and the linear regression using the R package *pwr* with a significance threshold of $p \leq .05$ (Champely, 2020; Selya et al., 2012). Only significant results with sufficient power (≥0.8) were considered meaningful, and the conclusions of this study were drawn from such results exclusively. Effects and measures of DNAmAA and dDNAmAA are reported in years.

Results

Cohort Description

A total of 95 same-sex twin pairs discordant for any cancer were identified (Table 1). Of these, 47 were monozygotic (MZ) and 48 were same-sex dizygotic (ssDZ) pairs. Within this cohort, 24 pairs were discordant for breast cancer and 71 pairs were discordant for nonbreast cancer (Table 1 and Supplementary Table S1). Nine individuals had more than one diagnosis, and in such cases, the first diagnosis was considered as the primary site. Age at first cancer diagnosis ranged from 33 to 87 years. The blood samples were collected at least 6 months before the cancer diagnosis in 46 pairs and at least 6 months after the diagnosis in the remaining 49 pairs. The interval between blood collection and the time of first cancer diagnosis ranged from 17 years before the diagnosis to 31 years after the diagnosis (Table 2).

Pan-Cancer

We first examined whether cancer diagnosis is associated with DNAmAA in a co-twin control design. To this end, we analyzed within-pair differences in DNAmAA using all 47 MZ and 48 ssDZ pairs discordant for any cancer as a pan-cancer analysis (Table 3 and Supplementary Figure S1). The twins with cancer from pairs whose blood samples were collected before diagnosis did not differ from their healthy co-twins for DNAmAA, the point estimates being small, and both negative and positive. However, among the twin pairs whose blood samples were collected after the diagnosis, all the DNAmAA models showed between 1.12 and 2.89 years within-pair difference in DNAmAA. The Horvath model and Hannum EEAA model showed slightly larger within-pair differences in DNAmAA after the cancer diagnosis compared with the other models (Table 3 and Supplementary Figure S1).

The within-pair differences measured by Horvath (2.48 years) and Horvath IEAA (2.18 years) predictions were highly similar, whereas the differences by the Hannum model (1.98 years) deviated from both the Hannum IEAA (1.12 years) and Hannum EEAA (2.89 years) models. These results may suggest that within-pair variations in predicted blood cell-type proportions did not have a large effect on the DNAmAA predictions of the Horvath model, whereas the Hannum models were affected by differences in blood cell-type proportions within twin pairs.

Next, we examined whether differences in DNAmAA within twin pairs depended on the time to cancer diagnosis by regressing within-pair differences in DNAmAA on the time to cancer diagnosis. According to all DNAmAA models investigated, statistically significant or not, in general, the closer to cancer diagnosis a twin pair was sampled and extending for years after the diagnosis, the higher the difference in DNAmAA within the twin pair was. This suggests that twins in a pair diverged in their DNAmAA toward the cancer diagnosis and afterward, where twins with a cancer diagnosis had higher DNAmAA. Rates for this divergence varied from 0.05 years to 0.17 years (dDNAmAA) per calendar year in this cohort. However, only the Horvath and Horvath IEAA models had statistically significant ($p \leq .05$) p values, with estimates of about two months (0.16 years and 0.14 years, dDNAmAA) per calendar year. The Hannum, Hannum EEAA and GrimAge models had low p values as well ($p \leq .11$), with estimates of one month (0.05 years to 0.11 years, dDNAmAA) per calendar year. Together, this could mean that the divergence of DNAmAA between the twins in a pair is captured differently by the different models. Further, for all DNAmAA models, a rather low fit of the models suggests that there were other factors besides cancer diagnosis that contribute to the observed within-pair differences in DNAmAA (Table 3 and Supplementary Figure S2).

Breast Cancer

As a next step, we analyzed separately, as the largest subgroup, the 24 twin pairs discordant for breast cancer. For the 10 twin pairs with their blood samples collected before breast cancer diagnosis, only the GrimAge model showed within-pair difference, where the twin with cancer is 1.22 years younger than their healthy co-twin ($p = .053$, Table 4). None of the other DNAmAA models showed that the twins in a pair differed for their DNAmAA. For the 14 twin pairs sampled after breast cancer diagnosis, the DNAmAA models predicted the cancer-diagnosed twin to be on average 1.88 years to 5.83 years older than their healthy co-twin (Table 4 and Supplementary Figure S3). The PhenoAge model for breast cancer resulted in the highest within-pair difference in DNAmAA (5.83 years, 95% CI [0.61 years, 11.04 years]), with the difference being much higher than in the pan-cancer analysis (1.66 years, 95% CI [0.69 years, 4.01 years). Thus, the morbidity captured by

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Table 4. Within-pair differences in DNAmAA in breast cancer-discordant twin pairs ($N = 24$). The presented units are in years

	Before the diagnosis ($n = 10$)				After the diagnosis ($n = 14$)				Time to diagnosis ($n = 24$)			
	Mean difference	95% CI	p value[a]	Power	Mean difference	95% CI	p value[a]	Power	Estimate	p value[b]	Power	adR^{2c}
Horvath	0.38	−1.72, 2.49	.69	0.19	3.29	0.64, 5.95	.02	1	0.17	.02	1	0.36
Horvath IEAA	0.54	− 1.25, 2.33	.51	0.34	3.10	0.73, 5.48	.01	1	0.16	.02	1	0.38
Hannum	−1.92	−5.21, 1.36	.22	1	3.18	1.84, 4.52	.0002	1	0.16	.08	1	0.23
Hannum IEAA	−1.66	−3.77, 0.44	.11	1	1.95	0.50, 3.41	.01	1	0.10	.12	1	0.19
Hannum EEAA	−2.35	−7.12, 2.42	.29	1	4.33	2.53, 6.13	.0002	1	0.22	.09	1	0.21
PhenoAge	1.11	−3.72, 5.94	.62	0.88	5.83	0.61, 11.04	.03	1	0.25	.29	0.98	0.07
GrimAge	−1.22	−2.46, 0.02	.053	1	1.88	−0.13, 3.87	.06	1	0.14	.02	1	0.38

Note: [a]t-test p value; [b]F-statistics p value; [c]adjusted coefficient of determination (R^2), proportion of variance in the data explained by the linear model.

Table 5. Within-pair differences in DNAmAA in nonbreast cancer-discordant twin pairs ($N = 71$). The presented units are in years

	Before the diagnosis ($n = 36$)				After the diagnosis ($n = 35$)				Time to diagnosis ($n = 71$)			
	Mean difference	95% CI	p value[a]	Power	Mean difference	95% CI	p value[a]	Power	Estimate	p value[b]	Power	adR^{2c}
Horvath	0.08	−1.65, 1.80]	.93	0.07	2.15	0.44, 3.86	.02	1	0.14	.02	1	0.14
Horvath IEAA	0.33	−1.25, 1.91	.68	0.48	1.81	0.18, 3.43	.03	1	0.13	.01	1	0.17
Hannum	0.23	−1.28, 2.23	.82	0.26	1.50	−0.30, 3.30	.10	1	0.09	.32	1	0.02
Hannum IEAA	0.97	−0.80, 2.74	.27	1	0.78	−0.57, 2.13	.25	1	0.03	.56	1	−0.02
Hannum EEAA	−0.19	−2.69, 2.31	.88	0.20	2.31	−0.24, 4.86	.07	1	0.15	.21	1	0.05
PhenoAge	−0.85	−3.46, 1.76	.51	1	−0.01	−2.51, 2.49	1	0.05	0.08	.43	1	0.002
GrimAge	0.28	−0.92, 1.49	.64	0.38	0.97	0.19, 1.76	.02	1	0.02	.26	1	0.03

Note: [a]t-test p value; [b]F-statistics p value; [c]adjusted coefficient of determination (R^2), proportion of variance in the data explained by the linear model.

the PhenoAge model might be particularly relevant for breast cancer (Table 4 and Supplementary Figure S3).

As in the pan-cancer analysis, the breast cancer analysis also showed similar effects with respect to the Horvath and Hannum models and their adjustments for predicted blood cell-type proportions. The predictions by the Horvath models were highly similar in their effect sizes, while the predictions by the Hannum models were affected by the blood cell-type proportion adjustments (Table 4).

In the regression of within-pair differences in DNAmAA over time to diagnosis, the Horvath, Horvath IEAA and GrimAge models showed a divergence of the twins with the cancer-diagnosed twin on average getting older faster than their healthy co-twin ($p \leq .05$). For these, the rate for this within-pair divergence in DNAmAA in breast cancer was two months (0.14 years to 0.17 years, dDNAmAA) per calendar year toward the diagnosis and afterward. However, the low fits of the regression models indicated the presence of additional unknown factors contributing to the within-pair difference in DNAmAA (Table 4 and Supplementary Figure S4).

Nonbreast Cancers

In the third analysis, we separately analyzed the 71 twin pairs from the cohort discordant for cancers other than breast cancer (nonbreast cancers). Here again, the 36 twin pairs sampled before diagnosis showed no within-pair difference in DNAmAA. For the 35 twin pairs sampled after diagnosis, only the Horvath, Horvath IEAA and GrimAge models showed that the twin diagnosed with cancer is 0.97 years to 2.15 years older than their healthy co-twin. The other models yielded point estimates of the same magnitude with the exception of PhenoAge (−0.01 years; Table 5 and Supplementary Figure S5). However, the observed mean differences were smaller compared to the breast cancer analysis (Table 4). In the case of PhenoAge, the lack of any difference (−0.01 years, 95% CI [−2.51 years, 2.49 years]) contrasts with the large effect size in breast cancer (5.83 years, 95% CI [0.61 years, 11.04 years]).

For the nonbreast cancer analysis, when regressing the within-pair differences in DNAmAA over time to diagnosis, only the Horvath and Horvath IEAA models showed substantive effects between the time to diagnosis and the within-pair difference in DNAmAA. This means that the co-twins diverged over time across the cancer types summarized here, where the twin with the cancer diagnosis aged faster. The rate of within-pair divergence of DNAmAA measured by the Horvath and Horvath IEAA models was two months (0.14 years and 0.13 years, dDNAmAA) per calendar year. While a good fit of the regression models was also not observed in the breast cancer group (Table 4), the fit was even lower in the nonbreast cancer group of discordant pairs, indicating an even greater impact of unknown factors on DNAmAA (Table 5 and Supplementary Figure S6).

Discussion

To our knowledge, this is the first study to report within-pair differences in DNAmAA in twin pairs discordant for cancer. The observed within-pair differences increased the closer the

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sample collection was to the time of cancer diagnosis and afterward, with the cancer-diagnosed twin having aged faster. However, significant and substantial within-pair differences in DNAmAA were observed mainly in twin pairs sampled after cancer diagnosis. Additionally, the observed within-pair differences for breast cancer-discordant twin pairs were notably high.

DNAmAA estimated by GrimAge was lower in the twins diagnosed with breast cancer compared with their healthy co-twins, while for the other DNAmAA estimates and cancer groups (pan-cancer and nonbreast cancer), the twins of pairs sampled before the cancer diagnosis did not differ by their DNAmAA. The within-pair differences, however, increased the closer to cancer diagnosis the pair was sampled, which may relate to the subclinical development of cancer prior to diagnosis. Nevertheless, the current study provided only minimal evidence for substantial within-pair differences in DNAmAA prior to cancer diagnosis. This observation is consistent with previous prospective studies on unrelated individuals with multiple cancer types, including breast cancer (Ambatipudi et al., 2017; Durso et al., 2017; Levine et al., 2015; Zheng et al., 2016), with comparable follow-up time to our cohort (≤20 years vs. ≤17 years) and with varying cohort sizes (43–451 cases and 424–2029 unrelated controls). Our findings of nonsubstantially elevated DNAmAA before diagnosis argue against the suitability of DNAmAA alone as a biomarker of cancer risk. Cancer risk predictors that are built directly on DNAm seem to be more promising cancer biomarkers (Terry et al., 2016). However, when the PhenoAge DNAmAA estimate was integrated into a model along with four other DNAm estimators (including estimates for blood cell types and age acceleration) and 19 breast cancer risk-associated CpGs, the resulting breast cancer risk score, mBCRS, was able to predict breast cancer risk with similar strength to polygenic risk scores (Kresovich et al., 2022). The mBCRS combined with the genetic risk data may enhance breast cancer prediction (Kresovich et al., 2022).

In general, larger within-pair differences in DNAmAA in twin pairs discordant for any cancer were observed in the pairs sampled after diagnosis compared with the pairs sampled before the cancer diagnosis. The observed elevated DNAmAA in the blood of the cancer-diagnosed twins sampled after the diagnosis may be due to the cancer or its treatment. In line with this, recent studies on breast (Sehl et al., 2020) and head and neck cancers (Xiao, Beitler et al., 2021) have shown that DNAmAA in blood increases significantly after cancer treatment. It has been further shown that the survivors of esophageal cancer have blood DNAmAA close to zero, whereas those who died within the 3-year follow-up had highly elevated DNAmAA (Beynon et al., 2022). Additionally, adult survivors of childhood cancers have higher DNAmAA than adults with no childhood cancer diagnosis (Qin et al., 2021). The increased DNAmAA after cancer diagnosis could be a response to treatment-related mutational stress, as many cancer treatments have mutagenic effects (Venkatesan et al., 2017). It has been hypothesized that epigenetic maintenance programs are hyperactivated to ensure genome stability as a reaction to mutational stress; thereby, the increased DNAmAA would mark an accumulated record of the mutational stress (Horvath & Raj, 2018; Levine et al., 2018). This could have led to the increased within-pair differences we observed after the cancer diagnosis. In our study, increased DNAmAA after diagnosis was associated especially with breast cancer. This may be due to hormonal treatment strategies common in breast cancer therapy (Barzaman et al., 2020). The association between female sex hormone exposures and DNAmAA is widely described (M. Chen et al., 2019; Horvath, 2013; Levine et al., 2016; Sehl et al., 2021). Therefore, hormone-targeted treatment of breast cancer may have resulted in the observed high within-pair differences in DNAmAA in the breast cancer-discordant pairs postdiagnosis.

Another cause of increased DNAmAA could be trauma and posttraumatic stress related to cancer diagnosis. Several studies have shown that trauma (Boks et al., 2015; Wolf, Maniates et al., 2018) and posttraumatic stress disorder (Wang et al., 2022; Wolf, Logue et al., 2018; Wolf, Maniates et al., 2018; Yang et al., 2020) are associated with DNAmAA in blood. Importantly for this study, traumatic life events have been shown to be associated with increased risk of breast cancer (Ginsberg et al., 1996; Lin et al., 2013; Santos et al., 2009), including a study from the Finnish Twin Cohort (Lillberg et al., 2003). A traumatic life event, such as the death of a close person or separation/divorce, significantly increases the risk of being diagnosed with breast cancer after such an event. It has been hypothesized that the traumatic event triggers changes in hormone metabolism (Lillberg et al., 2003; Lin et al., 2013; Santos et al., 2009) or the immune system (Lillberg et al., 2003), but a biological mechanism for this observation has not been demonstrated. Similar results have been reported for colorectal cancer (Kune et al., 1991) and for melanoma, lung cancer and hematopoietic malignancies (Levav et al., 2000). Overall, the traumatic event of a cancer diagnosis might increase DNAmAA in the twin diagnosed with cancer, or the twin with a cancer diagnosis might have been more likely to have been exposed to traumatic events prior to diagnosis, resulting in increased DNAmAA. Overall, the pathways from severe traumatic experiences to cancer are unclear and to which extent they involve biological aging processes picked up by epigenetic clocks are not established and outside the scope of the present work.

While the largest within-pair differences in DNAmAA were observed in breast cancer-discordant pairs, the Horvath and Horvath IEAA models captured significant associations between DNAmAA and time to diagnosis across all three cancer groups (pan-cancer, breast cancer and nonbreast cancer). Notably, the Horvath model was constructed using chronological age as a metric for aging, a common risk factor for most cancers. Furthermore, unlike the other models presented here, the Horvath model was developed for the prediction of DNAmAA in multiple tissues, including applicability to cancer tissues (Horvath, 2013). Both features may result in the Horvath models being able to robustly detect an increase in DNAmAA in the blood of individuals diagnosed with cancer. Our study further showed that the Hannum models were affected by estimated white blood cell percentages, as has been described before (Horvath & Raj, 2018). Hematologic changes with age are a well-known phenomenon (Groarke & Young, 2019), and moreover, chemotherapy and radiotherapy frequently lead to hematologic changes (Iorio et al., 2021; Spivak et al., 2009). This would suggest that cancer diagnosis-associated events, most likely the treatment, induce hematologic aging, which was captured by the Hannum models in the current study.

The main strength of the current study is the discordant twin study design. The fact that twins are highly matched on many factors — such as genetics, age, sex, early environment and lifestyle — that influence DNAm and aging leads to the best matched case–control setting available in humans (Ceribelli & Selmi, 2020). For the current study, especially the fact that the twins in a pair are of the same age is an important advantage, which is difficult to set up to this degree in studies investigating sibling

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pairs or unrelated individuals. Although DNAmAA is corrected for chronological age, this correction may not be complete and residual effects of chronological age may still confound the associations. Pairwise comparison of the co-twins sampled at the same time point, and thus at the same age, overcomes this problem.

Our study also has some limitations worth acknowledging. Although our prospective twin cohort covered a period of 48 years around the cancer diagnosis, repeated measurement of DNAm for individual twin pairs was not available. Therefore, we cannot draw any conclusions on individual epigenetic aging trajectories in relation to cancer diagnosis. Such investigations would be highly relevant, since DNAmAA of twins in a pair become less correlated over time (Li et al., 2020), and especially since the DNAmAA rate is not constant over time (Snir et al., 2019). Resources for conducting such longitudinal studies in genetically informative samples such as twin pairs are, unfortunately, rare.

Although to the best of our knowledge this is the largest DNAm study on cancer-discordant twin pairs to date, we only had sufficient power to examine breast cancer as a separate cancer group. Therefore, we may have missed associations between DNAmAA and other cancer types. This study served as a pilot study, and our results on the blood DNAmAA in breast cancer warrant further investigations on the value of DNAmAA as a biomarker for breast cancer risk.

In conclusion, DNAmAA as a proxy for biological aging is associated with cancer diagnosis in a manner dependent on cancer type and time to cancer diagnosis. However, only weak associations of DNAmAA with cancer were found before cancer diagnosis. Therefore, DNAmAA on its own may not serve as a reliable biomarker for individual cancer risk; however, larger sample sizes on different cancer types and longitudinal designs should be included in future studies to draw firm conclusions. Importantly, DNAmAA is increased after cancer diagnosis, potentially resulting from the cancer itself, from potential lifestyle changes and from cancer treatments, which makes DNAmAA a valuable tool to study cancer diagnosis-related aging and morbidity.

Supplementary Material. To view supplementary material for this article, please visit https://doi.org/10.1017/thg.2022.32.

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Conflicts of Interest. None.

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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