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Peripheral blood DNA methylation-based machine learning models for prediction of knee osteoarthritis progression: biospecimens and data from the Osteoarthritis Initiative and Johnston County Osteoarthritis Project

Christopher M. Dunn, MS[1,2], **Cassandra Sturdy, BS**[2], **Cassandra Velasco, BS**[1,2], **Leoni Schlupp, BS**[2], **Emmaline Prinz, BS**[2], **Vladislav Izda, BS**[3], **Liubov Arbeeva, MS**[4], **Yvonne M. Golightly, PT, PhD**[4,5], **Amanda E. Nelson, MD, MSCR**[4], **Matlock A. Jeffries, MD**[1,2]

[1.]University of Oklahoma Health Sciences Center, Department of Internal Medicine, Division of Rheumatology, Immunology, and Allergy, Oklahoma City, OK

[2.]Oklahoma Medical Research Foundation, Arthritis and Clinical Immunology Program, Oklahoma City, OK

[3.]Icahn School of Medicine at Mount Sinai, New York, NY

[4.]University of North Carolina at Chapel Hill, Thurston Arthritis Research Center, Chapel Hill, NC

[5.]University of Nebraska Medical Center, College of Allied Health Professions, Omaha, NE

Abstract

Objective: The lack of accurate biomarkers to predict knee osteoarthritis (OA) progression is a key unmet need in the OA field. The objective of this study was to develop baseline peripheral blood epigenetic biomarker models to predict knee OA progression.

Methods: Genome-wide buffy coat DNA methylation patterns from the Osteoarthritis Biomarkers Consortium (OABC, n=554) were determined using Illumina MethylationEPIC arrays. Data were divided into development and validation sets and machine learning models were trained to classify future knee pain, radiographic, dual (pain + radiographic), and any (pain, radiographic, or dual) progression. Parsimonious models, using the top 13 CpGs most frequently selected during development, were tested on independent samples from participants in the Johnston County Osteoarthritis Project (JoCoOA, n=141) and a previously published Osteoarthritis Initiative dataset (OAI, n=54).

Results: Full models accurately classified future radiographic (accuracy 87±0.8%, AUC=0.94±0.004, mean±SEM), pain (89±0.9%, 0.97±0.004), dual (72±0.7%, 0.79±0.006), and any progression (78±0.4%, 0.86±0.004). Pain-only and radiographic-only progressors were not distinguishable (accuracy 58±1%, AUC=0.62±0.001). Parsimonious models showed similar performance and accurately classified future radiographic progressors in OABC and in both

Contact information for correspondence: Matlock A. Jeffries, Oklahoma Medical Research Foundation, 825 NE 13th Street, Laboratory MC400, Oklahoma City, OK 73104, Ph. (405) 271-7438, matlock-jeffries@omrf.org.

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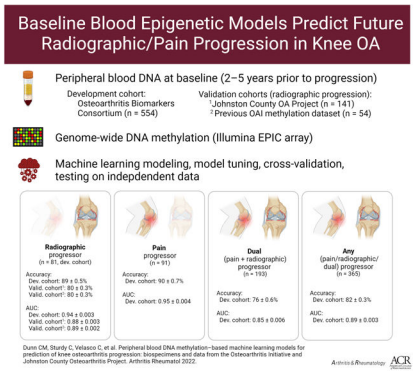
validation cohorts (JoCoOA: accuracy 80±0.3%, AUC=0.88±0.003, OAI: accuracy 80±0.8%, AUC=0.89±0.002).

Conclusions: Herein, we developed peripheral blood-based DNA methylation models to predict knee OA progression in the OABC cohort and validated our findings in two independent cohorts. Our data suggest that pain and structural progression share similar early systemic immune epigenotypes. Further work should focus on evaluating the pathophysiological consequences of differential DNA methylation and peripheral blood cell epigenotypes in individuals with knee OA.

Graphical Abstract



Introduction:

Osteoarthritis (OA) is the leading cause of chronic disability in the United States and among the most rapidly rising disability-associated medical conditions worldwide (1,2). Despite its widespread impact, neither the US Food and Drug Administration nor the European Medicines agency have yet approved any disease-modifying drug therapy for OA, although several agents are in late-stage clinical trials (3,4). One of the largest obstacles encountered in OA clinical trial design is the unpredictable nature of disease progression. Only a small minority of patients (~4–8%) will experience radiographic progression within 4 years (5,6), the median length of Phase 3 clinical trials in the US (7). Therefore, enriching OA clinical trial populations in individuals likely to experience rapid radiographic and/or pain progression will be critical to accelerate OA drug development and advance personalized, precision-guided OA therapies.

A number of recent studies have sought to identify biomarkers for prediction of rapid OA progression. The largest of these, the Foundation for the National Institutes of Health OA Biomarkers Consortium (OABC), performed an analysis of serum- and urine-protein and radiographic biomarkers using a nested case-control design in a subset of 600 patients from the Osteoarthritis Initiative cohort (8–12). Using multivariable analysis, Kraus and colleagues developed a model predictive of both pain and radiographic progression using baseline blood and serum markers with a receiver operator characteristic area under the curve (AUC) of 0.586 (8). Using a 24-month time-integrated concentration measure, based on the change in biomarker levels over 24 months to predict the subsequent 24 months, models improved to AUC=0.618 using 10-fold cross-validation. Other recent studies have also identified baseline radiographic characteristics that can predict future rapid radiographic

progression, including periarticular bone area (10), and 24-month change in effusion- and Hoffa-synovitis, meniscal morphometry, and cartilage thickness/surface area (AUC=0.740) (11). Additionally, a multivariable modeling analysis in the OABC has been performed, combining both radiographic and biochemical time-integrated concentration biomarkers that yielded an AUC of 0.712–0.832 to predict future radiographic progression (13).

A drawback of these biochemical and radiographic biomarker approaches is their inherent variability; that is, the best predictive capability comes from models integrating biomarker change over time rather than baseline values. By contrast, epigenetic patterns are among the first biological changes in disease pathogenesis, as an appropriately modified chromatin microenvironment is required prior to the expression of disease-associated gene transcripts. Additionally, epigenetic patterns are relatively stable over time (14), suggesting that single-timepoint (baseline) epigenetic assays may reflect disease-associated changes earlier than traditional protein-based approaches. Several studies, including our own, have demonstrated epigenetic changes in OA joint tissues (15–19). Furthermore, in 2019 our group published a pilot study where we developed peripheral blood DNA methylation-based models to predict future radiographic progression in a small cohort of participants (n=116) from the Osteoarthritis Initiative, yielding a mean accuracy of 73% and AUC=0.81 for prediction of progression within 48 months based on a single-timepoint baseline blood draw (20). In the present study, we sought to expand upon these data by developing and validating peripheral blood DNA methylation-based machine learning models to predict future radiographic and/or pain progression in the larger OABC cohort, allowing us to directly compare our outcomes with previous Foundation for the NIH biomarker projects. Additionally, we sought to validate our results in two independent cohorts, the Johnston County Osteoarthritis Project (JoCoOA) and our previous Osteoarthritis Initiative epigenetic biomarker data.

Methods:

Study Design, DNA methylation quantitation

Samples for initial model development were obtained from the OABC (21), a subset of the Osteoarthritis Initiative, including radiographic-only, pain-only, and ‘dual’ pain+radiographic progressors, with matched non-progressor controls. Although the OABC consists of a total of 600 patients, 46 samples were removed from our final analysis due to low DNA concentration or low-quality DNA methylation data. Our final discovery cohort consisted of 554 individuals, representing 193 dual (pain + radiographic), 91 pain-only, 81 radiographic-only progressors and 189 non-progressor controls. The validation cohorts consisted of 79 future radiographic-only progressors and 61 non-progressors from JoCoOA and 27 future radiographic-only progressors and 28 non-progressors from our previously published Osteoarthritis Initiative (OAI) study (20). There were no case or control overlaps between the OABC development cohort and the OAI validation cohort. Details of both studies have been previously published (8,21,22). All Osteoarthritis Initiative and JoCoOA participants provided written informed consent and the studies were approved by the Committee on Human Research of the Institutional Review Board (IRB) for the University of California San Francisco and the University of North Carolina at Chapel

Hill, respectively. The IRBs of the University of Oklahoma Health Sciences Center and Oklahoma Medical Research Foundation also reviewed and approved the present study.

In the OAI, participants had baseline and yearly follow-up knee radiographs and baseline buffy coat DNA available. Kellgren-Lawrence Grade (KLG) and quantitative joint space width (JSW) (23) were assessed by a central reading site using non-fluoroscopic fixed-flexion knee radiographs with a Synaflexer positioning device (Synarc, Newark, CA). In OABC and OAI patients, all buffy coat samples were derived from the baseline visit blood draw. Demographic variables including age, sex, body mass index (BMI), baseline KLG, baseline JSW, baseline Western Ontario and McMasters pain index (WOMAC pain), non-steroidal anti-inflammatory drug use, opiate pain medication use, and White, African-American, Asian-American, and Hispanic ancestry were obtained and compared among progressor groups.

JoCoOA samples were obtained from patients in whom radiographic progression occurred at both the initial and later timepoints (e.g. progression from baseline to second (T1 to T2), second to third (T2 to T3), or third to fourth (T3 to T4) visits). In each case, buffy coat samples were obtained from the visit immediately prior to radiographic progression, equating to 5.6±1.1 years, mean±S.D., prior to progression. In JoCoOA, fixed-flexion knee radiographs with a Synaflexer positioning device were graded by KLG. All participants in all cohorts had a baseline KLG of 2–3 in at least one knee without a history of previous total knee joint replacement. Demographic variables including age, sex, BMI, baseline KLG, baseline WOMAC pain, NSAID use, pain medication use, and Caucasian or African-American ancestry were obtained and compared between the two progressor groups.

DNA used in this study were derived from buffy coat. In OABC development and OAI validation samples, DNA was previously extracted by OAI personnel, stored within the OAI biobank, and shipped to our laboratory for further analysis. In the JoCoOA cohort, buffy coat was shipped to our laboratory and DNA was extracted using a Qiagen DNEasy kit (Qiagen, Hilden, Germany). In all cases, 500ng of DNA was treated with sodium bisulfite (EZ DNA methylation kit, Zymo) and loaded onto Illumina Infinium EPIC 850k Methylation arrays. Arrays were imaged by the Clinical Genomics Center at OMRF.

Progression definitions

In the OABC discovery cohort, the definitions of radiographic and pain progression were the same as a previous biochemical biomarker study in these same patients (8). Radiographic progressors were defined as a longitudinal loss in the minimum joint space width (JSW) of at least 0.7mm from baseline to 48-month follow-up in one index knee. In each case, the contralateral (non-index) knee had less or no progression over the follow-up period. Participants with a tibial plateau rim distance of 6.5mm at baseline, or with a change in the rim distance of >2.0mm between baseline and follow-up were excluded due to inappropriate and/or unreliable radiographic positioning. Non-progressors were defined as those with ≤0.5mm of JSW loss from baseline to 48 months in both knees. Pain progressors were defined by an increase of ≥9 points at 2 or more timepoints on the Western Ontario McMasters (WOMAC) pain subscale (normalized to a 0–100 scale) from the 24-month to 60-month pain assessment. In the JoCoOA validation cohort, radiographic progression

was defined as an increase of ≥1 Kellegren-Lawrence radiographic grade in the index knee between 2 visits, nonprogressors had no evidence for progression at any study timepoint. In the OAI validation cohort, radiographic progression was defined similar to the OABC, except that 0.7mm JSW loss had to occur faster, within the first 24 months, and remain narrowed at the 48-month follow-up visit (20).

Data preprocessing

Statistical analysis was performed using R (v. 4.0.2). Raw .IDAT files were loaded and processed using the *ChAMP* package (v.3.14). Raw array data were first loaded and CpG site methylation data converted to beta values (0–1 methylation value estimate representing the ratio of methylated to unmethylated probe intensities at a given CpG site). Normalization was then performed on beta values using the *champ.norm* function with default options using a beta-mixture quantile normalization procedure. From an initial set of 865,918 probes, the following were excluded: (1) probes with detection $P \geq 0.01$, probes targeting non-CpG positions, probes located on sex chromosomes, and probes with known single nucleotide polymorphisms within 5bp of the 3' end of the CpG probe with minor allele frequency ≥1% (24) (N=158,841), leaving 707,077 CpG probes available for further analysis. Data were batch corrected for methylation plate and array.

Mixed peripheral blood DNA methylation analyses can be confounded by changes in cellular composition among study groups, as immune cell subsets have distinct epigenetic signatures (25). Therefore, to exclude composition as a confounder in our modeling, we estimated cell composition using the *estimateCellCounts* function of *minfi* R package (26), (v.3.15). In the development cohort, B cells were lower in radiographic-only progressors vs. non-progressors (4.7±2.6%, mean±S.D. vs. 6.0±3.3%, $P=0.002$) (Supplementary Table 1). There were no differences in estimated cell composition in either validation cohort (Supplementary Tables 2 and 3). Models were generated both with and without correction of cell composition using surrogate variable analysis (27) via the *sva* package (v.3.28.0). This method has been previously shown to robustly correct for cell count variation and other batch effects in large-scale epigenomic studies (27–29). As outlined below, models demonstrated no differences after *sva* adjustment, suggesting no significant skewing of our findings by differences in cellular composition (Supplementary Table 4).

Model development

Data were first split into 70% development and 30% lockbox validation subsets and modeling performed on the development set (Figure 1). We first tested three methods for model development and feature selection. Elastic-net regularized generalized logistic models were developed using the *glmnet* package (v. 2.0–16) with feature selection. Models were tuned via 10-fold internal cross-validation. Further cross-validation was performed by looping random data splitting and model development for a total of 40 cycles. Models were then applied to the lockbox set and performance characteristics, including area under the receiver operator characteristic curve (AUC), diagnostic odds ratio, accuracy, sensitivity, specificity, and F_1 score (the weighted average of precision and recall) were recorded. DNA methylation sites (features) selected for inclusion in each model were recorded and compared.

Feature reduction and parsimonious model development

Our modeling approach used *glmnet* with an elastic net approach, which combines Ridge and LASSO regression to perform both feature selection and regularization. The resulting models included only those CpG sites important to discrimination of progressors vs. nonprogressors, pruning unnecessary features (Supplementary Table 5). The performance characteristics of development models (based on training, rather than test data) are presented in Supplementary Table 6. Models developed to discriminate 'any' progressors (dual + pain-only + radiographic-only) vs. non-progressors using the entire DNA methylation dataset selected a mean of 14±2 CpG sites (features, mean±SEM) during development, with 13 CpG features being chosen in at least 10 of 40 development rounds. Accordingly, we then reduced our dataset to include only these 13 features and derived new parsimonious models. Surprisingly, we were also able to derive parsimonious models for the remaining comparisons (dual-, pain-, and radiographic-progressors vs. non-progressors) based on these same 13 CpG sites; parsimonious models developed based on the top-most-frequently selected CpGs in each comparison were not superior to this core list of 13 CpGs. These newly derived parsimonious models were then tested on JoCoOA and OAI confirmation cohort datasets and performance tabulated.

Results

Baseline patient demographic, disease, and buffy coat composition characteristics were well matched. Models developed using only patient characteristics or cellular composition performed poorly.

Demographic and baseline clinical characteristics were generally well matched among various groups in both the development and confirmation cohorts (Table 1). Models developed using only baseline radiographic/pain data, age, sex, ethnicity, BMI, and baseline medication use performed poorly (accuracy 62–66%, AUC=0.49–0.68, Supplementary Table 4). DNA methylation data from mixed buffy coat samples may be skewed by variations in cellular composition between groups. As direct measures of cellular composition were not collected in any of the cohorts studied, we estimated these values computationally (25). We noted a decrease in B cells in radiographic-only progressors compared to non-progressors within the OABC (P=0.002, Supplementary Table 1) but no group differences were seen in the JoCoOA nor the Osteoarthritis Initiative confirmation cohorts (Supplementary Tables 2 and 3). Similar to demographic and clinical characteristics, models developed using cell composition data alone were unable to differentiate groups (Supplementary Table 4), suggesting no significant skewing of results owing to buffy coat composition heterogeneity. Methylation-based models developed after adjusting for demographic, clinical (including baseline pain/radiographic data and medication use), and cell composition data performed identically to models without covariate adjustment (detailed below).

Models developed using baseline peripheral blood DNA methylation data robustly discriminate future pain and radiographic progressors from non-progressors within the OABC.

First, we generated logistic regression models to discriminate various progressor groups from non-progressors. Radiographic-only vs. non-progressor models performed robustly

(accuracy 87±0.8%, mean±SEM, AUC=0.94±0.004, models tested on unseen lockbox data) (Table 2, Figure 2) and included 17±2 features (CpG sites, mean±SEM). Pain-only vs. non-progressors models also performed well (accuracy 89±0.9%, AUC=0.97±0.004, 13±2 CpG sites). Dual progressor vs. non-progressors models did not perform as well (accuracy 72±0.7%, AUC=0.79±0.006, 7±2 CpG sites). Models discriminating any progressor (pain, radiographic, or dual) from non-progressors performed moderately (accuracy 78±0.4%, AUC=0.86±0.004, 14±2 CpG sites). We were not able to distinguish pain-only from radiographic-only progressors (accuracy 58±1%, AUC=0.62±0.001); however, dual progressors were readily distinguishable from radiographic-only (accuracy 76±0.6%, AUC=0.82±0.007) and pain-only (accuracy 71±0.6%, AUC=0.79±0.007) progressors. We then added baseline demographic and clinical data, including age, sex, BMI, baseline joint space width, baseline pain (WOMAC), NSAID use, pain medication use, and/or estimated buffy coat cell composition to our dataset and re-developed multivariable models. None of these additional factors were chosen by *glmnet* for inclusion in models, indicating that DNA methylation data are stronger predictors for OA outcomes than these factors. Intriguingly, including baseline proteomic (serum and urine) biomarker data from previous biochemical biomarker studies on these same patients (8,13) also did not change modeling outcomes (data not shown). In a previous study, we noted improved performance in models when DNA methylation data were log-transformed to M values compared to untransformed beta values (20). However, in the present study we found no such differences (pain, radiographic, or pain+radiographic progressors vs. non-progressors model accuracy comparison, P=0.16, trend towards lower error rate in beta value-based models); therefore, beta value-based models were used for all analyses.

Parsimonious models retain the same discriminatory capabilities as models trained on the entire methylation dataset.

Next, we reduced our training dataset to the top 13 CpG sites most frequently included during initial model development (i.e., in ≥10 of 40 development rounds, Table 3, Supplementary Table 5) in ‘any progressor’ (pain, radiographic, and dual) models. We then re-derived parsimonious models for dual-, pain-, and radiographic-progressors using this reduced dataset (Table 2, Figure 2A). These parsimonious models demonstrated modest improvements in predictive capability compared to full models (accuracies: any progressor 82±0.3%, pain-only progressor 90±0.7%, radiographic-only progressor 89±0.5%, dual progressor 76±0.6%).

Parsimonious models trained on OABC data accurately classify future radiographic progressors in JoCoOA and OAI validation cohorts

Finally, we sought to validate our models on two independent datasets. First, we applied these models to buffy coat DNA methylation data from the JoCoOA cohort. Of note, both the definition of radiographic progression (≥1 radiographic Kellegren-Lawrence grade worsening at follow-up) and time to progression (mean 5.6 years) were different in this cohort than the model development cohort. We also validated these models in our previously published OAI methylation dataset (20). For the present analysis, we restricted our previous dataset to those samples run on MethylationEPIC arrays, as the previous generation 450k arrays did not include the necessary 13 CpG sites. Like the JoCoOA

cohort, the OAI cohort had slightly different progressor definitions compared to our model development samples (progression required at 24 months, maintained at 48 months) (Table 1, Figure 2A,B, Figure 3). Models demonstrated similar performance when tested on both validation cohorts (JoCoOA: accuracy 80±0.3%, AUC=0.88±0.003, OAI: accuracy 80±0.8%, AUC=0.89±0.002).

Discussion

The aim of this study was to evaluate whether peripheral blood-based epigenetic biomarkers could be used to differentiate future rapid radiographic and/or pain OA progressors from non-progressors using a single-timepoint baseline sample. To do this, we first determined genome-wide DNA methylation patterns using Illumina MethylationEPIC arrays in 554 patients from the OABC cohort of the OAI, consisting of patients who experienced radiographic and/or pain progression within 48 months of blood draw, and matched non-progressor controls. We then applied machine learning techniques to develop models to differentiate pain, radiographic, dual (pain + radiographic), and any (pain, radiographic, pain + radiographic) progressors from non-progressors. We used a generalized logistic modeling approach with feature reduction which allowed us to determine the subset of DNA methylation sites most predictive of progression status. All models were trained on a 70% development data split and tested on a 30% validation data split, and random development/validation data splits followed by model development were performed in a looped fashion over the course of 40 development cycles (Figure 1). Then, we re-derived models based on a reduced dataset incorporating only the 13 CpG methylation sites most frequently selected during initial development. These parsimonious models were then tested on two validation cohorts: JoCoOA and a previously generated methylation dataset from the OAI.

Predictions based on clinical characteristics alone were not accurate. Including additional baseline serum and urine biomarker data from the OABC study to our models did not improve discrimination capability. We found parsimonious models to be roughly equivalent in predicting pain and/or radiographic progression within OABC data. Furthermore, the radiographic-prediction subset of these parsimonious models remained accurate when applied to the independent JoCoOA and OAI validation cohorts, despite differences in both OA progression definition and time-to-progression. Prediction of pain and radiographic progression within OABC was highly accurate, although surprisingly, we were not able to discriminate pain from radiographic progression. This a curious finding; it may be artifactual or it may reflect an underlying inflammatory process shared among early OA pain and radiographic progressors cohort itself, although we encountered a similar inability to distinguish pain-only progressors vs. radiographic-only progressors within the JoCoOA cohort (data not shown, only a small number of patients met these criteria: 14 pain-only and 45 radiographic-only progressors). Also curious is our finding that dual pain+radiographic patients were less accurately distinguished from nonprogressors than single-domain (pain or radiographic) progressors. As above, this may be artifactual and related to our particular dataset or might suggest that dual progressors represent an independent OA phenotype/epigenotype when compared to pain or radiographic progressors. This is supported by the lack of overlap in CpG sites selected for modeling of dual progressors compared to

single-domain progressors. This intriguing finding should be confirmed in additional cohorts of dual OA progressors.

This study builds on our previous pilot data modeling peripheral blood DNA methylation data as a predictor of radiographic progression (20) while adding several important additional features. First, we evaluated significantly more patients, including different OA phenotypes, using a newer and more complete DNA methylation array (Illumina EPIC). Additionally, the development cohort of our current study was performed on the same patients as the Phase 1 FNIH-OABC analyses, meaning that we can directly compare our findings to those previously published for both biochemical (8) and radiographic (9,10,30) models. Our models compare favorably to previously published OA predictive models. In the same cohort of patients, the OABC developed models to differentiate future dual (radiographic + pain) progressors from pain-only, radiographic-only, and non-progressors (8). Models based on baseline proteomics data performed relatively poorly with an AUC of 0.586, whereas time-integrated concentration (TIC) models comparing the change in biomarkers over 12 or 24 months (compared to baseline) to predict disease status at 48 months were better, reaching AUC=0.668 without cross-validation and AUC=0.618 with cross-validation. No confirmation of these models in an independent cohort was performed.

Progression prediction using radiographic features has also been performed within this same cohort. In 2015, Eckstein and colleagues determined that central medial femorotibial compartment thickness loss by MRI between baseline and 12- or 24-month follow-up was predictive of future progression with an odds ratio of 4 (9). Similarly, in 2016 Hunter et al. demonstrated 24-month changes in periarticular bone area and shape were associated with both pain and radiographic progression at 48 months with odds ratios in the ~1.25 to 2.62 range for each 1 S.D. increase in area or shape (10). A multivariable radiographic model including cartilage thickness, surface area, effusion-synovitis, Hoffa-synovitis, and meniscal morphology change over 24 months to predict dual pain + radiographic progression reached AUC=0.740 within the OABC cohort (13). A 2021 study by Hunter and colleagues combined TIC biochemical and radiographic biomarkers from the OABC to develop models to predict dual progressors vs. controls (AUC=0.680–0.724) and radiographic-only progressors (AUC=0.716–0.832), although similar to previous studies, TIC biomarker change over 24 months was used to predict outcomes at 48 months. Using a multivariate approach to OABC data, a 2019 study implemented a distance weighted discrimination linear machine learning analysis and found that progressor status was more strongly related to baseline MR imaging features than with either demographic/clinical variables or with baseline biochemical biomarkers (31).

Relatively little has been published regarding peripheral blood epigenomic or transcriptomic biomarkers in human OA. A 2018 analysis of publicly available blood gene expression profiles from a small set of OA patients and controls, identifying a 23-gene set with a reported accuracy of 0.971 to distinguish OA patients from controls, although no confirmation was performed (32). A recent study by Attur et al. developed a multivariate model including a 3-gene inflammatory gene expression panel from mixed peripheral blood samples and radiographic data to predict radiographic OA progression within 24 months, yielding an AUC of 0.75 with an odds ratio of 19.10 (33). This study included only

data from a single baseline timepoint, and the authors confirmed their findings in two independent cohorts, although models were not able to predict pain outcomes.

Of the 13 CpG sites included in our parsimonious models, 12 are within known genes or regulatory elements, but only 2 of these have previous links to OA (Table 3). *ERC1* is an antiapoptotic gene found downregulated in OA fibroblast-like synoviocytes (34). *TRIP12* is an E3 ubiquitin ligase that complexes with tankyrase in chondrocytes; tankyrase inhibitors reduce surgically-induced OA in mice (35). These findings are not surprising; given the lack of previous epigenetic analyses of circulating immune cells in OA patients, we would expect that the majority of epigenetic dysregulation within immune cells or cell subsets would be distinct from patterns seen within joint tissues. Considering our findings, future pathogenesis-focused OA studies should specifically evaluate immune cell epigenetic dysregulation. These should focus on individual immune cell subsets, as relatively little pathogenic information can be gleaned from the biomarker-focused mixed buffy coat methylation data we have generated in the present study.

Our study has several strengths. Most importantly, we have found superior predictive capability for future radiographic and/or pain progression from a single baseline blood sample. This predictive capability appears to be valid as early as 2 years (OAI validation cohort) and as late as 5 years (JoCoOA validation cohort). The fact that predicted cell counts and baseline demographic information did not alter model performance broadens applicability, as does the fact that DNA processed by independent institutions demonstrated similar performance. Also striking was the clear distinguishability of pain-only and radiographic-only progressors from dual (pain and radiographic) progressors, which were more difficult to distinguish from non-progressor controls, suggesting a distinct epigenetic endophenotype in dual progressors that should be further evaluated in future studies. Parsimonious models we developed retained high levels of accuracy, including in validation cohorts, when data were reduced to a relatively small number (N=13) of CpG features. This suggests that our findings may be translatable to higher-throughput, inexpensive, sequencing-based assays rather than requiring genome-wide DNA methylation arrays.

Our analysis does have several limitations. Our development and validation cohorts were not ideally matched; specifically, follow-up periods and radiographic measurements differed between the 3 cohorts. Although this mismatch reduces the ability for us to fine-tune our development models, it may also be considered a strength, as accurate predictions were still able to be made despite these differences. We have not yet examined the predictive capability of baseline blood epigenetic patterns for other radiographic endpoints beyond joint space narrowing; future analyses should focus on radiographic markers previously identified as strongly predictive of progression, including synovitis (13). Furthermore, our study focused exclusively on knee OA outcomes, despite the likelihood that a number of our samples included individuals with additional joint involvement (e.g., hand, hip), which may also be amenable to a systemic immune epigenetic modeling approach (36). This is a limitation shared by most of the OA systemic biomarker studies published to date and should be further evaluated in future studies designed to investigate potential epigenetic biomarkers of multi-joint OA. A major limitation of our study (as in any biomarker study) is that our findings are correlative rather than reflective of underlying disease mechanisms.

The CpG methylation sites we identified as strongly predictive of OA progression, therefore, may or may not be directly related to OA pathophysiology and caution must be taken when interpreting these findings.

In summary, our study offers compelling evidence that peripheral blood epigenetic-based models may be used as baseline predictive biomarkers for future rapid progression in symptomatic knee OA patients. We have identified a small subset of CpG sites that appear to be highly correlated with both pain and radiographic progression, although we were unable to differentiate between the two. The AUC we describe for radiographic progression, confirmed in two independent validation sets, is the highest yet published for a baseline biomarker and is similar to the best models produced by multivariate TIC biochemical and radiographic biomarkers from the OABC. Our results should be applied to other large OA datasets to confirm validity. Additional work should be done both to translate our findings into an inexpensive and high-throughput clinical assay. Future studies should also evaluate the pathophysiological implications, if any, of epigenetic changes in individual immune cell subsets in OA patients. Nonetheless, our results offer hope for an easily accessible, blood-based single-timepoint biomarker to enrich OA clinical trials in patients likely to experience rapid progression, thereby improving the care of adults with knee OA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement:

The DNA methylation datasets generated during the current study are available from the OAI and JoCoOA cohorts upon reasonable request.

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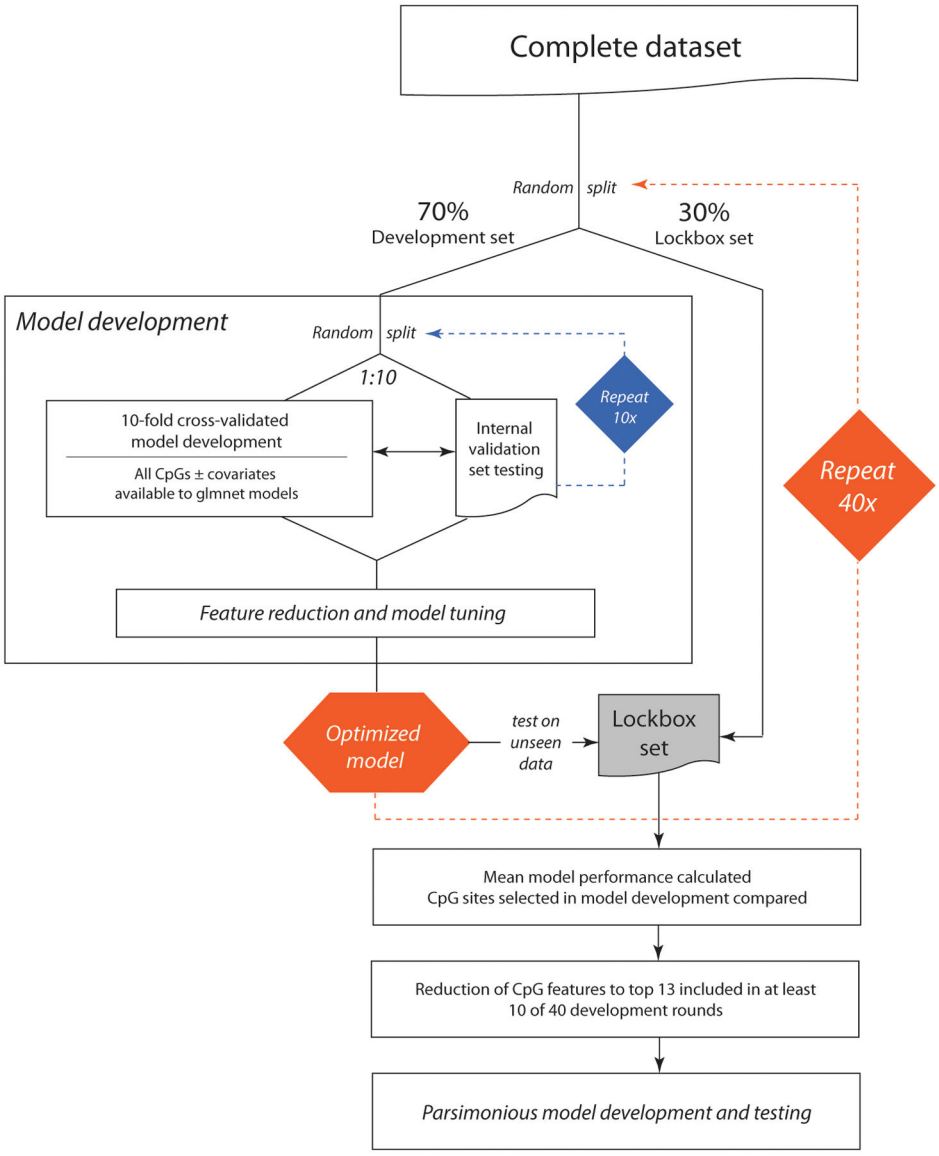

Figure 1:
Diagram of machine learning model development workflow.

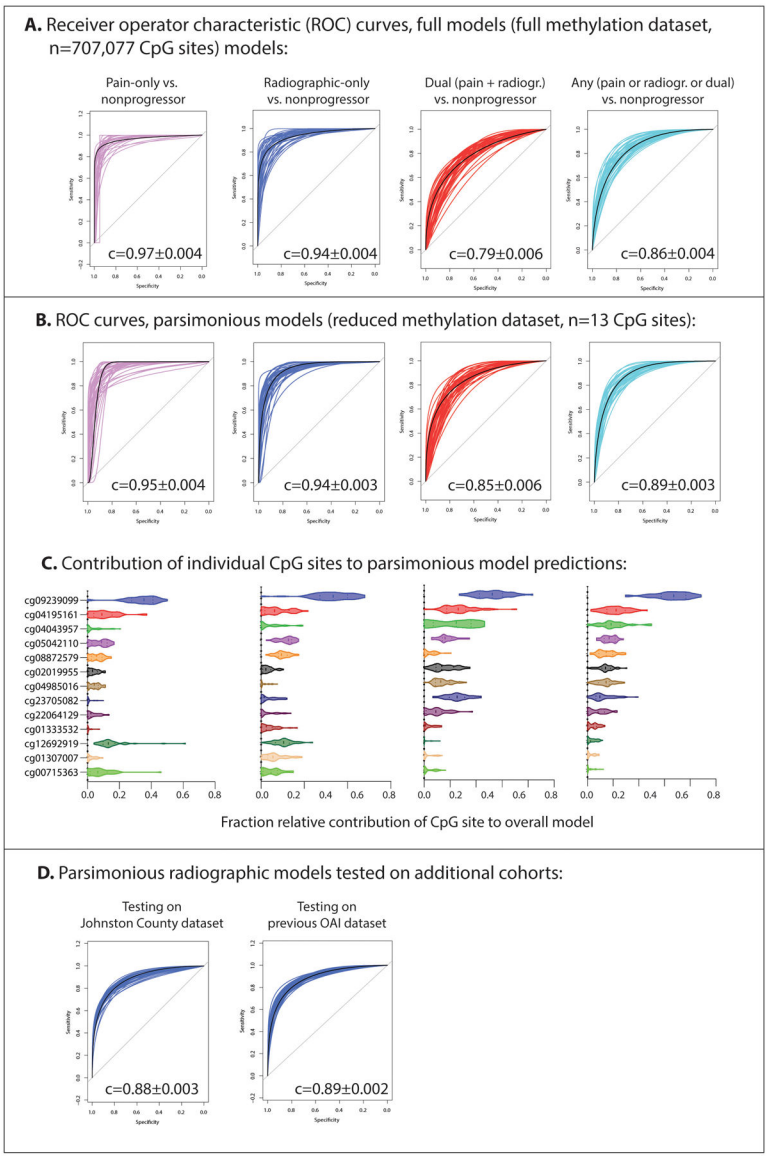

Figure 2:

Performance of peripheral blood DNA methylation machine learning models to predict future knee OA progression. (A) Receiver operator characteristic (ROC) curves for full models. (B) ROC curves for parsimonious models following reduction in dataset to 13 CpG sites most frequently selected during full model development. (C) Relative contribution of individual CpG sites (features) to model predictions in 40 rounds of parsimonious model development (D) ROC curves for parsimonious models tested on independent datasets including the Johnston County OA project (JoCoOA) and a previously published OAI buffy coat DNA methylation dataset

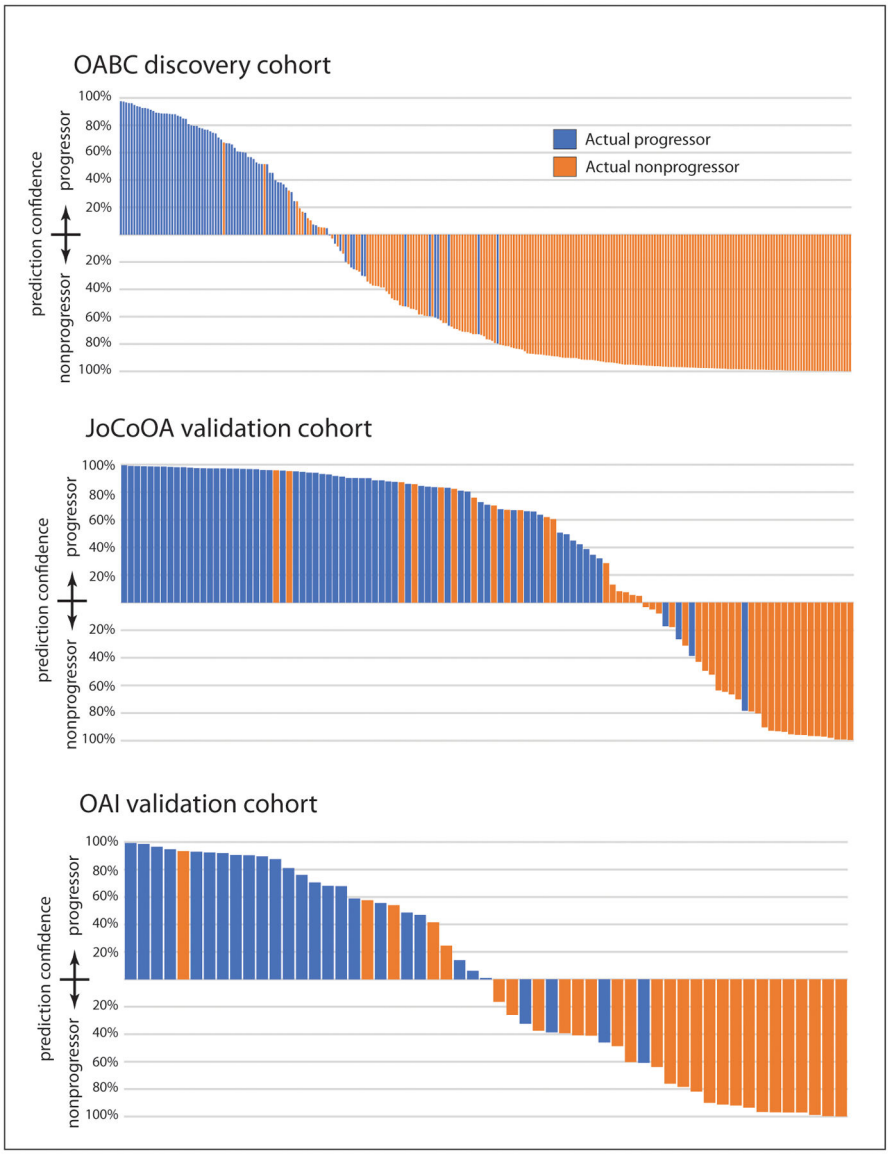

Figure 3:

Plot of prediction confidence in radiographic-only vs. non-progressor parsimonious models for all development and validation samples (mean of 40 rounds of model development or validation). Y axis represent model confidence for prediction of progressor status, X axis represents individual patient samples. Blue bars are actual radiographic progressors, orange bars are actual non-progressors.

Table 1:

Participant Characteristics

Discovery cohort (OAI FNIH-OABC, n=554 individuals)					
	Non-progressors (NP, n=189)	**Radiographic-only progressors (RP, n=81)**	**Pain-only progressors (PP, n=91)**	**Dual (radiographic + pain) progressors (DP, n=193)**	**Significant group differences**
Age (mean±SD), years	61±9	63±8	59±9	62±9	PP vs. NP: *P*=0.02
Sex (%female)	64%	44%	63%	56%	RP vs NP: *P*=0.003
BMI (mean±SD), kg/m^2	31±5	31±5	31±5	31±5	
Baseline KLG (mean±SD)	1.8±0.9	2.0±0.9	1.9±0.9	2.2±0.9	DP vs. NP: *P*<0.0001
Baseline JSW (mean±SD), mm	4.0±1.3	4.0±1.3	3.9±1.4	3.9±1.4	
Baseline WOMAC pain (mean±SD)	12.0±16.0	14.0±19.2	14.0±17.6	13.3±16.7	
NSAID use (%)	16%	23%	22%	35%	DP vs NP: *P*=0.02
Pain medication use (%)	13%	10%	12%	10%	
African-American	21%	10%	30%	17%	RP vs NP: *P*=0.04
Asian-American	2%	0%	0%	1%	
Hispanic	0%	1%	0%	2%	
White	79%	79%	70%	80%	
Validation cohort (JoCoOA, n=128 individuals)					
	Non-progressors (n=51)	**Radiographic-only progressors (n=77)**	**Significant group differences**		
Age (mean±SD), years	66±9	64±8			
Sex (%female)	73%	80%			
BMI (mean±SD), kg/m^2	34±8	36±8			
Baseline KLG (mean±SD)	2.5±0.5	2.3±0.5	*P*=0.03		
Baseline WOMAC pain (mean±SD)	33±20	30±20			
NSAID use (%)	19%	32%			
Pain medication use (%)	12%	4%			
African-American	27%	47%	*P*=0.03		
White	73%	53%	*P*=0.03		
Time between blood-draw visit and 'progression' visit (years, mean±SD)	n/a	5.6±1.1			
Validation cohort (OAI, n=55 individuals)					
Age (mean±SD), years	60±8	60±8			
Sex (%female)	50%	52%			

BMI (mean±SD), kg/m²	30±4	31±5			
Baseline KLG (mean±SD)	2.3±0.7	1.9±0.7			
Baseline JSW (mean±SD), mm	4.0±1.3	4.2±1.2			
Baseline WOMAC pain (mean±SD)	17.0±5.3	21.0±4.6	*P*=0.004		
NSAID use (%)	7%	22%			
Pain medication use (%)	7%	30%	*P*=0.04		
African-American	11%	11%			
White	89%	89%			

Table 2:

Model performance characteristics

Comparison groups	Accuracy (mean±SEM)	AUC-ROC (mean±SEM)	Sensitivity (mean±SEM)	Specificity (mean±SEM)	Diagnostic odds ratio (mean±SEM)	F_1 score (mean±SEM)	Number of features (CpGs) included (mean±SEM)
Osteoarthritis Biomarkers Consortium (OABC) Discovery Cohort, Full models 707,077 CpGs available for modeling							
Radiographic-only vs. non-progressor	87±0.8%	0.94±0.004	0.88±0.01	0.88±0.01	83±12	0.78±0.01	17±2
Pain-only vs. non-progressor	89±0.9%	0.97±0.004	0.92±0.01	0.89±0.01	120±20	0.83±0.02	13±2
Dual- vs. non-progressor	72±0.7%	0.79±0.006	0.70±0.01	0.74±0.01	7.4±0.4	0.74±0.01	7±2
Any (pain/ radiographic/ dual) vs. non-progressor	78±0.4%	0.86±0.004	0.78±0.004	0.78±0.01	15±1.0	0.85±0.003	14±2
Radiographic-only vs. pain-only	58±1%	0.62±0.01	0.54±0.02	0.60±0.01	2.2±0.2	0.51±0.02	14±3
Dual- vs. radiographic-only progressor	76±0.6%	0.82±0.007	0.78±0.06	0.72±0.03	12±1.3	0.86±0.003	10±2
Dual- vs. pain-only progressor	71±0.6%	0.79±0.007	0.74±0.006	0.76±0.03	7.8±0.7	0.84±0.003	5±1
OABC Discovery Cohort, parsimonious models 13 CpGs available for modeling							
Radiographic-only vs. non-progressor	89±0.5%	0.94±0.003	0.83±0.01	0.91±0.004	77±11	0.81±0.01	n/a
Pain-only vs. non-progressor	90±0.7%	0.95±0.004	0.88±0.01	0.92±0.01	125±21	0.86±0.01	n/a
Dual- vs. non-progressor	76±0.6%	0.85±0.006	0.75±0.01	0.78±0.01	12±0.8	0.77±0.01	n/a
Any (pain/ radiographic/ dual) vs. non-progressor	82±0.3%	0.89±0.003	0.84±0.004	0.78±0.008	20±1.0	0.87±0.002	n/a
Applying OABC-developed parsimonious models to other independent datasets:							
JoCoOA: Radiographic-only vs. non-progressor:	80±0.3%	0.88±0.003	0.78±0.004	0.88±0.004	28±1	0.86±0.002	n/a
Previous OAI dataset: Radiographic-only vs. non-progressor:	80±0.8%	0.89±0.002	0.80±0.008	0.82±0.01	20±1.4	0.80±0.005	n/a

Table 3:

CpG sites chosen for reduced models

CpG site	Chromosome	Gene symbol	CpG location	CpG Island	Relative contribution to pain- only progressor model	Relative contribution to radiographic-only progressor model	Relative contribution to dual-progressor model	Relative contribution to any- (pain/ radiographic/ dual) progressor model
cg09239099	12	*ERC1*	TSS200	Island	0.34±0.02	0.32±0.02	0.26±0.01	0.33±0.01
cg04195161	17	*ARHGAP23*	1stExon	S_Shelf	0.11±0.01	0.07±0.01	0.14±0.01	0.11±0.008
cg04043957	11	*TNNI2*	TSS1500		0.03±0.009	0.04±0.01	0.11±0.01	0.10±0.009
cg05042110	8	*PKIA*	5'UTR		0.08±0.008	0.12±0.006	0.09±0.005	0.08±0.004
cg08872579	1	*TTC22*	Body	N_Shore	0.06±0.007	0.10±0.005	0.02±0.004	0.07±0.005
cg02019955	9				0.04±0.005	0.03±0.004	0.08±0.007	0.07±0.005
cg04985016	2	*TRIP12*	5'UTR		0.04±0.005	0.01±0.003	0.07±0.005	0.07±0.005
cg23705082	22	*TBC1D22A*	Body		0.01±0.003	0.03±0.006	0.13±0.007	0.06±0.007
cg22064129	18	*SLC14A2*	TSS1500		0.03±0.006	0.03±0.006	0.05±0.007	0.04±0.006
cg01333532	16	*PHLPP2*	TSS1500		0.01±0.003	0.04±0.006	0.02±0.004	0.02±0.003
cg12692919	8	*TSNARE1*	Body	N_Shore	0.16±0.02	0.10±0.007	0.01±0.002	0.02±0.003
cg01307007	19	*FZR1*	TSS1500		0.02±0.004	0.06±0.008	0.01±0.003	0.01±0.003
cg00715363	17	*MIR548W*	Body		0.08±0.01	0.05±0.007	0.02±0.004	0.01±0.002