CONCEPT PAPER

Provisional Paper Title:	DNA Methylation Analysis of Age Appearance Rated from Facial Photographs
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Objective of the study:

(NOTE: all data to be analyzed by Grey House team at Duke U)

Aging is associated with DNA methylation (DNAm) changes, which can be used to estimate chronological age. Various epigenetic signatures – alternatively called "epigenetic clocks" – have been described (Hannum et al., 2013; Horvath, 2013; Koch and Wagner, 2011; Weidner et al., 2014). There is evidence that the deviation of predicted age and chronological age (delta age) is indicative for parameters of biological aging (Marioni et al., 2015a; Marioni et al., 2015b). For example accelerated epigenetic age is associated higher all-cause mortality in later life (Chen et al., 2016; Lin et al., 2016; Marioni et al., 2015a). For the Dunedin cohort, it has recently been observed that epigenetic age-predictions are modestly correlated (r=0.12, P=0.001) with perceived age based on facial photographs (bioRxiv 2016, doi:http://dx.doi.org/10.1101/071373). Facial aging – as a surrogate for biological aging (Gunn et al., 2013) – might be used to identify an epigenetic photo-age-signature that is independent from chronological age.

In this study, we want to further analyze photo-age-associated DNAm patterns using two complementary cohorts:

1) The Lothian Birth Cohort 1921 (LBC1921) (Deary et al., 2012) with facial age appearance determined in about 230 patients at an age of 83 years.
2) The Dunedin Cohort with facial age appearance determined at an age of 38 years.

The combination of these two cohorts provides possibilities for validation and it may elucidate the relevance of age appearance in middle-aged donors as compared to elderly donors.

Our study has the following specific objectives:

1) To further determine if delta age based on epigenetic age-predictors is related to age-appearance in facial photographs in LBC1921 (as compared to Dunedin cohort).
2) To identify CpGs that correlate with photo age estimations and to determine whether these CpGs are also associated with chronological age.
3) To establish photo-aging methylation signatures that can be used to estimate photo-age.
4) To determine if the photo-aging signatures are indicative for life expectancy or biological aging.

Data analysis methods:

1) **Epigenetic aging and photo-age**. The epigenetic age of the LBC1921 and Dunedin cohorts will be estimated by different predictors (Horvath, Hannum et al., and Weidner et al.,) to determine the difference between predicted and chronological age (delta age). Subsequently, we will analyze how delta age is related to facial photo age estimations in the LBC1921 and compare associations with associations observed in the Dunedin cohort (Belsky et al. BioRxiv, Under Review).

   Pilot Data: The 71-CpG Clock developed by Hannum et al. is associated with photo-age in the Dunedin cohort (r=0.12, Belsky et al. BioRxiv)

2) **Correlation Analysis.** Compute Spearman correlations between each probe and facial aging. We will compare correlations between CpG methylation and photo-age in LBC and Dunedin cohorts with correlations between CpG methylation and chronological age in reference datasets that are publicly available (Lin et al., 2016). Furthermore, we will perform enrichment studies (CpG islands, gene regions, TF binding sites, chromosomal locations etc.) for CpGs that correlate with photo-age. We expect to find similarities with DNAm changes that correlate with chronological age.

   Pilot Data: In LBC1921 age-associated CpGs and photo-age-associated CpGs revealed overall only very moderate correlation, whereas some CpGs seemed to be coherently modified. Comparison with results from the younger Dunedin cohort will provide new data to address the question of whether the same CpGs are associated with photo-age in younger and older adults.

3) **Prediction model development and testing**. LASSO regression models will be fitted to the Dunedin epigenetic data to develop a predictive model of facial aging for testing in LBC1921. In addition, predictive models developed in LBC1921 will be tested in the Dunedin data. (for a explanation of LASSO models, see http://statweb.stanford.edu/~tibs/lasso/simple.html). Models will include covariates for sex, white blood cell counts, technical variation, and smoking history.

   Pilot Data: In analysis of LBC1921 cohort, LASSO predictive model based on n=150 participants predicted photo-age in a hold out sample of n=80 (r=0.40).

4) **Follow-up of prediction model.** If the epigenetic prediction model for photo age trained in the Dunedin sample is predictive of photo age in the LBC1921 sample, we will conduct a follow-up analysis. We will test if Dunedin Study based epigenetic predictions of photo-age are (a) associated with lifespan, and (b) associated with risk factors for early onset mortality (childhood social class and childhood IQ) in LBC1921.

Variables Needed at Which Ages:

NOTE: All Dunedin data will be analyzed at Duke University

Facial Age at age 38
Pack Years at age 38
White blood cell counts at age 38
Sex

Genome-wide methylation data at age 38 years

(NOTE: all data to be analyzed by Grey House team at Duke U)

Significance of the Study (for theory, research methods or clinical practice):

The primary significance of the study will be to test if there are replicable epigenetic signatures of facial photo age and if these epigenetic signatures are associated with chronological, epigenetic, and biological aging.

References cited:

Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Horvath S, Levine ME, Prinz J, Schaefer J, Sugden K, Williams B, Poulton R, Caspi A. Telomere, epigenetic clock, and biomarker-composite quantifications of biological aging: Do they measure the same thing? Posted to BioRxiv August 25, 2016. doi: http://dx.doi.org/10.1101/071373

Chen, B.H., Marioni, R.E., Colicino, E., Peters, M.J., Ward-Caviness, C.K., Tsai, P.C., Roetker, N.S., Just, A.C., Demerath, E.W., Guan, W.*, et al.* (2016). DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging (Albany NY) *8*, 1844-1865.

Deary, I.J., Gow, A.J., Pattie, A., and Starr, J.M. (2012). Cohort profile: the Lothian Birth Cohorts of 1921 and 1936. Int J Epidemiol *41*, 1576-1584.

Gunn, D.A., de Craen, A.J., Dick, J.L., Tomlin, C.C., van Heemst, D., Catt, S.D., Griffiths, T., Ogden, S., Maier, A.B., Murray, P.G.*, et al.* (2013). Facial appearance reflects human familial longevity and cardiovascular disease risk in healthy individuals. J Gerontol A Biol Sci Med Sci *68*, 145-152.

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., Klotzle, B., Bibikova, M., Fan, J.B., Gao, Y.*, et al.* (2013). Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. Mol Cell *49*, 459-367.

Horvath, S. (2013). DNA methylation age of human tissues and cell types. Genome Biol *14*, R115.

Koch, C.M., and Wagner, W. (2011). Epigenetic-aging-signature to determine age in different tissues. Aging (Albany NY) *3*, 1018-1027.

Lin, Q., Weidner, C.I., Costa, I.G., Marioni, R.E., Ferreira, M.R., Deary, I.J., and Wagner, W. (2016). DNA methylation levels at individual age-associated CpG sites can be indicative for life expectancy. Aging (Albany NY) *8*, 394-401.

Marioni, R.E., Shah, S., McRae, A.F., Chen, B.H., Colicino, E., Harris, S.E., Gibson, J., Henders, A.K., Redmond, P., Cox, S.R.*, et al.* (2015a). DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol *16*, 25.

Marioni, R.E., Shah, S., McRae, A.F., Ritchie, S.J., Muniz-Terrera, G., Harris, S.E., Gibson, J., Redmond, P., Cox, S.R., Pattie, A.*, et al.* (2015b). The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. Int J Epidemiol *44*, 1388-1396.

Weidner, C.I., Lin, Q., Koch, C.M., Eisele, L., Beier, F., Ziegler, P., Bauerschlag, D.O., Jockel, K.H., Erbel, R., Muhleisen, T.W.*, et al.* (2014). Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol *15*, R24.

Data Security Agreement

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Proposing Author	Riccardo Marioni, Daniel W Belsky, Wolfgang Wagner
Today's Date	Jan 27, 2017

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__X__ My project is covered by Duke or Otago IRB OR I have /will obtain IRB approval from my home institution.

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Some journals are now requesting the data file as part of the manuscript submission process. The Dunedin Study cannot be shared because the Study Members have not given informed consent for unrestricted open access. Speak to Terrie or Avshalom for strategies for dealing with data sharing requests from Journals.

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Proposing Author	Riccardo Marioni, Daniel W Belsky, Wolfgang Wagner
Other Contributors	David Corcoran, Joseph Prinz, Karen Sugden, Avshalom Caspi, Terrie Moffitt, Richie Poulton
Today's Date	Jan 27, 2017
Intended Submission Date	August 1, 2017

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☐ Approved ☐ Not Approved ☐ Let's discuss, I have concerns

Comments:

Please check your contribution(s) for authorship:

☐Conceptualizing and designing the longitudinal study

☐Conceptualizing and collecting one or more variables

☐Data collection

☐Conceptualizing and designing this specific paper project

☐Statistical analyses

☐Writing

☐Reviewing manuscript drafts

☐Final approval before submission for publication

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