Searching for improvements in predicting human eye colour from DNA

Magdalena Kukla-Bartoszek¹,² · Paweł Teisseyre³,⁴ · Ewelina Pośpiech² · Joanna Karłowska-Pik² · Piotr Zielinski⁶ · Anna Woźniak⁷ · Michał Boroń⁷ · Michał Dąbrowski⁸ · Magdalena Zubańska⁹,¹⁰ · Agata Jarosz² · Rafał Płoski¹¹ · Tomasz Grzybowski¹² · Magdalena Spółnicka⁷ · Jan Mielniczuk³,⁴ · Wojciech Branicki²,⁷

Received: 31 March 2021 / Accepted: 17 June 2021 / Published online: 14 July 2021
© The Author(s) 2021

Abstract
Increasing understanding of human genome variability allows for better use of the predictive potential of DNA. An obvious direct application is the prediction of the physical phenotypes. Significant success has been achieved, especially in predicting pigmentation characteristics, but the inference of some phenotypes is still challenging. In search of further improvements in predicting human eye colour, we conducted whole-exome (enriched in regulome) sequencing of 150 Polish samples to discover new markers. For this, we adopted quantitative characterization of eye colour phenotypes using high-resolution photographic images of the iris in combination with DIAT software analysis. An independent set of 849 samples was used for subsequent predictive modelling. Newly identified candidates and 114 additional literature-based selected SNPs, previously associated with pigmentation, and advanced machine learning algorithms were used. Whole-exome sequencing analysis found 27 previously unreported candidate SNP markers for eye colour. The highest overall prediction accuracies were achieved with LASSO-regularized and BIC-based selected regression models. A new candidate variant, rs2253104, located in the \textit{ARFIP2} gene and identified with the HyperLasso method, revealed predictive potential and was included in the best-performing regression models. Advanced machine learning approaches showed a significant increase in sensitivity of intermediate eye colour prediction (up to 39%) compared to 0% obtained for the original IrisPlex model. We identified a new potential predictor of eye colour and evaluated several widely used advanced machine learning algorithms in predictive analysis of this trait. Our results provide useful hints for developing future predictive models for eye colour in forensic and anthropological studies.

Keywords Whole-exome sequencing · Eye colour · DNA phenotyping · Predictive modelling · Machine learning algorithms

© The Author(s) 2021

International Journal of Legal Medicine (2021) 135:2175–2187
Introduction

Increasing understanding of human genome variability is enabling better use of DNA’s predictive potential [1]. Besides clinical applications, predictive DNA analysis can be useful in forensics for intelligence purposes [2], in molecular anthropology [3] and in identification of historical figures [4–6]. In recent years, intensive research has been carried out on the prediction of various human appearance characteristics [e.g. 7–15]. The most significant progress was made in the prediction of pigmentation characteristics, and eye colour in particular [16]. Nevertheless, the genetic architecture of some categories of pigmentation phenotypes remains elusive, their prediction is still inaccurate and research to improve accuracy continues. One such category is intermediate eye colour, which in the most commonly used IrisPlex model is predicted with low sensitivity [16]. Because of the very complex genetic basis of the appearance traits, a promising direction is building predictive tools that take into account markers based on the criterion of improved prediction and not genetic association, and the use of more advanced mathematical methods in prediction modelling [17]. There are many machine learning (ML) methods available for developing predictive models, and their effectiveness may depend on the type and amount of data used; some of them may be more suitable than others for taking into account diverse genetic phenomena, including epistasis. First, we can distinguish linear and nonlinear methods [18]. The linear methods in their basic form are limited to detecting the linear dependency between a class variable and attributes. Representative examples are logistic and multinomial regression, linear discriminant analysis (LDA), the basic linear version of support vector machines (SVM) or perceptron. The nonlinear methods are designed to detect more complex dependencies between a class variable and attributes. Examples include various tree-based methods, multivariate adaptive regression splines (MARS) and multilayer neural networks (NN). The advantage of the first group is the relatively low computational cost of fitting the model as well as simplicity and interpretability. On the other hand, nonlinear models usually achieve greater predictive power, especially in the case of complex classification issues. Moreover, they are also able to detect interactions among attributes [19]. In addition to single models, ensemble techniques, which combine multiple learning algorithms, have gained great popularity. It has been proved that ensemble methods such as random forest (RF) or extreme gradient boosting (XGB) are among the most powerful classification models; they usually achieve significantly higher accuracy when compared to simple models. The price for this is the higher computational cost and more complicated interpretation. An important line of research in ML is focused on combining classification methods with feature selection techniques. Feature selection plays a crucial role in many analyses, especially when the number of attributes is large compared with the sample size. Selection of relevant attributes improves the understandability of the considered model and allows one to discover the relationship between attributes and the class variable. Secondly, it helps to devise approaches with better generalization and larger predictive power [20]. In the case of some classification methods, feature selection is an integral element of learning the model; for example, in tree-based methods, relevant attributes are chosen during the building of the tree. Another solution is using regularization techniques [18], such as least absolute shrinkage and selection operator (LASSO) regularization, which ensure sparsity in the parameter vector and allow one to find attributes influencing the class variable.

In this study, we explored the possibility of increasing the accuracy in predicting eye colour. To this end, we adopted the following strategies: (1) quantitative characterization of samples using high-quality images of the iris analysed with Digital Iris Analysis Tool (DIAT) software; (2) whole-exome sequencing (WES)-based identification of new potential predictors in a group of 150 phenotypically diverse Polish samples using the HyperLasso method and regression-based single-SNP association testing; (3) predictive modelling conducted based on the literature and WES-identified markers, using various machine learning algorithms and independent sets of samples in order to find the most accurate method for eye colour in a moderate dimensional dataset.

Materials and methods

Sample collection and DNA extraction

The study cohort consisted of 999 unrelated individuals (673 males and 326 females), collected together within the NEXT project, funded by the National Centre for Research and Development, grant number DOB-BIO7/17/01/2015. The study was approved by the Ethics Committee of the Jagiellonian University in Kraków (decision no. KBET/122/6120/11/2016), and all volunteers gave written informed consent prior to their inclusion in the study. Recruitment of the participants was carried out in the Police Academy in Szczecin.

Whole blood was collected from the volunteers and subjected to DNA extraction using the PrepFiler Express™ Forensic DNA Extraction Kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. Quantification
of the extracted samples was performed using the Quantifiler™ Human DNA Quantification Kit or the Plexor® HY System.

Phenotype assessment

Phenotyping of the investigated samples for eye colour was conducted in two independent ways: quantitative measurements, used for identification of new SNP candidates from WES analysis, and categorization, used at the predictive modelling stage. The evaluation was performed based on collected high-resolution photographic documentation. Photos of both eyes were taken in identical conditions for all volunteers using a Nikon D5300 camera with an R1C1 Wireless Close-up Speedlight System (Nikon, Tokyo, Japan). Images of the irises were taken from a distance of about 20–30 cm, with the following settings: shutter speed 1/125, aperture f/22, ISO 200, flash A = 1/8, B = 1/4. Eye colour was classified into 3 categories: blue, intermediate (green, green-hazel) and brown. Classification was performed based on photographic documentation of both irises, by one assessor. The assignment to a specific category was carried out in two independent rounds of classification, or three, when there was an inconsistency between the first and the second round of assessment. The second approach consisted in an objective, quantitative characterization of eye colouration. Eye colour quantitative evaluation was conducted based on high-quality images and with DIAT software [21]. Blue and brown pixels in the area of the iris are counted and the Pixel Index of Eye (PIE score) is calculated as a measure of eye pigmentation. The PIE score ranges between – 1 (which corresponds to perfectly brown eye colour) and 1 (which corresponds to perfectly blue eyes). Additional information that was used in statistical analyses included age and sex. The studied group was divided into two sets: the discovery cohort consisted of 150 phenotypically diverse samples, used for candidate markers selection based on WES analysis, and the predictive modelling cohort consisted of the remaining 849 samples, used to develop and evaluate predictive models.

Whole-exome sequencing of the discovery cohort

Exonic sequences (66 Mbp) enriched in regulatory regions of > 160 loci with a known association with human appearance traits (1.5 Mbp) extracted from Nencki Genomics Database and FANTOM [22, 23] were sequenced and bioinformatically analysed in the group of 150 carefully selected, phenotypically diverse individuals, as described in detail in [14]. As a result, genetic data for 77,485 SNPs with less than 20% of missing data and global minor allele frequency ≥ 5% were extracted for further statistical analyses.

Selection of potential DNA predictors

Taking into consideration the high importance of precise phenotype characterization and the fact that the studied trait exhibits continuous distribution, WES-based marker selection for eye colour was performed based on quantitative measurements, which provide an objective and accurate trait description. Two different statistical approaches were applied for candidate marker selection. As it is still the most common concept, especially when handling large numbers of tested variables, single marker testing was applied. In order to increase the chance of identification of powerful predictors, we decided to set the suggestive threshold of P-value < 1 × 10–4 for candidate SNP selection. Because of the character of the data, linear regression for quantitatively described eye colour was used. Results were adjusted for age and sex. In addition, the HyperLasso method (https://www.ncbi.nlm.nih.gov/projects/BARGEN) was applied as an alternative approach for feature selection. It is a highly attractive method that addresses the computational challenge of simultaneous SNP analysis from large-scale experiments. It is a model selection method that utilizes a Bayesian-based penalized maximum likelihood approach, which can handle high-dimensional inputs [24]. Various penalty and shape parameters were tested. The best ones were selected empirically, based on the assumption that the model should consist of a reasonable number of predictors (p), i.e. 0 < p < 100. All newly selected candidate SNP markers were subjected to linkage disequilibrium (LD) pruning and one SNP from each LD block (r² > 0.7) was kept for further analyses. LD analysis was conducted using PLINK 1.9, while remaining analyses were conducted with R v3.5.2 using ‘ordinal’ package and ‘HyperLasso’ code. Since many variants associated with human pigmentation traits have already been identified, an intensive literature review was conducted and 114 SNP markers, previously correlated with pigmentation in general, were selected for further statistical analyses (Supplementary Information; Table S4).

Targeted sequencing of DNA candidates

Genetic data for 141 DNA variants in a population of 849 individuals were collected using targeted high-throughput DNA sequencing with Ion AmpliSeq™ technology and an Ion S5™ or Ion Proton™ platform. Two independent Ion AmpliSeq™ custom panels were designed using Ion AmpliSeq™ Designer tool (https://www.ampliseq.com) with Thermo Fisher Scientific support, and covered DNA markers for various human appearance traits, including pigmentation, hair morphology, hair greying, earlobe, monobrow and other traits investigated within the NEXT project. Because of technical problems, four SNPs were replaced.
by SNPs in LD (rs2004775—> rs60247077 in RBFOX1, rs7762830—> rs743589 in MYB, rs224223—> rs224219 in MEFV and rs12052928—> rs9636495 in ANKRD36). DNA libraries were prepared manually and sequenced as described previously [11]. Missing SNP data were at the level of 0.2% and were imputed using the ‘missForest’ method in R v3.5.2 (with a total number of trees equal to 500).

**Predictive modelling**

All candidate SNP variants selected from the literature and WES analysis were used in prediction modelling except four variants (rs1800414 in OCA2, and rs3212355, rs312262906 and rs201326893 in MC1R), which were monomorphic in our dataset and were excluded from statistical analyses. The final list of variables also included age and sex. Various machine learning algorithms were evaluated for the most accurate model development. Models were developed to predict eye colour categorized in three classes.

**Regression models**

Marker selection for regression models was performed by the forward selection method with two classical statistical approaches, the Akaike Information Criterion (AIC) [25] and the Bayesian Information Criterion (BIC) [26]. They are used to find a trade-off between the goodness of fit of a model and its complexity and are suitable in the case where \( p < n \) (\( p \)-total number of variables, \( n \)-total number of cases). In order to determine their robustness, two additional regression models, i.e. (1) developed using only one marker—the most important one—chosen in the first round of SNP selection (LOG 1-STEP), and (2) using all the analysed in this study SNPs (LOG FULL), were added for comparison purposes. In addition, we used LASSO, in which the penalized log-likelihood function is considered [27]. This regularized regression method is particularly popular in high-dimensional statistical data analysis. LASSO shrinks some coefficients of the model to zero, and therefore, it can be regarded as a feature selection method. Analyses were carried out using RStudio (v 1.1.456) and the *glmnet* package.

**Other machine learning algorithms**

The performance of seven additional machine learning algorithms was also tested. They included Classification and Regression Trees (TREE), Random Forests (RF), Extreme Gradient Boosting (XGB), Multivariate Adaptive Regression Splines (MARS), Neural Network (NN), Support Vector Machine (SVM) and Naïve Bayes (NB). A random naïve classifier, which assigned observations randomly to classes according to apriori probabilities (Naive), was used as a benchmark. Default settings were applied to tested algorithms. Feature selection for TREE, RF, XGB and MARS algorithms is embedded in the learning algorithm, i.e. relevant features were selected during fitting the models. Analyses were carried out using R (v 4.0.3) and the following packages: *infotheo*, *missForest*, *cvTools*, *rpart*, *randomForest*, *xgboost*, *glmnet*, *class*, *ROCR*, *earth*, *nnet* and *caret*. All tested algorithms with their abbreviations used in the text are listed in Table 1.

**Evaluation of the models’ performance**

In order to assess the performance of the fitted models, the predictive modelling cohort was divided into training (70%) and testing (30%) datasets, which is a commonly used strategy, close to optimal for reasonable sized datasets (\( n \geq 100 \)) with strong signals (\( \geq 85\% \) accuracy) [28]. Additionally, to reduce randomness of data splitting, the process was repeated 100 times, with seed numbers 1–100, using the function set.seed in R. The final performance of the models

| Algorithm/model                                           | Abbreviation |
|-----------------------------------------------------------|--------------|
| Random classifier                                         | Naive        |
| Logistic/multinomial regression with LASSO regularization  | LOG REG      |
| Logistic/multinomial regression with AIC-based model selection | LOG AIC      |
| Logistic/multinomial regression with BIC-based model selection | LOG BIC      |
| Logistic/multinomial regression with 1 step (1 SNP)       | LOG 1-STEP   |
| Logistic/multinomial regression model with all SNPs       | LOG FULL     |
| Classification and Regression Tree                        | TREE         |
| Random forest                                             | RF           |
| Extreme gradient boosting                                 | XGB          |
| Multivariate and adaptive regression splines              | MARS         |
| Neural networks                                           | NN           |
| Support vector machine                                    | SVM          |
| Naïve Bayes                                               | NB           |
was determined based on the results collected from 100 testing sets, by calculation of the mean values of the following measures:

- **AUC** — area under the ROC curve — expressing the overall prediction accuracy and ranges between 0.5, which corresponds to random classification, and 1.0, which corresponds to perfect classification
- **Accuracy (acc.)** — the percentage of individuals correctly classified into a specific category over the total number of individuals in the analysis, expressed by the equation:

\[
\text{Accuracy} = \frac{\text{True Positives} + \text{True Negatives}}{\text{True Positives} + \text{True Negatives} + \text{False Positives} + \text{False Negatives}}
\]

- **Sensitivity (sens.)** — true positive rate, expressed by the equation:

\[
\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}
\]

- **Specificity (spec.)** — true negative rate, expressed by the equation:

\[
\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}
\]

We calculated the above measures for each class (eye colour), i.e. the observations corresponding to the considered class are treated as positive examples, whereas observations corresponding to the two remaining classes are treated as negative examples.

### Results

#### Characteristics of the study group

The study cohort consisted of 673 (67.4%) males and 326 (32.6%) females in the age range 19–77 years (mean = 30.6; SD = 9.0). Considering the categorized pigmentation phenotype, most individuals had blue eyes (62.8%) followed by brown (18.1%) and intermediate (15.0%). Due to difficulties in unambiguous eye colour categorization, forty-one individuals’ eye colour was not determined (4.1%) mainly due to varying degrees of heterochromia. There was no statistically significant correlation between categorized eye colour and age or sex (P-value = 0.349 and P-value = 0.582, respectively). Quantitative measurements of eye colour revealed a whole range of possible phenotypes, from PIE score = −1 to 1. Mean PIE score was 0.2 (SD = 0.8). The borderline statistical significance was noted for correlation between quantitatively described eye colour and age (P-value = 0.041) but not sex (P-value = 0.321). Nevertheless, although statistically

|                          | Discovery cohort [N = 150] | Predictive modelling cohort [N = 849] | Total [N = 999] |
|--------------------------|---------------------------|--------------------------------------|-----------------|
| **Sex**                  |                           |                                      |                 |
| Females                  | 67                        | 259                                  | 327             |
| Males                    | 83                        | 590                                  | 673             |
| **Age**                  |                           |                                      |                 |
| min                      | 19                        | 19                                   | 19              |
| max                      | 77                        | 62                                   | 77              |
| mean value               | 31.5                      | 30.4                                 | 30.6            |
| SD                       | 10.3                      | 8.7                                  | 9.0             |
| **Eye colour**           |                           |                                      |                 |
| Blue                     | 76                        | 551                                  | 627             |
| Intermediate             | 28                        | 122                                  | 150             |
| Brown                    | 42                        | 139                                  | 181             |
| NA                       | 4                         | 37                                   | 41              |
| PIE score, min           | −1.0                      | −1.0                                 | −1.0            |
| PIE score, max           | 1.0                       | 1.0                                  | 1.0             |
| PIE score, mean value    | 0.0                       | 0.2                                  | 0.2             |
| PIE score, SD            | 0.9                       | 0.8                                  | 0.8             |
significant, correlation between PIE score and age was negligible in the studied sample set ($r = 0.065$). Information about eye colour of the whole studied cohort, and divided into discovery and predictive modelling cohorts, is provided in Table 2.

**Selection of DNA markers**

Univariate association testing conducted on WES data generated for 150 samples included in the discovery cohort allowed the selection of 14 candidates for eye colour ($P$-value $< 1 \times 10^{-4}$) (Supplementary Information; Table S1). The HyperLasso method identified an additional 20 candidate SNPs and age as important variables for eye colour (Supplementary Information; Table S2). Subsequent analysis of the linkage disequilibrium (Supplementary Information; Table S3) led to a set of 30 independent ($r^2 < 0.7$) candidate DNA markers for eye colour. A literature search revealed a further 114 DNA markers previously associated with pigmentation (all listed in Supplementary Information; Table S4) and three SNPs (rs12896399 in SLC24A4, rs7495174 in OCA2 and rs11636232 in HERC2) overlapped with WES-based selected variants. Overall, WES analysis discovered twenty-seven novel candidates for eye colour. As the four SNPs were monomorphic in the studied dataset, prediction modelling finally involved analysis of 137 SNPs, age and sex (Fig. 1).

**Prediction modelling using regression methods**

**Development of the models**

AIC, BIC and LASSO were used for marker selection, resulting in unique sets of variables selected in each of 100 data splits. Analysis revealed that the BIC method produced the most parsimonious models while the most extensive models were developed using AIC. Further analysis showed that these extensive models contained variables that were selected for parsimonious models, which means that in many cases, markers selected by BIC were a subset of AIC- and LASSO-based developed models. The most important predictors were chosen on the basis of two criteria: (1) selected in $> 50$ out of 100 data splits and (2) selected by at least two selection methods. Among them, besides well-known literature predictors, was the novel marker rs2253104 in ARFIP2. The most important variants fulfilling both conditions are listed in Table 3. Figure 2 shows all ‘stable’ variants, i.e. those that were selected with each selection method in $> 50$ out of 100 data splits.

**Testing of models’ performance**

Five different approaches were analysed in order to find the most accurate methods for regression model development. The highest prediction accuracies were achieved using the BIC and LASSO regularization methods. These values reached acc. = 0.84 and 0.85, respectively, which means that 84–85% of individuals were classified into the correct eye colour category. Slightly lower accuracies were achieved with the AIC method and for the 1-STEP model (for both acc. = 0.79), and the lowest for the LOG FULL model (acc. = 0.74). Moreover, high AUC values were noted for all categories, including intermediate eye colour (AUC = 0.85), equal for models developed with the help of the BIC and LASSO approaches. Using these methods, blue and brown eye colours were predicted with an

**Table 3** The most important SNP variants selected in $> 50$ out of 100 data splits, by at least two of variables selection methods for regression models

| SNP_ID | Chromosome position (GRCh38) | Gene     | Selection method |
|--------|-----------------------------|----------|-----------------|
| rs10874518 | 1:101,806,756 | OLFM3    | LASSO, AIC      |
| rs166891982 | 5:33,951,588 | SLC45A2  | LASSO, AIC, BIC |
| rs2253104  | 11:6,479,079  | ARFIP2   | LASSO, AIC      |
| rs12913832 | 15:28,120,472 | HERC2    | LASSO, AIC, BIC |
| rs1800407  | 15:27,985,172 | OCA2     | LASSO, AIC, BIC |
| rs74653330 | 15:27,983,407 | OCA2     | LASSO, AIC, BIC |
| rs885479   | 16:89,919,746 | MC1R     | LASSO, AIC      |
| rs8049897  | 16:89,957,794 | DEF8     | LASSO, AIC      |
AUC of 0.96 and 0.93–0.94, respectively. Interestingly, a 1-STEP model (which was always based on one of the key variants in the HERC2 gene, selected as the best predictor) achieved slightly better AUC values than both AIC-based and FULL models and only marginally lower compared to BIC- and LASSO-based developed models for all eye colour categories. The differences were mostly observed for the intermediate category (AUC = 0.83 for the 1-STEP model, 0.75 for LOG AIC, 0.67 for LOG FULL and 0.85 for both LOG REG and LOG BIC), while for blue and brown eye colours, the differences were less pronounced. Most importantly, the sensitivity of intermediate eye colour prediction reached high values, especially in the case of LOG AIC: sens. = 0.40 and LOG FULL: sens. = 0.41. LOG BIC and LOG REG also showed relatively good values of 0.29 and 0.17, respectively, but not LOG 1-STEP: 0.00. At the same time, the specificity of the LOG BIC and LOG REG models was very high, reaching values of 0.96 and 0.97, respectively, while LOG FULL and LOG AIC remained reasonably high: 0.85–0.88. Detailed results of prediction performance analysis are shown in Table 4.

**Discussion**

Accuracy of phenotype prediction from genetic data is essential for the successful application of predictive methods in biomedical studies including anthropology, paleogenetics and forensics [29]. Several factors determine good accuracy of DNA-based predictive methods, including high heritability of a trait, identification of appropriate predictors and selection of the best mathematical approach to model development. Even highly heritable traits are often difficult to predict, due to polygenicity, epistasis, and allelic and locus heterogeneity. In this study, we used quantitative assessment of eye colour phenotypes and whole exome/
Table 4  Detailed results of predictive analysis of eye colour with various machine learning approaches

|          | Naive | LOG REG | LOG AIC | LOG BIC | LOG 1-STEP | LOG FULL | TREE | RF | XGB | MARS | NN | SVM | NB |
|----------|-------|---------|---------|---------|------------|----------|------|----|-----|------|----|-----|----|
| Accuracy |       |         |         |         |            |          |      |    |     |      |    |     |    |
| Mean     | 0.33  | 0.85    | 0.79    | 0.84    | 0.79       | 0.74     | 0.82 | 0.83| 0.81| 0.82 | 0.77| 0.79| 0.77|
| SD       | 0.03  | 0.02    | 0.03    | 0.03    | 0.02       | 0.03     | 0.03 | 0.02| 0.02| 0.03 | 0.03| 0.03| 0.02|
| AUC      |       |         |         |         |            |          |      |    |     |      |    |     |    |
| Mean     | 0.50  | 0.96    | 0.91    | 0.96    | 0.95       | 0.92     | 0.95 | 0.96| 0.96| 0.95 | 0.93| 0.95| 0.94|
| SD       | 0.04  | 0.01    | 0.03    | 0.01    | 0.01       | 0.02     | 0.01 | 0.01| 0.01| 0.03 | 0.05| 0.01| 0.02|
| Mean     | 0.51  | 0.85    | 0.75    | 0.85    | 0.83       | 0.67     | 0.82 | 0.84| 0.82| 0.80 | 0.77| 0.82| 0.80|
| SD       | 0.05  | 0.03    | 0.07    | 0.03    | 0.03       | 0.04     | 0.04 | 0.03| 0.03| 0.06 | 0.06| 0.03| 0.03|
| Mean     | 0.49  | 0.94    | 0.88    | 0.93    | 0.91       | 0.82     | 0.91 | 0.92| 0.91| 0.91 | 0.87| 0.91| 0.89|
| SD       | 0.05  | 0.02    | 0.07    | 0.02    | 0.01       | 0.03     | 0.03 | 0.01| 0.02| 0.03 | 0.08| 0.02| 0.02|
| Sensitivity |      |         |         |         |            |          |      |    |     |      |    |     |    |
| Mean     | 0.17  | 0.96    | 0.93    | 0.97    | 0.96       | 0.86     | 0.96 | 0.96| 0.97| 0.96 | 0.95| 0.96| 0.94|
| SD       | 0.03  | 0.01    | 0.03    | 0.01    | 0.01       | 0.04     | 0.01 | 0.01| 0.01| 0.02 | 0.02| 0.01| 0.02|
| Mean     | 0.17  | 0.17    | 0.40    | 0.29    | 0.00       | 0.41     | 0.35 | 0.11| 0.34| 0.39 | 0.18| 0.10| 0.16|
| SD       | 0.06  | 0.09    | 0.09    | 0.13    | 0.00       | 0.08     | 0.11 | 0.05| 0.09| 0.09 | 0.24| 0.09| 0.16|
| Mean     | 0.16  | 0.62    | 0.59    | 0.76    | 0.37       | 0.56     | 0.62 | 0.42| 0.61| 0.63 | 0.55| 0.46| 0.67|
| SD       | 0.06  | 0.17    | 0.09    | 0.19    | 0.42       | 0.09     | 0.11 | 0.09| 0.08| 0.10 | 0.37| 0.16| 0.18|
| Specificity |      |         |         |         |            |          |      |    |     |      |    |     |    |
| Mean     | 0.84  | 0.94    | 0.85    | 0.93    | 0.94       | 0.88     | 0.93 | 0.94| 0.93| 0.92 | 0.82| 0.88| 0.79|
| SD       | 0.04  | 0.02    | 0.05    | 0.03    | 0.02       | 0.04     | 0.03 | 0.03| 0.03| 0.04 | 0.14| 0.04| 0.06|
| Mean     | 0.83  | 0.97    | 0.88    | 0.96    | 1.00       | 0.85     | 0.91 | 0.98| 0.92| 0.91 | 0.94| 0.97| 0.95|
| SD       | 0.03  | 0.02    | 0.03    | 0.03    | 0.00       | 0.03     | 0.02 | 0.01| 0.02| 0.03 | 0.08| 0.03| 0.04|
| Mean     | 0.83  | 0.93    | 0.93    | 0.90    | 0.94       | 0.88     | 0.90 | 0.94| 0.90| 0.92 | 0.89| 0.92| 0.86|
| SD       | 0.02  | 0.03    | 0.02    | 0.03    | 0.09       | 0.03     | 0.03 | 0.02| 0.02| 0.02 | 0.07| 0.03| 0.05|
regulated sequencing to identify additional predictors, and additionally, we verified multiple machine learning methods to assess their impact on prediction accuracy, focusing especially on more complex intermediate phenotypes. The studied cohort of Polish individuals shows a relatively large diversity of pigmentation phenotype compared to some other European populations, which makes it useful for studying the genetics of pigmentation traits. Objective phenotyping of eye colour for finding new loci provided quantitative measurements. The analysis using DIAT software [21] confirmed that the calculated PIE score reflecting the ratio of blue to brown pixels highly correlates with human evaluation of eye colour (Spearman correlation $= -0.82$, $P$-value $= 5.46 \times 10^{-235}$). Using the single-SNP association testing of the WES/regulated data under $P$-value $< 1 \times 10^{-4}$, and the HyperLasso algorithm, which aimed to select the subset of SNPs that best predicted the trait under study simultaneously controlling the type I error of the selected variants [24], we identified 34 SNPs and age as important factors for eye colour prediction. In the next step, we moved directly to the extensive predictive modelling.

A large number of algorithms have been developed to deal with a variety of increasingly demanding and computationally challenging data analyses. Analysis of AIC, BIC and LASSO methods of marker selection conducted in this study revealed that all of them are robust, since they produced models with better performance compared to models without any selection method applied (i.e. LOG FULL). We confirmed that BIC, which more heavily penalizes the introduction of additional variables, produced the most parsimonious models. Together with BIC, LASSO yielded models with the best predictive performance. Interestingly, focusing on SNPs selected by at least two out of three feature selection methods and in at least 50% of data splits, we found the well-known pigmentation markers and the intronic variation methods and in at least 50% of data splits, we found on SNPs selected by at least two out of three feature selection methods. Together with BIC, LASSO yielded models with the best predictive performance. Interestingly, focusing on SNPs selected by at least two out of three feature selection methods, we found the well-known pigmentation markers and the intronic variation methods.

Our study confirms importance of rs74653330 for eye colour prediction and further indicates that allelic heterogeneity altogether with the population-specific differences in allele frequencies may be important factors in predictive DNA analysis. Other SNPs for eye colour prediction included rs12913832 (HERC2) and rs1689182 (SLC45A2) [16, 42] as well as others, previously associated with eye patterning (rs10874518, OLFM3; [43]), or other pigmentation traits (rs885479; MC1R, rs8049897, DEFB8; [44]) (Table 3).

Importantly, the accuracy of predicting intermediate eye colour achieved a high level (e.g. regression model developed with BIC approach or with LASSO regularization: $AUC = 0.85$, higher than reported for IrisPlex [16] and Snipper [45], the two most widely used eye colour predictive tools. In data analysed here, the sensitivity of intermediate eye colour prediction was also better (LOG BIC sens. = 0.29) compared to the results obtained with the original IrisPlex model (sens. = 0.00). In previous research, a significant increase in the sensitivity of intermediate eye colour prediction was achieved due to
additional variation in the HERC2 gene included in the predictive model. The positive effect was, however, reversible, since the addition of other HERC2 variants decreased the ability of the model to predict intermediate eye colours [45]. A small increase in accuracy of intermediate eye colour was also reported in a study that involved genetic interactions [46].

Besides classical regression, several more advanced machine learning algorithms were evaluated. The study demonstrated that advanced machine learning methods showed even higher sensitivity values of intermediate eye colour prediction (i.e. TREE, XGB and MARS with \( \text{sens}_{\text{interm.}} = 0.34–0.39 \)): however, a slightly reduced sensitivity of brown eye colour prediction was observed for these models when compared to the regression model. It is well known that more advanced machine learning methods may better cope with recognition of complex phenotypes, including intermediate eye colour, due to the ability to identify possible nonlinear dependencies between variables, such as interactions. Nevertheless, while some advanced methods were found to demonstrate increased sensitivity or specificity in predicting certain categories, none of these approaches outperformed the regression method developed following prior features selection using BIC or LASSO, when AUC or accuracy metrics were compared. Moreover, differences between the tested methods were modest. These results suggest that more sophisticated learning algorithms may need larger datasets to demonstrate their superiority and do not reveal their potential in low- and medium-dimensional data. Also, a systematic review [47] of logistic regression and other machine learning methods (among which the most common were classification trees, random forests, artificial neural networks and support vector machines) showed that in the group of low risk of biased study, no performance benefit of machine learning over logistic regression methods was reported for clinical prediction models. Further, evaluation of deep learning methods (multilayer perceptron and convolutional neural networks) conducted on high-dimensional data (~100k individuals and ~500k SNPs) did not provide any proof that these methods outperform simple linear methods and improve complex human trait prediction by a sizeable margin [48]. Although our analysis did not involve advanced machine learning hyperparameters tuning aimed at improving the obtained prediction accuracies, there is evidence in the literature that such tuning may still not be helpful for significantly improving accuracy [49]. Nevertheless, it was found that the superiority of the advanced ML approaches (random forests) depends on the dataset and tends to be more pronounced for an increasing number of analysed features or an increase in the ratio of the number of features to the number of cases [50]. Indeed, it has been shown that some of the advanced algorithms can be very successful in predicting complex traits if applied to very high-dimensional data [51]. It is also worth noting that advanced machine learning methods outperform basic linear regression in age prediction using DNA methylation data. In the evaluation of 17 different machine learning approaches performed by Aliferi et al., the support vector machine with the polynomial function method was chosen as highly robust, generalizable and the best-performing modelling approach [52], as it was in another previous study [53]. Also, neural networks (e.g. [54]) and random forest regression [55] were successfully applied to accurate human age prediction. This demonstrates the superiority of some ML approaches over classical regression methods in data with observed nonlinear correlation effects and also suggests a possible dependence of ML methods’ efficiency on the data type: discrete for SNP vs. quantitative for DNA methylation.

In summary, whole-exome sequencing of 150 individuals has allowed identification of 27 DNA variants that are relevant for eye colour prediction which have not been reported before in pigmentation predictive studies. Besides well-known pigmentation-associated variants, rs2253104 in ARFIP2 was selected by at least two different feature selection methods for regression predictive models, which turned out to be the most accurate. None of the sophisticated machine learning algorithms outperformed the overall prediction accuracy of regression models developed following prior features selection using BIC or LASSO regularization, indicating that medium-dimensional data does not use the whole potential of these more advanced algorithms.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00414-021-02645-5.

Acknowledgements We would like to thank Dr. Susan Walsh and Krystal Breslin for their great help with phenotypic data collection, including assistance in choosing the most appropriate equipment for iris photographic documentation, and for sharing guidance on camera settings. Moreover, many thanks are due to Dr. Jeppe Dyberg Andersen for his support with DIAT software. Furthermore, the authors wish to thank all volunteers who contributed to this study.

Funding This research was supported by the Polish National Centre for Research and Development [grant number DOB-BIO7/17/01/2015].

Availability of data and material Raw and additional data are available upon request.

Code availability Not applicable.
Declarations

Ethics approval The study was approved by the Ethics Committee of the Jagiellonian University in Kraków (decision no. KBET/122/6120/11/2016).

Consent to participate All volunteers gave written informed consent prior to their inclusion in the study.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Lippert C, Sabatini R, Maher MC, Kang EY, Lee S, Arikan O, Harley A, Bernal A, Garst P, Lavrenko V, Yocom K, Wang M, Yang WY, Chang C, Lu T, Lee C, Hicks B, Ramakrishnan S, Tang H, Venter JC (2017) Identification of individuals by trait prediction using whole-genome sequencing data. Proc Natl Acad Sci U S A 114(38):10166–10171. https://doi.org/10.1073/pnas.1711251114
2. Phillips C, Prieto L, Fondervila M, Salas A, Gómez-Tato A, Alvarez-Dios J, Alonso A, Blanco-Verrea A, Brión M, Montesino M, Carracedo A, Lareu MV (2009) Ancestry analysis in the 11-M Madrid bomb attack investigation. PLoS ONE 4(8):e6583. https://doi.org/10.1371/journal.pone.0006583
3. Lalueza-Fox C, Römler H, Caramelli D, Stäubert C, Catalano G, Hughes D, Rohland N, Pilli E, Longo L, Condiem S, de la Rasilla M, Fortea J, Rosas A, Stoneking M, Schöneberg T, Bertranpetit J, Hofreiter M (2007) A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. Science (New York, NY) 318(5855):1453–1455. https://doi.org/10.1126/science.1147417
4. Bogdanowicz W, Allen M, Branicki W, Lembrin M, Gajewska M, Kupiec T (2009) Genetic identification of putative remains of the famous astronomer Nicolaus Copernicus. Proc Natl Acad Sci USA 106(30):12279–12282. https://doi.org/10.1073/pnas.09001848106
5. King TE, Fortes GG, Balaresque P, Thomas MG, Balding D, Maisano Delser P, Neumann R, Parson W, Knapp M, Walsh S, Tonasso L, Holt J, Kayser M, Appleby J, Forster P, Ekserdjian D, Hofreiter M, Schürer K (2014) Identification of the remains of King Richard III. Nat Commun 5:5631. https://doi.org/10.1038/ncomms6631
6. Kukla-Bartoszek M, Szargut M, Pośpiech E, Diepenbrock M, Zielińska G, Jarosz A, Piwieńska-Róg D, Ariszewska J, Cytaucza S, Spólnicka M, Branicki W, Osowski A (2020) The challenge of predicting human pigmentation traits in degraded bone samples with the MPS-based HiirisPlex-S system. Forensic Sci Int Genet 47:10232. https://doi.org/10.1016/j.fsigen.2020.102301
7. Chaitanya L, Breslin K, Zuniga S, Wirken L, Pośpiech E, Kukla-Bartoszek M, Sijen T, Knijff P, Liu F, Branicki W, Kayser M, Walsh S (2018) The HiirisPlex-S system for eye, hair and skin colour prediction from DNA: introduction and forensic developmental validation. Forensic Sci Int Genet 35:123–135. https://doi.org/10.1016/j.fsigen.2018.04.004
8. Claes P, Roosenboom J, White JD, Swigut T, Sero D, Li J, Lee MK, Zaidi A, Mattern BC, Liebowitz C, Pearson L, González T, Leslie EJ, Carlson JC, Orlova E, Suetens P, Vandermeulen D, Feingold E, Marazita ML, Shaffer JR, … Weinberg SM (2018) Genome-wide mapping of global-to-local genetic effects on human facial shape. Nat Genet 50(3):414–423. https://doi.org/10.1038/s41588-018-0057-4
9. Hagenauers SP, Hill WD, Harris SE, Ritchie SJ, Davies G, Liewald DC, Gale CR, Porteous DJ, Deary IJ, Marioni RE (2017) Genetic prediction of male pattern baldness. PLoS Genet 13(2):e1006594. https://doi.org/10.1371/journal.pgen.1006594
10. Hysy PG, Valdes AM, Liu F, Furlotte NA, Evans DM, Bataille V, Visconti A, Hemani G, McMahon G, Ring SM, Smith GD, Duffy DL, Zhu G, Gordon SD, Medland SE, Lin BD, Willemsen G, Jan Hottenga J, Vuckovic D, Girotto G, … Spector TD (2018) Genome-wide association meta-analysis of individuals of European ancestry identifies new loci explaining a substantial fraction of hair color variation and heritability. Nat Genet 50(5):652–656. https://doi.org/10.1038/s41588-018-0100-5
11. Kukla-Bartoszek M, Pośpiech E, Woźniak A, Boroń M, Karłowska-Pik J, Teisseyre P, Zubańska M, Bronikowska A, Grzybowsi T, Ploski R, Spólnicka M, Branicki W (2019) DNA-based predictive models for the presence of freckles. Forensic Sci Int Genet 47:102301. https://doi.org/10.1016/j.fsigen.2019.07.012
12. Marouli E, Graff M, Medina-Gomez C, Lo KS, Wood AR, Kjaer TR, Fine RS, Lu Y, Schurmann C, Highland HM, Rieger S, Thorleifsson G, Justice AE, Lamparter D, Stirrups KE, Turcot V, Young KL, Winkler TW, Esko T, Karaderi T, … Lettre G (2017) Rare and low-frequency coding variants alter human adult height. Nature 542(7640):186–190. https://doi.org/10.1038/natur e21039
13. Pośpiech E, Chen Y, Kukla-Bartoszek M, Breslin K, Aliferi A, Andersen JD, Ballard D, Chaitanya L, Freire-Aradas A, van der Gaag KJ, Girón-Santamaría L, Gross TE, Gysi M, Huber G, Mosquera-Miguel A, Muralidharan C, Skowron M, Carracedo A, Haas C, Morling N, … EUROFORGEN-NoE Consortium (2018) Towards broadening DNA Phenotyping beyond pigmentation: improving the prediction of hair head shape from DNA. Forensic science international. Genetics 37:241–251. https://doi.org/10.1016/j.fsigen.2018.08.017
14. Pośpiech E, Kukla-Bartoszek M, Karłowska-Pik J, Zieliński P, Woźniak A, Boroń M, Dąbrowski M, Zubańska M, Jarosz A, Grzybowsi T, Ploski R, Spólnicka M, Branicki W (2020) Exploring the possibility of predicting human head hair greying from DNA using whole-exome and targeted NGS data. BMC Genomics 21(1):538. https://doi.org/10.1186/s12864-020-06926-y
15. Walsh S, Liu F, Wollstein A, Kovatsi L, Ralf A, Kosiniak-Kamysz A, Branicki W, Kayser M (2013) The HiirisPlex system for simultaneous prediction of hair and eye colour from DNA. Forensic Sci Int Genet 7(1):98–115. https://doi.org/10.1016/j.fsigen.2012.07.005
16. Walsh S, Wollstein A, Liu F, Chakravartthy U, Rahu M, Seland JH, Soubrane G, Tomazzoni L, Topouzis F, Vingerling JR, Vioque J, Fletcher AE, Ballantyne KN, Kayser M (2012) DNA-based eye colour prediction across Europe with the IrisPlex system. Forensic Sci Int Genet 6(3):330–340. https://doi.org/10.1016/j.fsigen.2011.07.0009
17. de Los Campos G, Vazquez AI, Hsu S, Lello L (2018) Complex-trait prediction in the era of big data. Trends Genet 34(10):746–754. https://doi.org/10.1016/j.tig.2018.07.004
18. Haste T, Tibshirani R, Friedman J (2001) The elements of statistical learning. Springer, New York Inc, New York
19. Winham SJ, Colby CL, Freimuth RR, Wang X, de Andrade M, Huebner M, Biernacka JM (2012) SNP interaction detection with Random Forests in high-dimensional genetic data. BMC Bioinformatics 13:164. https://doi.org/10.1186/1471-2105-13-164
20. Guyon I, Elisseeff A (2003) An introduction to variable and feature selection. J Mach Learn Res 3:1157–1182
21. Andersen JD, Johansen P, Harder S, Christoffersen SR, Delgado MC, Henriksen ST, Nielsen MM, Sorenson E, Ullum H, Hansen T, Dahl AL, Paulsen RB, Bøtstrup C, Morling N (2013) Genetic analyses of the human eye colours using a novel objective method for eye colour classification. Forensic Sci Int Genet 7(5):508–515. https://doi.org/10.1016/j.fsigen.2013.05.003
22. Kryskowiak I, Lenart J, Debksi K, Kuterba P, Petas M, Kaminska B, Dabrowski M (2013) Nencki genomics database—Ensembl function enhanced with intersections, user data and genome-wide TFBS motifs. Database J Biol Databases Curation 2013:bat069. https://doi.org/10.1093/database/bat069
23. FANTOM Consortium and the RIKEN PMI and CLST (DGT), Forrest AR, Kawai H, Rehli M, Baille J, de Hoon M, Haberle V, Lassman T, Kulakovskiy IV, Lizio M, Itoh M, Andersson R, Mungall CJ, Mechan TF, Schreiner S, Bertin N, Jørgensen M, Dimont E, Arner E, Schmidl C, ... Hayashizaki Y (2014) A promoter-level mammalian expression atlas. Nature 507(7493):462–470. https://doi.org/10.1038/nature13182
24. Hoggart CJ, Whittacker JC, De Iorio M, Balding DJ (2008) Simultaneous analysis of all SNPs in genome-wide and sequencing association studies. PLoS Genet 4(7):e1000130. https://doi.org/10.1371/journal.pgen.1000130
25. Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19(6):716–723. https://doi.org/10.1109/TAC.1974.1100705
26. Schwarz GE (1978) Estimating the dimension of a model. Ann Stat 6(2):461–464. https://doi.org/10.1214/aos/1176344136
27. Tibshirani R (1996) Regression shrinkage and selection via the Lasso. J Roy Stat Soc: Ser B (Methodol) 58:267–288
28. Dobbin KK, Simon RM (2011) Optimally splitting cases for training and testing high dimensional classifiers. BMC Med Genomics 4:31. https://doi.org/10.1186/1755-8794-4-31
29. Lee HY (2021) Application of SNP-based DNA phenotyping to archaeological and forensic cases. In: Shin DH, Bianucci R (eds) The Handbook of Mummy Studies. Springer, Singapore.
30. D’Souza-Scott C, Bosshans RL, McDonough M, Stahl PD, Van Aelst L (1997) A role for POR1, a Rac1-interacting protein, in ARF6-mediated cytoskeletal rearrangements. EMBO J 16(7):5455–5454. https://doi.org/10.1093/emboj/16.17.5455
31. Scott GA, Cassidy L (1998) Rac1 mediates dendrite formation in response to melancortin stimulating hormone and ultraviolet light in a murine melanoma model. J Invest Dermatol 111(2):243–250. https://doi.org/10.1046/j.1523-1747.1998.00276.x
32. You DJ, Park CR, Furlong M, Koo O, Lee C, Ahn C, Seong JY, Hwang Ji (2015) Dimer of arafaptin 2 regulates NF-kB signaling by interacting with IKKβ/NEMO and inhibiting IKKβ kinase activity. Cell Signal 27(11):2173–2181. https://doi.org/10.1016/j.cellsig.2015.08.012
33. Fu C, Chen J, Lu J, Yi L, Tong X, Kang L, Pei S, Ouyang Y, Jiang L, Ding Y, Zhao X, Li S, Yang Y, Huang J, Zeng Q (2020) Rules of inflammation in melanogenesis (Review). Mol Med Rep 21(3):1421–1430. https://doi.org/10.3892/mmr.2020.10950
34. Cheng SL, Liu RH, Sheu JN, Chen ST, Sinchaikul S, Tsay GJ (2007) Toxicogenomics of A375 human malignant melanoma cells treated with arbutin. J Biomed Sci 14(1):87–105. https://doi.org/10.1007/s11373-006-9130-6
35. Sarkar R, Arora P, Garg KV (2013) Cosmeceuticals for hyper-pigmentation: what is available? J Cutan Aesthet Surg 6(1):4–11. https://doi.org/10.4103/2007-2077.110089
36. Boyle EA, Li YI, Pritchard JK (2017) An expanded view of complex traits: from polygenic to omnigenic. Cell 169(7):1177–1186. https://doi.org/10.1016/j.cell.2017.05.038
37. Zhang R, Zhao Y, Chu M, Wu C, Jin G, Dai J, Wang C, Hu L, Gou J, Qian C, Bai J, Wu T, Hu Z, Lin D, Shen H, Chen F (2013) Pathway analysis for genome-wide association study of lung cancer in Han Chinese population. PLoS ONE 8(3):e57763. https://doi.org/10.1371/journal.pone.0057763
38. Yuasa I, Umetsu K, Harihara S, Miyoshi A, Saitou N, Park KS, Dashyam B, Jin F, Lucotte G, Chattopadhyay PK, Henke L, Henke J (2007) OCA2 481Thr, a hypofunctional allele in pigmentation, is characteristic of northeastern Asian populations. J Hum Genet 52(8):690–693. https://doi.org/10.1007/s10038-007-0167-9
39. Sviderskaya EV, Bennett DC, Ho L, Bailin T, Lee ST, Spritz RA (1997) Complementation of hypopigmentation in p-mutant (pink-eyed dilution) mouse melanocytes by normal human P cDNA, and defective complementation by OCA2 mutant sequences. J Invest Dermatol 108(1):30–34. https://doi.org/10.1111/j.1523-1747.ep12285021
40. Andersen JD, Pietroni C, Johansen P, Andersen MM, Pereira V, Bøtstrup C, Morling N (2016) Importance of non synonymous OCA2 variants in human eye color prediction. Mol Genet Genomic Med 4(4):420–430. https://doi.org/10.1002/mgg3.213
41. Meyer OS, Salvo NM, Kjerbye A, Kjersem M, Andersen MM, Sorenson E, Ullum H, Janssen K, Morling N, Bøtstrup C, Olsen GH, Andersen JD (2021) Prediction of Eye Colour in Scandinavians Using the EyeColour 11 (EC11) SNP Set. Genes 12(6):821. https://doi.org/10.3390/genes1206082
42. Liu F, van Duijn K, Vingerling JR, Hofman A, Uitterlinden AG, Janssens AC, Kayser M (2009) Eye color and the prediction of complex phenotypes from genotypes. Curr Biol 19(5):R192–R193. https://doi.org/10.1016/j.cub.2009.01.027
43. Larsson M, Duffy DL, Zhu G, Liu JZ, Macgregor S, McRae AF, Wright MJ, Sturm RA, Mackey DA, Montgomery GW, Martin NG, Medland SE (2011) GWAS findings for human iris patterns: associations with variants in genes that influence normal neurlural pattern development. Am J Hum Genet 89(2):334–343. https://doi.org/10.1016/j.ajhg.2011.07.011
44. Eriksson N, Macpherson JM, Tung JY, Hon LS, Naughton B, Saxonov S, Avey L, Wojcicki A, Pe’er I, Mountain J (2010) Web-based, participant-driven studies yield novel genetic associations for common traits. PLoS Genet 6(6):e1000993. https://doi.org/10.1371/journal.pgen.1000993
45. Ruiz Y, Phillips C, Gomez-Tato A, Alvarez-Dios J, Casares de Cal M, Cruz R, Maroñas O, Söchting J, Fondeliva M, Rodriguez-Cid MJ, Carracedo A, Lareu MV (2013) Further development of forensic eye color predictive tests. Forensic Sci Int Genet 7(1):28–40. https://doi.org/10.1016/j.fsigen.2012.05.009
46. Pośpiślic E, Wojas-Pelec A, Walsh S, Liu F, Maeda H, Ishikawa T, Skowron M, Kayser M, Branicki W (2014) The common occurrence of epistasis in the determination of human pigmentation and its impact on DNA-based pigmentation phenotype prediction. Forensic Sci Int Genet 11:64–72. https://doi.org/10.1016/j.fsigen.2014.01.012
47. Christodoulou E, Ma J, Collins GS, Steyerberg EW, Verbakel JY, Van Calster B (2019) A systematic review shows no performance benefit of machine learning over logistic regression for
clinical prediction models. J Clin Epidemiol 110:12–22. https://doi.org/10.1016/j.jclinepi.2019.02.004

48. Bellot P, de Los Campos G, Pérez-Enciso M (2018) Can deep learning improve genomic prediction of complex human traits? Genetics 210(3):809–819. https://doi.org/10.1534/genetics.118.301298

49. Lynam AL, Dennis JM, Owen KR, Oram RA, Jones AG, Shields BM, Ferrat LA (2020) Logistic regression has similar performance to optimised machine learning algorithms in a clinical setting: application to the discrimination between type 1 and type 2 diabetes in young adults. Diagn Prognostic Res 4:6. https://doi.org/10.1186/s41512-020-00075-2

50. Couronné R, Probst P, Boulesteix AL (2018) Random forest versus logistic regression: a large-scale benchmark experiment. BMC Bioinformatics 19(1):270. https://doi.org/10.1186/s12859-018-2264-5

51. Lello L, Avery SG, Tellier L, Vazquez AI, de Los Campos G, Hsu S (2018) Accurate genomic prediction of human height. Genetics 210(2):477–497. https://doi.org/10.1534/genetics.118.301267

52. Aliferi A, Ballard D, Gallidabino MD, Thurtle H, Barron L, Syndercombe Court D (2018) DNA methylation-based age prediction using massively parallel sequencing data and multiple machine learning models. Forensic Sci Int Genet 37:215–226. https://doi.org/10.1016/j.fsigen.2018.09.003

53. Xu C, Qu H, Wang G, Xie B, Shi Y, Yang Y, Zhao Z, Hu L, Fang X, Yan J, Feng L (2015) A novel strategy for forensic age prediction by DNA methylation and support vector regression model. Sci Rep 5:17788. https://doi.org/10.1038/srep17788

54. Spólnicka M, Pośpiech E, Peplońska B, Zbieć-Piekarska R, Makowska Z, Pięta A, Karlowska-Pik J, Ziemkiewicz B, Wężyk M, Gagerowicz P, Bednarzuk T, Barcikowska M, Żekanowski C, Płoski R, Branički W (2018) DNA methylation in ELOVL2 and C1orf132 correctly predicted chronological age of individuals from three disease groups. Int J Legal Med 132(1):1–11. https://doi.org/10.1007/s00414-017-1636-0

55. Nauj J, Hoefsloot H, Mook O, Rijlaarsdam-Hoekstra L, van der Zwalm M, Henneman P, Kloosterman AD, Verschure PJ (2017) Chronological age prediction based on DNA methylation: massive parallel sequencing and random forest regression. Forensic Sci Int Genet 31:19–28. https://doi.org/10.1016/j.fsigen.2017.07.015

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.