Epigenetic age prediction drifts resulting from next-generation methylation arrays

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Abstract

Background

Epigenetic clocks based on DNA methylation data are routinely used to obtain surrogate measures of biological age and estimate epigenetic age acceleration rates. These tools are mathematical models that rely on the methylation state of specific sets of CpG islands quantified using microarrays. The set of CpG islands probed in the microarrays differed between the models. Thus, as new methylation microarrays are developed and older models are discontinued, existing epigenetic clocks might become obsolete. Here, we explored the effects of the changes introduced in the new DNA methylation array from Illumina (EPICv2) on existing epigenetic clocks.

Methods

We compiled a whole-blood DNA methylation dataset of 10835 samples to test the performance of four epigenetic clocks on the probe set of the EPICv2 array. We then used the same data to train a new epigenetic age prediction model compatible across the 450k, EPICv1 and EPICv2 microarrays. We compiled a validation dataset of 2095 samples to compare our model with a state-of-the-art epigenetic clock. We used a dataset of cancer survivors who had undergone different types of therapy and a dataset of breast cancer patients and controls to test the ability of our model to detect alterations in epigenetic age acceleration.

Results

We found that the results of the four epigenetic clocks tested are significantly distorted by the absence of specific probes in the EPICv2 microarray, causing an average drift of up to 25 years. We developed an epigenetic age prediction model compatible with the 450k, EPICv1 and EPICv2 microarrays. Our model produced highly accurate chronological age predictions that were comparable to those of a state-of-the-art epiclock. This finding reproduced previous results showing increased epigenetic age acceleration in cancer patients and in survivors who had been treated with radiation therapy.

Conclusion

Our work demonstrated that existing epigenetic clocks need to be updated to be applicable to data generated with the new EPICv2 microarray, which has phased out the 450k and EPICv1 models. To overcome this technical hurdle, we developed a new model that translates the capabilities of state-of-

the-art epigenetic clocks to the new EPICv2 platform and is cross-compatible with the 450k and EPICv1 microarrays.

Keywords

Epigenetics, epigenetic clock, DNA methylation

Introduction

In the rapidly evolving field of epigenomics, the development of epigenetic clocks has revolutionized our ability to gauge biological aging through DNA methylation patterns. Changes in the methylation state of CpG sites have proven to be highly correlated with chronological age[1,2]. Thus, DNA methylation patterns are being increasingly used to gain insights into aging and associated pathologies[2] and have led to the development of several epigenetic clocks, which are predictive models that estimate the age of subjects based on methylation markers[3]. In these epigenetic clocks, the model prediction is interpreted as the "biological" or "epigenetic" age. Subjects whose biological age is greater than their chronological age are considered to have epigenetic age acceleration[4] (EAA). EAAs have been linked to increased risk for various health issues and mortality, independent of traditional risk factors[5–8]. Epigenetic clocks have thus not only underscored the potential of DNA methylation as a biomarker of aging[9] but also represent a valuable tool to gain insight into the complexities of multiple pathologies[4]. In cancer, EAAs have been linked to an increased risk of developing breast and colon cancer[10,11]. Moreover, breast tissue from breast cancer patients has been shown to exhibit EAA[12]; likewise, cancer survivors do present an accelerated epigenetic age compared to noncancer patients, with an acceleration rate dependent on the treatment intensity received[13].

From the initial model of Bockland *et al*. published in 2011[1], the field quickly evolved toward more robust models, such as the Hannum[14] and the Horvath[15] clocks, and later toward "second generation" approaches, which included features beyond DNA methylation with the promise of providing a "biological age" prediction that could better reflect age-related health status[16–18]. The field continues to evolve, with the implementation of new predictive models[19–21] and the development of species-[22,23] and tissue-specific[24–27] epigenetic clocks. Most recently, Bernabeu *et al*. conducted a large-scale study to refine the predictive ability of both first (methylation-based) and second (including other features) generation epigenetic clocks, which yielded a significant improvement over existing models in the ability to predict chronological age from DNA methylation data across multiple cohorts with subjects of all ages[28].

The evolution of epigenetic clocks has run in parallel with that of methylation microarrays, whose coverage of DNA methylation sites increased from 27578 in 2008[29,30] to 866836 in 2015[31]. In 2023, Illumina released the newest iteration of its series of methylation arrays, EPICv2, which targets 936866 methylation sites[32]. Although the vast majority of probes have been conserved across different microarray models over the years[32–34], some of them have been lost. The probes on the latest (and currently the only commercially available) methylation microarray version cover more than 80% of the probes used by the most popular epigenetic clocks (PhenoAge[16], Horvath (2013)[15], Horvath (2018)[35],

Hannum[14], DunedinPACE[17], etc.), but they do not offer complete coverage for any of them[36]. Given that earlier microarray models are now discontinued while there is still a need to use epigenetic clocks, it is necessary to evaluate whether the existing models can offer reliable results when using EPICv2 data.

In this study, we examined the performance of existing epigenetic clocks using the CpG probes available in the new EPICv2 methylation array in human blood samples. We observed that the biological age predictions of these models were distorted due to missing probes in the EPICv2 array. To overcome this hurdle, we trained a model that can be applied to data obtained with 450k, EPICv1 or EPICv2 chips and whose performance is at least on par with that of state-of-the-art models. Finally, we further validated our model by demonstrating that its predictions reflect the influence of radiation therapy on epigenetic aging and that it attributes significantly increased EAA values to sporadic breast cancer patients with respect to control subjects.

Results

DNA methylation datasets

We explored public repositories to assemble a large and diverse DNA methylation dataset to examine the performance of existing epigenetic clocks and to train and test new models. We used public data to generate two separate datasets: 1) a main dataset for evaluating existing models and training new ones and 2) a validation dataset to test the generalizability of new models.

The main dataset included whole blood DNA methylation data of human subjects from 24 previous studies (Supplementary Table 1). We filtered the data to retain exclusively subjects labeled as controls, with reported age and sex values, resulting in a total of 11825 individuals. To be able to split the data in a stratified fashion into training and test sets, we kept only the samples with an age value present at least twice in the dataset, leaving a total of 10835 subjects (Figure 1A). The subjects in this main dataset had ages between 8 and 96 years, with a mean of 47.68 years and an almost even distribution of sexes (5456 females and 5379 males) (Figure 1B).

The validation dataset was composed of data from 6 additional studies (Supplementary Table 1). We selected only control subjects with reported age and sex values, which resulted in a total of 2095 individuals. The age range in this validation set was between 14 and 94 years, and the sex distribution was slightly biased, with 828 females and 1267 males (Figure 1C-D).

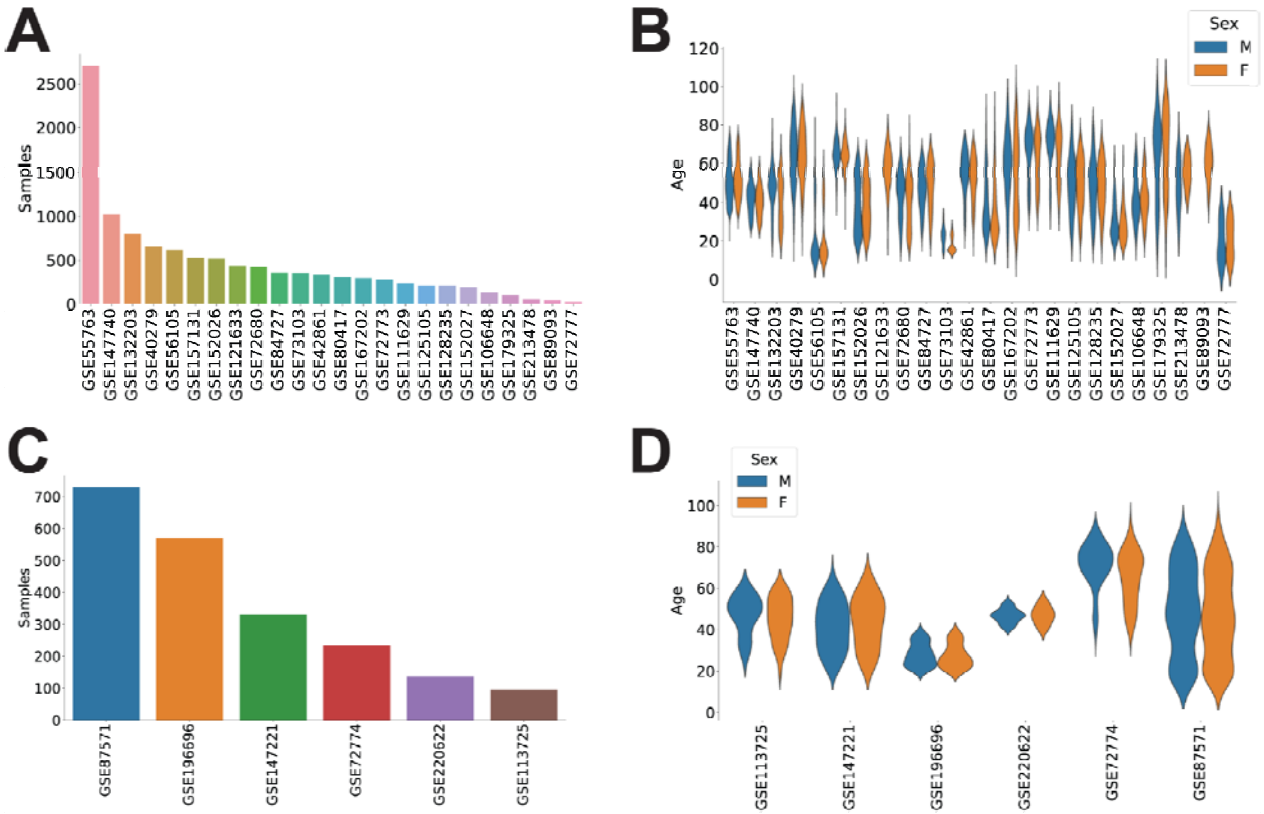


Figure 1 - Public DNA methylation datasets compiled for the study. **A**. Number of subjects present in each of the datasets included in the main set. **B.** Distribution of ages and sex in the datasets included in the main set. **C** and **D**. Distribution of the number of subjects and their age and sex in the datasets used for the validation set.

Existing epigenetic clocks generate distorted results from EPICv2 data

Although more than 80% of the probes used by existing epiclocks are conserved in the EPICv2 methylation array[36], applying these models to data limited to the probes present in the EPICv2 array results in significant differences in epigenetic age predictions. We applied four different models (Horvath[15], Hannum[14], PhenoAge[16] and the recent chronological age prediction model (cAge) from Bernabeu *et al.*[28]) to the main dataset of 10835 individuals, using either all the required probes or only those present in the new EPICv2 array. The proportion of probes relevant for these models in the EPICv2 ranged from 84.5% (Hannum) to 97.1% (cAge).

We observed a high degree of correlation between chronological age and the predicted biological age in all cases, with cAge obtaining the highest value (r=0.979). After the models were restricted to the probes available in the EPICv2 chip, they maintained this correlation, which was still strong (>0.88) in all cases (Figure 2A).

However, limiting the number of probes distorted the epigenetic age predictions in all the models, causing a drift. In all four epiclocks, we observed a significant difference ($p<0.001$) in the biological age predicted using all the CpG probes compared to the predictions made using only probes from the EPICv2

array (Figure 2B). As expected, this effect was greater for the models with fewer parameters: the average drift in the Hannum clock, which uses only 71 parameters, was 25.45 years on average. The larger models PhenoAge (513 parameters) and cAge (4058 parameters) had average drifts of -1.11 and -1.67 years, respectively.

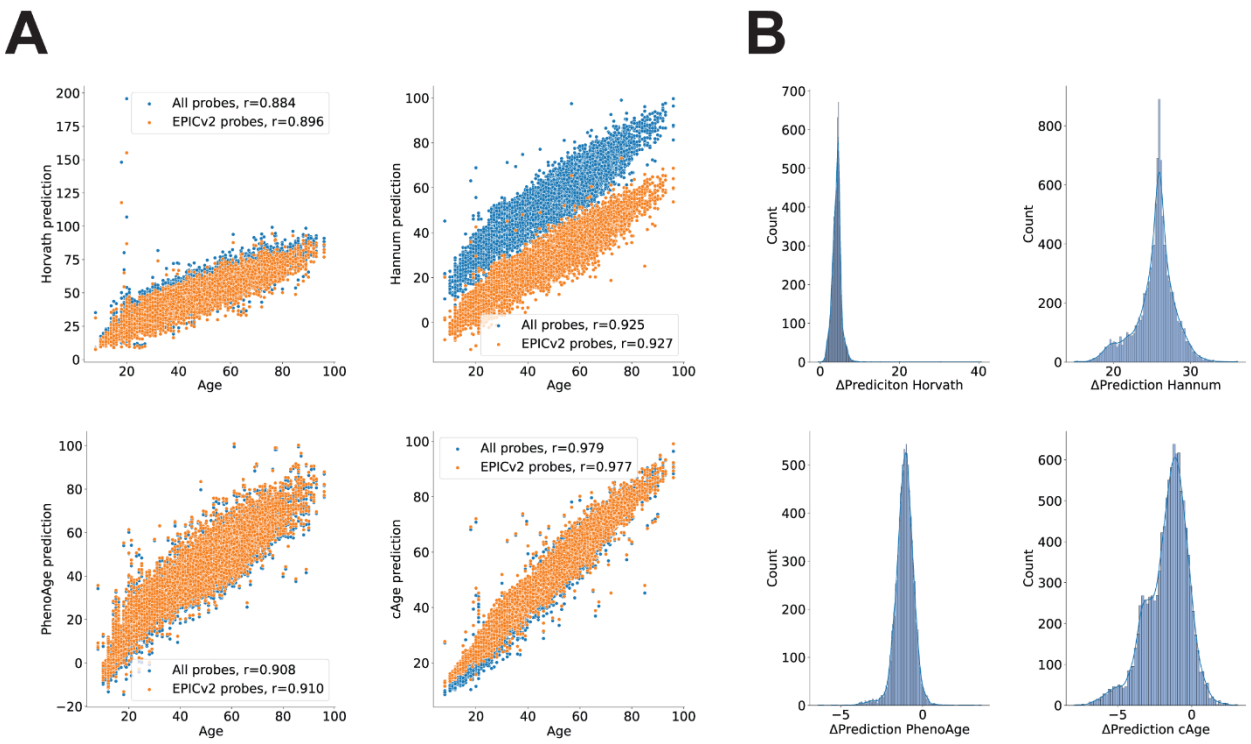


Figure 2 – Epigenetic clock age prediction drifts caused by the loss of probes in the EPICv2 microarray. **A**. Epigenetic age predictions by four existing epigenetic clocks in 10835 subjects using either their complete sets of CpG probes (blue) or only those available in the EPICv2 array (orange). **B**. Distribution of differences between the values predicted using the full sets of probes or only those present in the EPICv2 array (drift) for the same four epigenetic clocks.

A new epigenetic clock compatible with the EPICv2 array

The changes in the CpG probes present in the EPICv2 array produce significant alterations in the results of existing epigenetic clocks. To overcome this limitation and to produce robust biological age predictions using EPICv2 data, we trained a new epiclock model using CpG probes common to the 450k, EPICv1 and EPICv2 methylation arrays. Based on the epigenome-wide association study (EWAS) from Bernabeu *et al.*[28], we found 42728 probes with a significant linear association with chronological age that were present in the three different methylation arrays. Similarly, there were 63324 CpG probes with significant quadratic associations with age according to the EWAS were present in the three microarray models (Figure 3A).

Following the strategy employed by Bernabeu *et al.*[28], we ranked the probes by their p-value on the EWAS and preselected the first 10000 with linear and the first 300 with quadratic associations to chronological age to train an epiclock using elastic net regression[37] (Supplementary Table 2).

We split the main dataset with 10835 subjects into training and test sets using a 70/30 split, resulting in 7259 and 3576 samples, respectively, and trained a general age-prediction model. The model used an L1 ratio of 0.5[28], and the alpha value was optimized to 0.001 through 5-fold cross-validation. This general model obtained a low prediction error (mean squared error (MSE)=3.14 years) and a high correlation between the predicted and chronological ages (r=0.982) in the test set (Figure 3B).

Previous works have suggested that the relationship between methylation state and age is nonlinear[14,28,38–41]. Therefore, we decided to stratify our training data into three age groups and train separate predictors for "young", "middle-aged", and "old" subjects. The predictors were intended to work on nonoverlapping age groups, but we did use overlapping age ranges during training to limit the inconsistencies between models assigned to contiguous groups. We produced a combined model in which the general model assigned a sample to one of the three age groups, and then the corresponding age-stratified model generated the age prediction (Figure 3C). This combined model reduced the age prediction error significantly with respect to the general model (Figure 3D), lowering the MSE to 3.06 years in the test set and increasing the correlation between the predicted and chronological ages to 0.983. Although the combined model reduced the error in all age groups, the difference was statistically significant only in the younger subjects (Figure 3E). Weights for the general and age-specific models are provided in Supplementary Table 3.

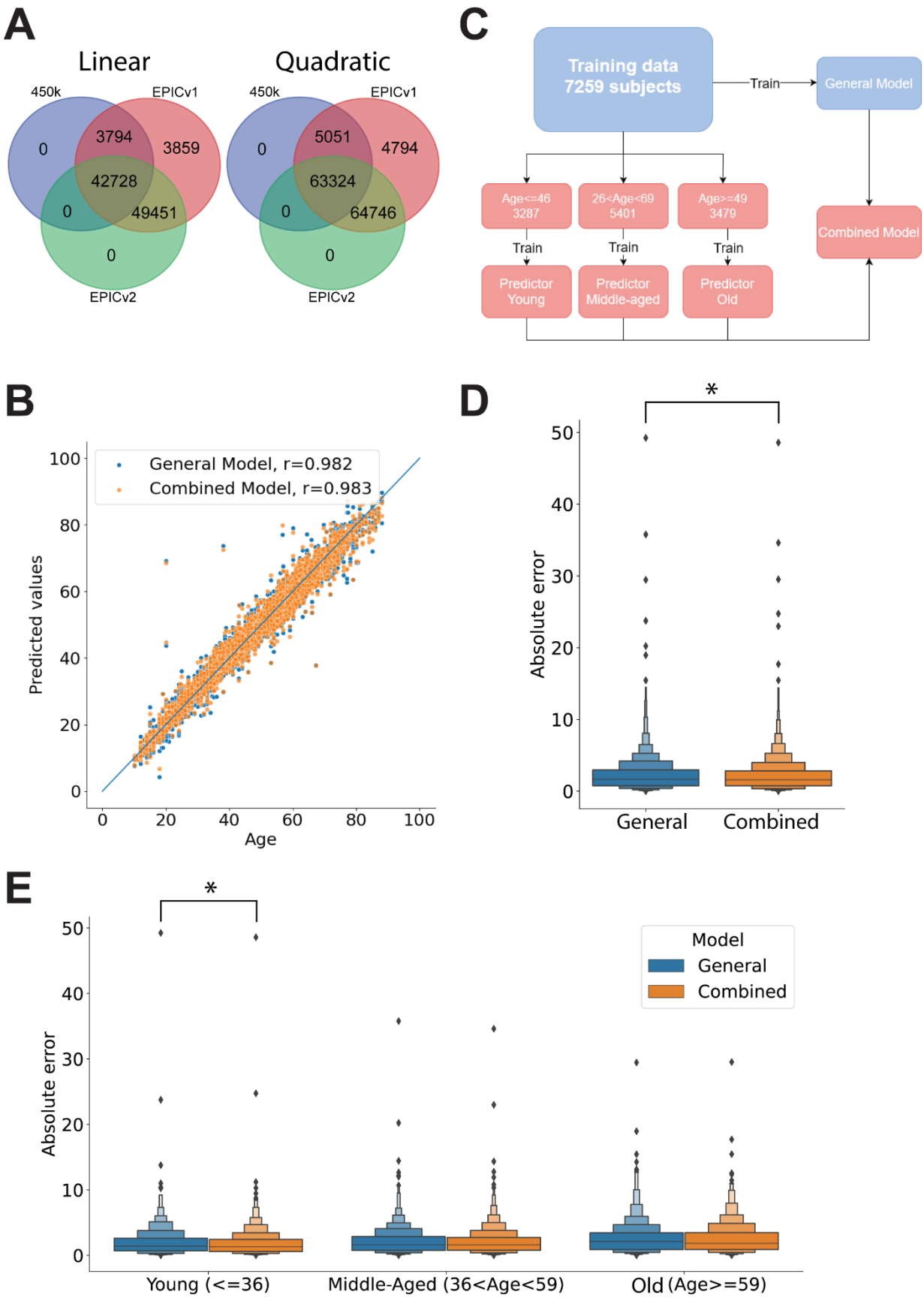

A
Linear
450k
EPICv1
EPICv2
0
3794
3859
42728
0
49451
0
Quadratic
450k
EPICv1
EPICv2
0
5051
4794
63324
0
64746
0
C
Training data
7259 subjects
Train
General Model
Age<=46
3287
26<Age<69
5401
Age>=49
3479
Train
Train
Train
Predictor
Young
Predictor
Middle-aged
Predictor
Old
Combined Model
B
General Model, r=0.982
Combined Model, r=0.983
Predicted values
Age
D
*
Absolute error
General
Combined
E
*
Absolute error
Model
General
Combined
Young (<=36)
Middle-Aged (36<Age<59)
Old (Age>=59)

Figure 3 – Results of the new epigenetic clocks in the test dataset. A. Methylation probes with significant linear (left) and quadratic (right) associations with chronological age in the EWAS from Bernabeu *et al.* present in the 450k, EPICv1 and EPICv2 arrays. **B**. Prediction results on the test set by the General and Combined models. The blue line indicates the 1:1 correspondence. **C**. Schematic overview of the training of the different age prediction models. **D**. Absolute error of the ages predicted in the test set by the general and combined models. **F**. Absolute error from the predictions on the test set by each model, broken down by age group.

The performance of the new clock is on par with that of a state-of-the-art model

To examine the performance of our model, we compared it against cAge, a refined model that has shown significant improvement over previous epigenetic clocks[28]. We tested cAge and our general and combined models on a validation dataset with 2095 samples from 6 studies. We included all the probes used for cAge and those required by our models and predicted the age of each sample based on the methylation data. In all cases, the predicted ages were strongly correlated with the chronological ages (Figure 4A).

Our general model performed as well as cAge, with no significant difference in the distribution of errors between the two, whereas the error from the predictions of our combined (age-segregated) model was significantly lower (Figure 4B). By breaking down the data by age group, we could observe differences in the prediction error. In the young (<=36) and old (>=59) groups, the error from the combined model was significantly lower than that of the cAge and the general models. In the group of middle-aged subjects, the error of the general model was significantly greater than that of the other two models (Figure 4C).

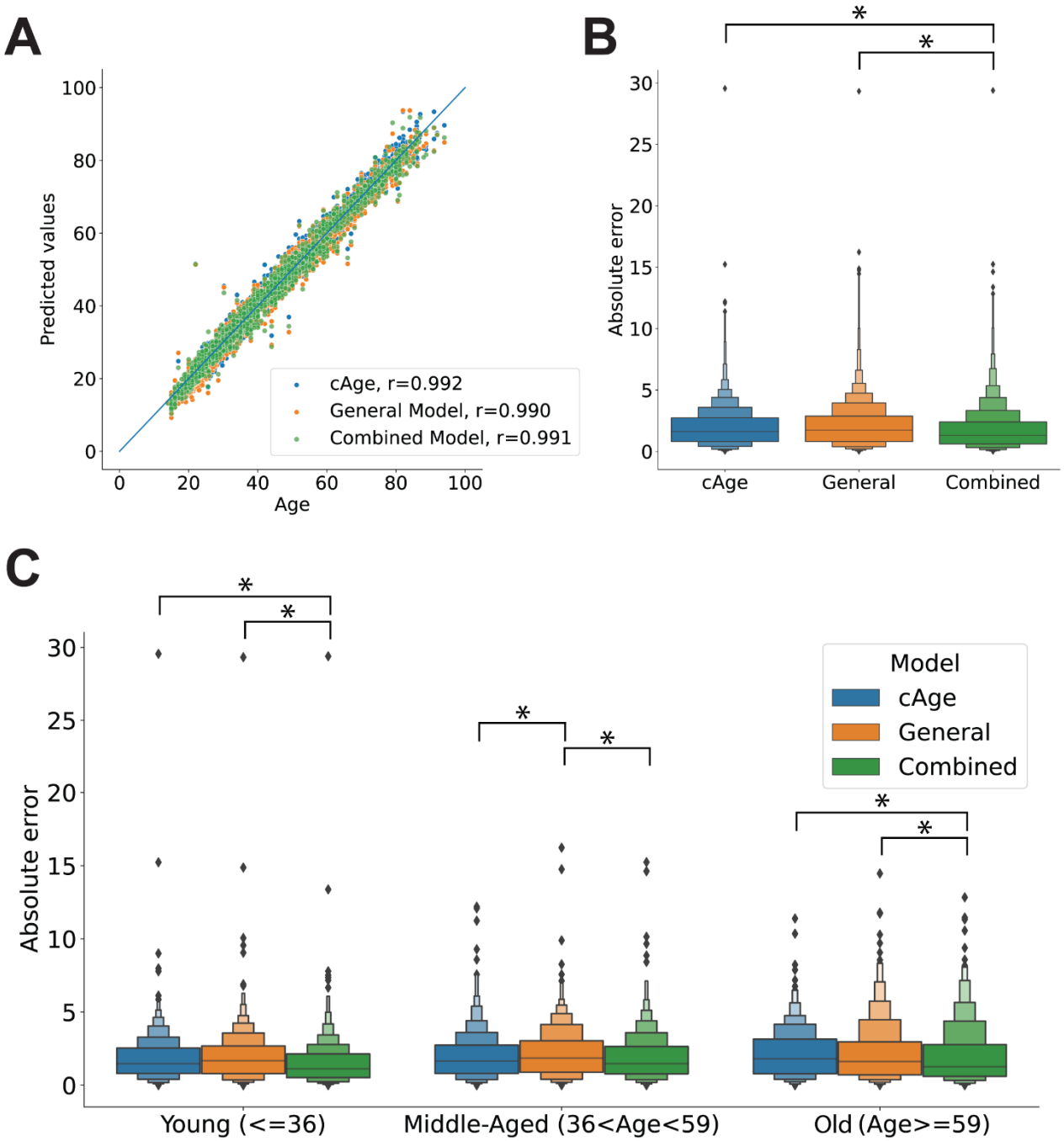


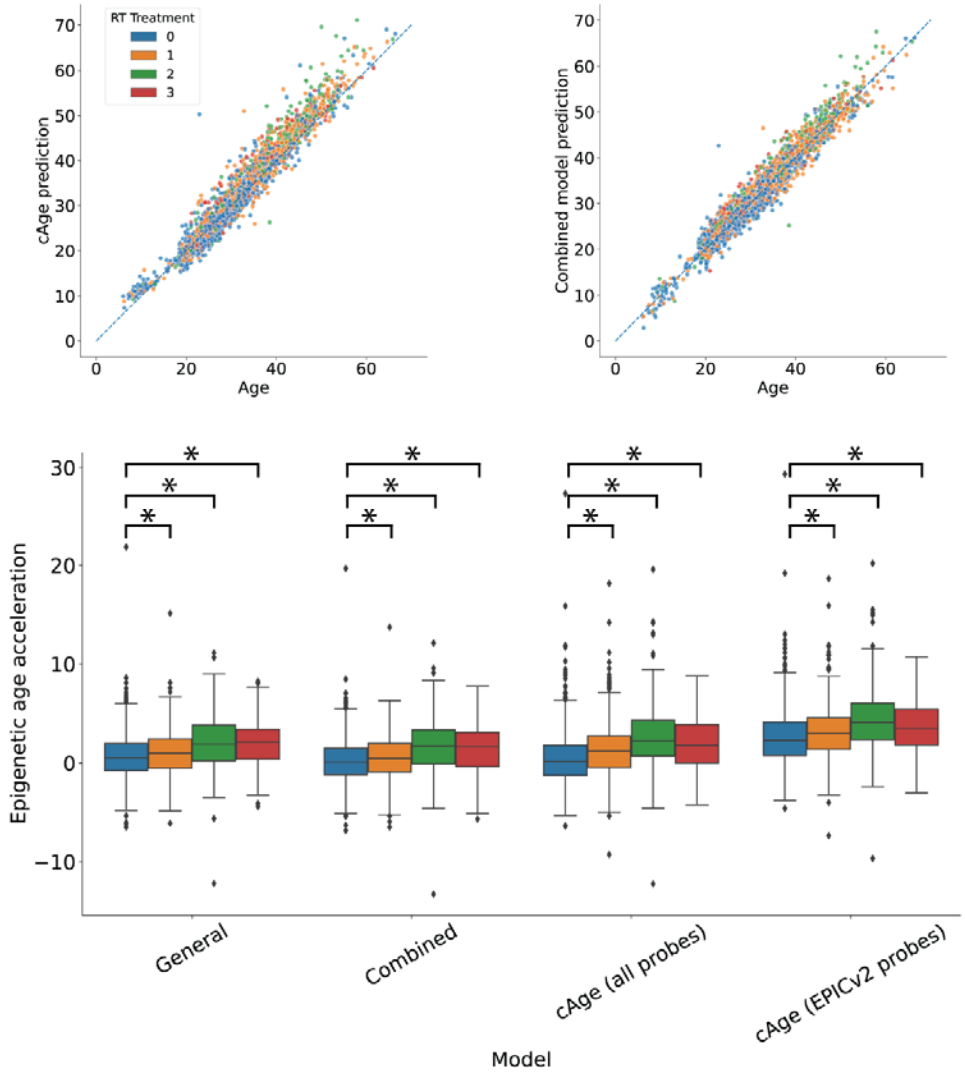
Figure 4 – **Age predictions on the validation set**. **A**. Chronological age and age values predicted by cAge and the general and combined models in the validation set. **B**. Distribution of the absolute prediction error for each of the three models. **C**. Distribution of the absolute error of each model in the validation set broken down by age group.

The new epigenetic clock reflects the influence of radiation therapy and breast cancer

Several studies have employed different epigenetic clocks to assess the impact of pathologies and environmental factors on epigenetic age acceleration (EAA)[42]. Using PhenoAge[16], Qin *et al*. demonstrated that exposure to different anticancer treatments had a significant influence on the EAA of childhood cancer survivors[43]. To validate the performance of our model, we tested it in public datasets that have already shown increased EAA in cancer patients. Using data made public in a subsequent study by Dong *et al*.[44] (GEO accession number GSE197674) which contained data from 2138 childhood cancer survivors, we studied the influence of radiation therapy (RT) on the EAA determined by cAge and our models. In this dataset, all the models produced epigenetic age predictions highly correlated with chronological age, with r values above 0.9 in all cases (Figure 5A). When comparing subjects who had received RT in one or more body areas (chest, abdomen or pelvis, brain) to those who had not been exposed to RT, all models revealed that the latter group had a significantly lower EAA (Figure 5A). This is in line with the results reported in the original study[43], and similar observations have been made in other studies in which radiation exposure was associated with an increased EAA[45,46].

Next, we leveraged a dataset with DNA methylation data from peripheral blood leukocytes of sporadic breast cancer patients and control subjects[47] (GEO accession number GSE148663) to study the effect of the disease on the EAA determined by the different models. We computed the predicted epigenetic age for 22 sporadic breast cancer patients and 10 controls using our models and cAge. Although the correlation between the epigenetic age predictions and the chronological ages of the subjects remained high (0.914-0.951), our models predicted a significantly greater EAA in the cancer group (Figure 5B). As reported in the original study[47], the cancer patients had no previous cancer history and were sampled at the time of diagnosis, so the difference in EAA cannot be attributed to anticancer therapy but rather to the disease itself.

A


RT Treatment
0
1
2
3
cAge prediction
Age
Combined model prediction
Age
Epigenetic age acceleration
General
Combined
cAge (all probes)
cAge (EPICv2 probes)
Model

B

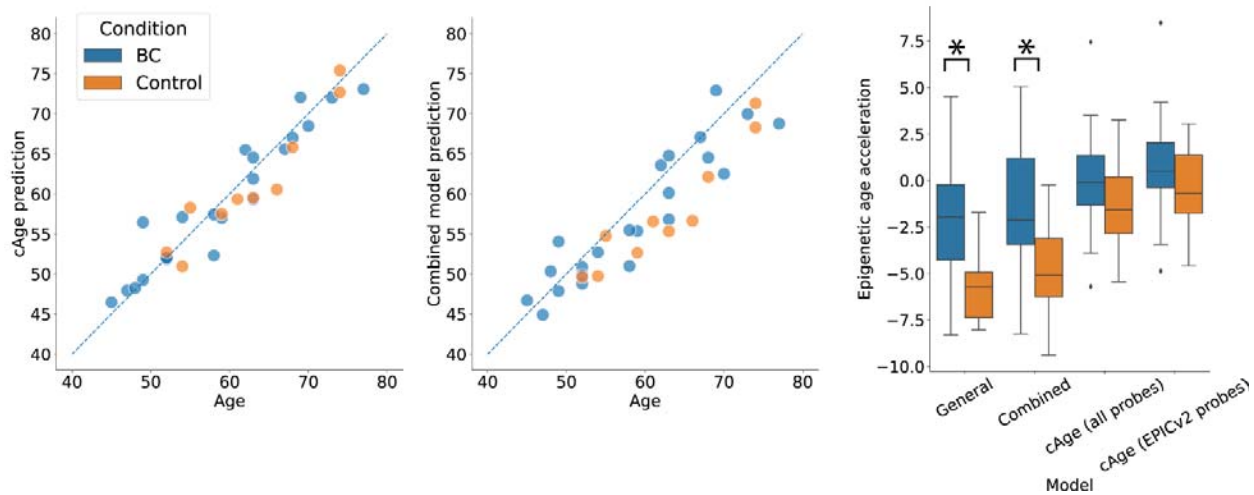

Condition
BC
Control
cAge prediction
Age
Combined model prediction
Age
Epigenetic age acceleration
General
Combined
cAge (all probes)
cAge (EPICv2 probes)
Model

Figure 5 - Effects of radiation therapy and spontaneous breast cancer in EAA. **A.** Chronological age and predicted age for childhood cancer survivors, colored by the number of body areas (brain, chest, abdomen/pelvis) in which they received radiation therapy (top). Distribution of the EAA for each group of subjects determined by each of the models (bottom). **B.** Chronological ages of spontaneous breast cancer patients (BC) and control subjects and the corresponding epigenetic ages determined by the combined model and cAge (left). Distribution of the EAA estimated for each group by the different models (right).

Discussion

Epigenetic clocks are valuable tools for research on aging and pathological states. In this work, we evaluated the applicability of existing epigenetic clocks to the data generated by EPICv2, the newest methylation microarray model from Illumina. The EPICv2 will phase out previous microarray models (namely, 450k and EPIC), which were used for the development of some widely used epigenetic clocks (e.g., Hannum, Horvath). As the EPICv2 discontinued the use of some of the probes used by existing epiclocks[36], we felt urged to test whether this would affect the performance of the models.

We generated a large DNA methylation dataset by compiling data from whole blood samples obtained in 24 different studies. This dataset allowed us to quantify the effect of running 4 different epiclocks (Hannum[14], Horvath[15], PhenoAge[16] and the cAge model from Bernabeu *et al.*[28]) restricted to the set of methylation probes present in the EPICv2 methylation array. Our observations indicate that the results of these 4 models are significantly altered by probes absent from the EPICv2 array.

Epigenetic age models are routinely applied in epigenetic research and are also exploited commercially[48–50]. In the face of commercial discontinuation of the 450k and EPICv1 arrays, our findings suggest that both researchers and commercial vendors alike will need to update their epigenetic clock models to make them compatible with EPICv2 data. Future studies and commercial solutions using this new microarray will require readjusted or new epigenetic age models, which are currently lacking.

As our results show, *none* of the epiclocks tested work as intended on data generated by the EPICv2 microarrays, showing significant drifts between the epigenetic ages predicted using data from different chip models. Therefore, we sought to produce a model compatible across the 450k, EPICv1 and EPICv2 microarray platforms, aiming to obtain results in accordance with those of existing methods rather than outperforming them. The approach that we used closely followed the one applied by Bernabeu *et al.* in the development of their cAge model[28], so that we would rely on established methodologies. Our results on the training and test datasets indicate that our epiclock offered very high performance in the prediction of ages from DNA methylation values. Similar to Bernabeu's model, these results highlight the benefit of using feature preselection, nonlinear terms, and age-based stratification. We followed a simple strategy regarding these aspects, as we relied on a prior EWAS study[28] to preselect features, we considered only quadratic features, and we stratified our subjects into three arbitrary age groups. We are confident that follow-up work can improve the results presented here by, for example, conducting more thorough feature selection, deriving more features from the original beta values and/or exploring different data stratification strategies.

Despite its simplicity, our implementation outperformed cAge in the validation dataset, where it obtained significantly lower prediction errors. These results demonstrate that our model can be generalizable (as it is applicable to new data) and that its performance is on par with that of state-of-the-art models.

To demonstrate the ability of our model to detect EAA alterations, we first applied it to methylation data from cancer survivors[43]. The results of our epiclock successfully reflected the increased EAA induced by radiation therapy, a known phenomenon that has been observed in multiple studies[45,46]. Next, we applied our model to data obtained from breast cancer patients. Our epiclock revealed a significant increase in the EAA of cancer patients compared to that of control subjects, suggesting that the epigenetic age predicted by the model is sensitive to pathological states. Notably, the cAge model did not detect this difference and did not reveal a significant difference in the EAA between the two groups.

As a relevant limitation, we would like to highlight that our model is limited to methylation data obtained from whole blood samples, a constraint that is also shared by multiple other models[28,51,52]. Its applicability to data obtained from other tissues has not been assessed. Likewise, its ability to reflect pathological states beyond the cases we have shown (radiation therapy, breast cancer) remains to be explored.

Taken together, our results demonstrate that our epigenetic clock is compatible with data generated using the 450k, EPICv1 and EPICv2 microarray platforms. Its epigenetic age predictions are highly correlated with chronological age in control subjects of all ages. The model assigns increased EAA values to subjects exposed to radiation therapy and to breast cancer patients, agreeing with previous findings and mimicking the ability of existing epiclocks to reflect pathological states in epigenetic age predictions. Our work solves a technical barrier derived from technological development that has not yet been addressed and has important implications both for aging research and for biotechnology companies offering services in this field.

Methods

Data collection and preprocessing

Methylation data from previous studies were collected from the GEO database[53,54]. In all cases, the data were preprocessed: we used beta values provided by the original authors when available; otherwise, we computed the beta value from the methylated and unmethylated signals using the following formula:

$$\beta = \frac{M}{M+U+100},$$

where M and U are the intensities of the methylated and unmethylated signals respectively. The main dataset consisted of 11825 subjects with reported methylation, age and sex data. We discarded 147 with nonnumerical age values, one with a reported age of 891 years, and one with a negative epigenetic age according to the cAge model. To generate training and test sets with even age distributions, we kept only samples with an age value present at least twice in the dataset. This process left a total of 10835 samples on the main dataset, which were split into training and test sets with 7259 and 3576 subjects, respectively.

On the main dataset, we computed the average beta value of each probe across the whole dataset (Supplementary Table 4) and used this average to impute missing values. Among the probes used to train our model (see below) or for running other epigenetic clocks, we found 1.58% missing values. The number of probes missing per dataset is provided in Supplementary Table 5.

The validation dataset included data from 2098 subjects. Excluding subjects with missing or invalid sex and/or age annotations reduced the data to 2095 subjects. A total of 0.23% of the beta values were missing for the set of probes used to run our epiclcok and the cAge models. The missing values were imputed using the average values derived from the main dataset. Supplementary Table 5 reports the number of probes missing per dataset.

The cancer survivor dataset (GSE197674, 2138 subjects) and the data from the breast cancer study (GSE148663, 32 subjects) were also retrieved from the GEO database[53,54]. We used the average beta values derived from the main datasets to impute beta values for 25 missing probes in the cancer survivor dataset.

Epigenetic age prediction using existing models

We applied the Horvath[15], Hannum[14], PhenoAge[16] and cAge[28] epiclocks to the methylation data in the main dataset by using the methods and parameters reported by the authors for each case. In the case of the Horvath clock, this implies computing an initial value using a linear model and then applying additional transformations for the "young" (<=20) and "adult" (>20) subjects[15]. Similarly, the cAge model uses one model to predict *age* and another to predict log(*age*); if the *age* prediction is <=20, then it is replaced by the exponential of the log(*age*) prediction[28].

Training of the new epigenetic clock

We trained a general epigenetic age prediction model using the beta values of 10,000 probes and the squared beta values of 300 probes to perform a regularized linear regression of chronological age using elastic net[37]. The linear and quadratic features were chosen based on an EWAS study[28]: we chose the 10,000 probes with a significant linear association with age with the lowest association p-value and the 300 probes with a significant quadratic association with age with the lowest association p-value.

To train our general epigenetic age prediction model, we separated the main dataset into training and test sets using a 70/30 split stratified by age (i.e., all ages present in the dataset were sampled in both the training and test sets). An L1 ratio of 0.5 was chosen based on its performance in previous studies[15,55,] and the alpha value was optimized to 0.001 through 5-fold cross-validation.

Age-specific models were trained using the same approach on 3 different batches of data with subjects of specific ages: age<=46 (young), 26<age<69 (middle-aged), and age>=59 (old). The alpha value was set to 0.001 in all cases. The three age-specific models were then used to perform predictions on nonoverlapping *putative* age (age values assigned by the general model) groups: age<=36 (young), 36<age<59 (middle-aged), and age>=59 (old).

List of abbreviations

Epigenetic age acceleration (EAA)

Chronological age prediction model (cAge)

Epigenome-wide association study (EWAS)

Mean squared error (MSE)

Radiation therapy (RT)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in the GEO repository. All accession numbers are reported in Supplementary Table 1.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

L.G. and M.Q. designed the study. L.G. generated and analyzed the data. All authors read and approved the final manuscript.

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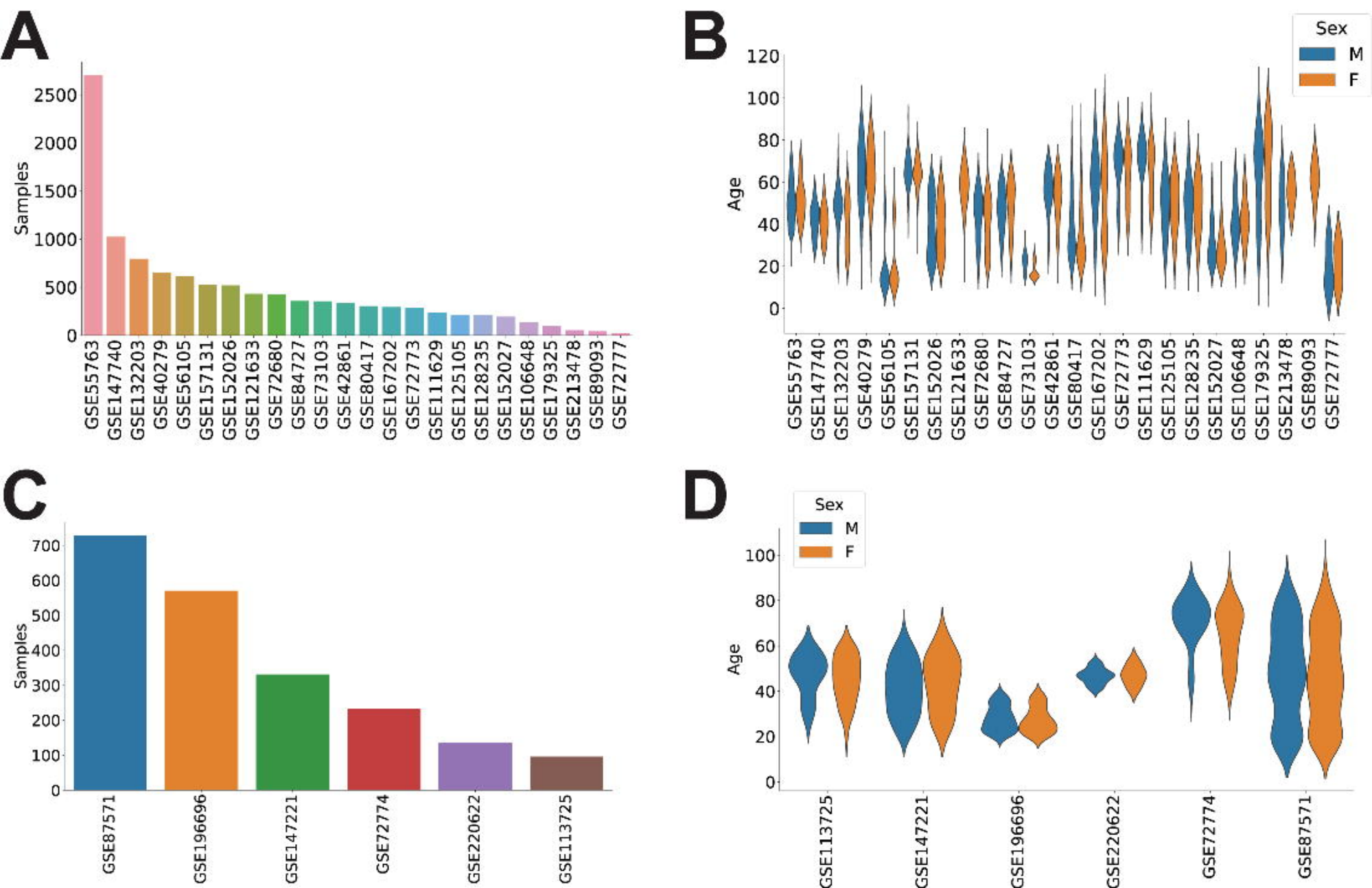
A
Samples
0
500
1000
1500
2000
2500
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GSE132203
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GSE56105
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GSE152026
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GSE72680
GSE84727
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GSE111629
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GSE128235
GSE152027
GSE106648
GSE179325
GSE213478
GSE89093
GSE72777
B
Sex
M
F
Age
0
20
40
60
80
100
120
GSE55763
GSE147740
GSE132203
GSE40279
GSE56105
GSE157131
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GSE72777
C
Samples
0
100
200
300
400
500
600
700
GSE87571
GSE196696
GSE147221
GSE72774
GSE220622
GSE113725
D
Sex
M
F
Age
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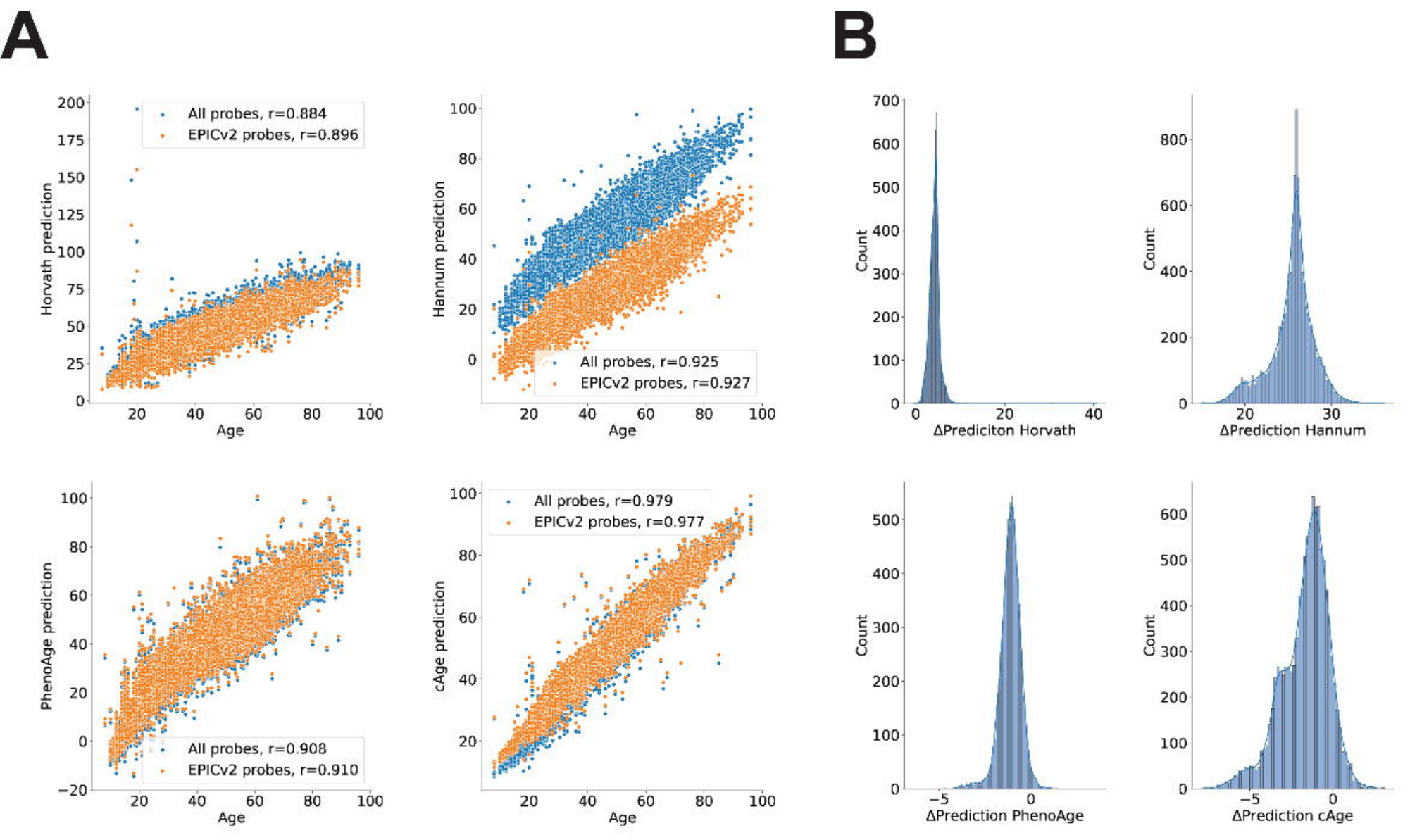
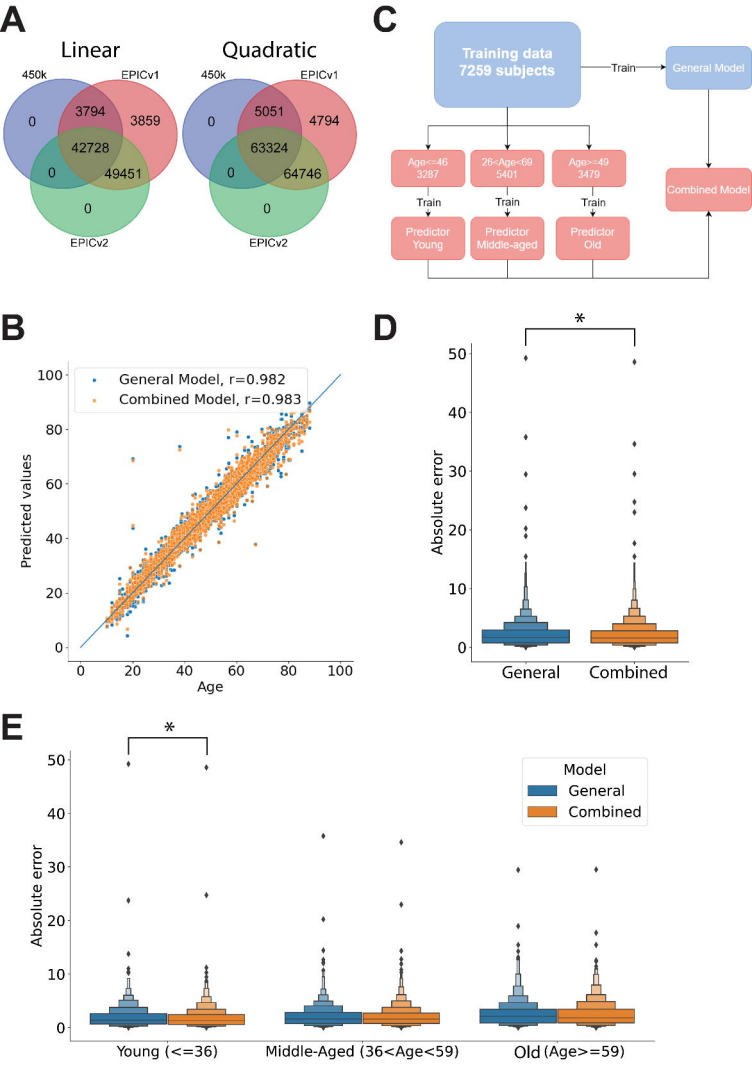
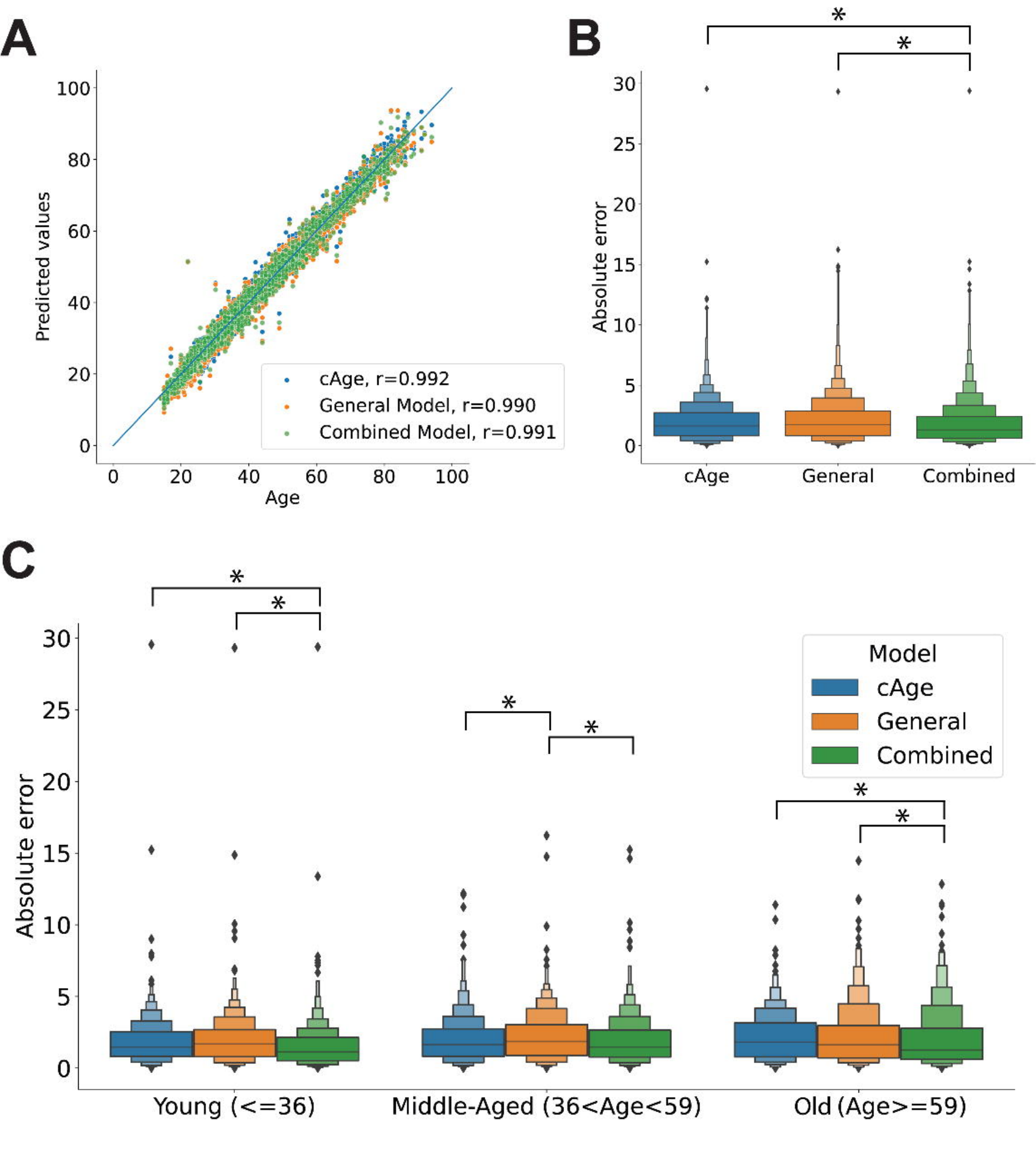
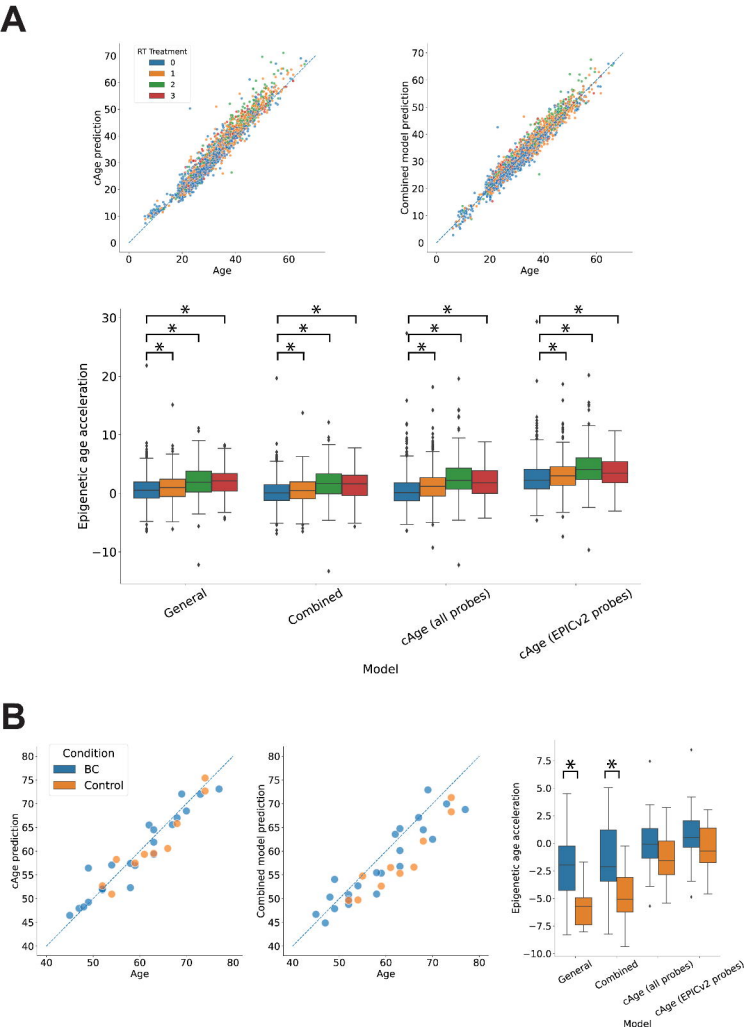

A
B
All probes, r=0.884
EPICv2 probes, r=0.896
Horvath prediction
Age
All probes, r=0.925
EPICv2 probes, r=0.927
Hannum prediction
Age
All probes, r=0.908
EPICv2 probes, r=0.910
PhenoAge prediction
Age
All probes, r=0.979
EPICv2 probes, r=0.977
cAge prediction
Age
Count
ΔPrediciton Horvath
Count
ΔPrediction Hannum
Count
ΔPrediction PhenoAge
Count
ΔPrediction cAge


A
Linear
450k
EPICv1
EPICv2
0
3794
3859
42728
0
49451
0
Quadratic
450k
EPICv1
EPICv2
0
5051
4794
63324
0
64746
0
C
Training data
7259 subjects
Train
General Model
Age<=46
3287
26<Age<69
5401
Age>=49
3479
Train
Train
Train
Predictor
Young
Predictor
Middle-aged
Predictor
Old
Combined Model
B
General Model, r=0.982
Combined Model, r=0.983
Predicted values
Age
D
*
Absolute error
General
Combined
E
*
Absolute error
Model
General
Combined
Young (<=36)
Middle-Aged (36<Age<59)
Old (Age>=59)


A
Predicted values
Age
cAge, r=0.992
General Model, r=0.990
Combined Model, r=0.991
B
Absolute error
cAge
General
Combined
C
Absolute error
Model
cAge
General
Combined
Young (<=36)
Middle-Aged (36<Age<59)
Old (Age>=59)


A
RT Treatment
0
1
2
3
cAge prediction
Age
Combined model prediction
Age
Epigenetic age acceleration
General
Combined
cAge (all probes)
cAge (EPICv2 probes)
Model
B
Condition
BC
Control
cAge prediction
Age
Combined model prediction
Age
Epigenetic age acceleration
General
Combined
cAge (all probes)
cAge (EPICv2 probes)
Model