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Original paper

Pair Matcher (PaM): fast model-based optimisation of treatment/case-control matches

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Abstract

Motivation: In clinical trials, individuals are matched using demographic criteria, paired, and then randomly assigned to treatment and control groups to determine a drug's efficacy. A chief cause for the irreproducibility of results across pilot to Phase III trials is population stratification bias caused by the uneven distribution of ancestries in the treatment and control groups.

Results: Pair Matcher (PaM) addresses stratification bias by optimising pairing assignments a priori and/or a posteriori to the trial using both genetic and demographic criteria. Using simulated and real datasets, we show that PaM identifies ideal and near-ideal pairs that are more genetically homogeneous than those identified based on competing methods, including the commonly used principal component analysis (PCA). Homogenising the treatment (or case) and control groups can be expected to improve the accuracy and reproducibility of the trial or genetic study. PaM's ancestral inferences also allow characterizing responders and developing a precision medicine approach to treatment.

Availability: PaM is freely available via R <https://github.com/eelhaik/PAM> and a web-interface at <http://elhaik-matcher.sheffield.ac.uk/ElhaikLab/>.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

It is well recognized that pharmaceutical research and development (R&D) is in crisis. The number of new drugs approved per billion US dollars spent on R&D has halved roughly every nine years since 1950 (Scannell et al. 2012) as spending in the industry has inflated to an average of ~\$5.8 billion per drug in 2011 compared to \$1.3 billion per drug in 2005 (Roy 2012). The latter phases of clinical trials test the drug's efficacy compared to a placebo or other treatments in a randomised trial setting and require assessing tens, hundreds (Phase II trials), and eventually tens of thousands (Phase III trials) of volunteers over a long period of time to prove that there is substantial evidence of a clinical benefit of the drug. Only one in 12 drugs that enters human clinical trials ends up gaining approval from the FDA. It is acknowledged that one of the biggest drivers of the increase in R&D costs is the regulatory process governing Phase-III clinical trials of new pharmaceuticals (Roy 2012). As the regulatory environment is unlikely to relax (Scannell et al. 2012), it is important to

understand why randomised control trials may be more successful in smaller trials.

Matching treatment (or case) with control groups is the most elementary and critical part of any trial (or study). Mismatched groups introduce genetic heterogeneity that may obscure performance of the trialed drug, for example, due to genetic predisposition to response to the treatment, and result in reduced reproducibility between different cohorts (Scannell et al. 2012). Currently, individuals are matched based on demographic criteria (e.g., age, gender, and self-reported "race") and then randomly assigned to treatment and controls groups. It is well acknowledged that due to the significant heterogeneity among humans, demographic-based matching alone is inadequate. Trials are, thereby, vulnerable to 'stratification bias,' i.e., differences in genetic ancestry between individuals, which are not factored in when trial participants are grouped based on demographics alone. This undetected bias may contribute to biased interpretation of trial results due to lack of genetic information that may confound interpretation, lead-

ing to alterations in the false negative or false positive results, with subsequent financial and patient health consequences (Fig S1). In large groups, the stratification bias may be less pronounced, however, it is practically unavoidable in the case of rare diseases due to the difficulties in recruiting genetically homogeneous participants (Yusuf and Wittes 2016). Crucially, this bias is more severe in small cohorts, leading to an applied misinterpretation of the drug's efficacy that will be difficult to replicate in larger trials.

Population stratification can be addressed by optimising the treatment-control matches a priori or/and a posteriori to the trial using a variety of tools applied to the genotype data and selecting matched pairs for downstream analyses. Due to the historically high cost of genotyping and sequencing, a priori methods rely heavily on demographic-based matching criteria followed by statistical corrections made a posteriori, if at all. A priori methods have long been considered biased, inaccurate, and unhelpful (De Bono 1996; McAuley et al. 1996; Fustinoni and Biller 2000) due to their reliance on self-reported "race" ("Africans," "Asians," and "European-Americans" or "Whites") or regional similarity, which does not eliminate the bias (Campbell et al. 2005; Wang, Localio, and Rebbeck 2006; Chikhi et al. 2010; Elhaik et al. 2014; Yusuf and Wittes 2016). Unable to completely account for choices made at the a priori stage, a posteriori methods may make over-simplified, unrealistic, or problematic assumptions (Kimmel et al. 2007), particularly concerning population structure. Computing the principal components (PCs) of the genotype matrix and adjusting the genotype vectors by their projections on the PCs is a popular method of accounting for population structure (Price et al. 2006). However, linear projections cannot be assumed to sufficiently correct for the effect of stratification due to other unaccounted confounders (Kimmel et al. 2007). PCs also ignore the complexity of population structure, are influenced by uneven sampling, and cannot properly represent individuals of mixed origins (McVean 2009; Yang et al. 2012; Elhaik et al. 2014; Lacour et al. 2015). Even newer tools (Epstein, Allen, and Satten 2007; Kimmel et al. 2007; Lacour et al. 2015) can make only basic assumptions concerning population structure and may ignore admixture or demographic criteria.

We developed Pair Matcher (PaM) – A genetic-based tool that optimises pairing assignments a priori and/or a posteriori to the trial. PaM matches samples by demographic and genetic criteria and allows trial designers to make informed decisions in real time (Fig S1). PaM models individual genomes as consisting of gene pools (or admixture components) that correspond to their recent demographic history (Elhaik et al. 2014; Das et al. 2016). PaM then matches individuals based on their age, gender, and the similarity of their admixture components. We first compared the accuracy of PaM_{simple} and PaM_{full} and then the accuracy of the best performing tool to pairings made either at random, based on racial-criteria, or through PC analysis (PCA). Finally, we compared PaM's pairing accuracy to that of clustering tools used in population genetic and genome-wide association studies (GWAS) in analyzing unmixed and mixed individuals. We also assessed reproducibility.

Optimising the trial design can be expected to homogenize the treatment (or case) and control pairs and improve the accuracy and reproducibility of the trial or genetic study. This can be expected to lower drug developmental costs and benefit patients. Together with biogeographical tools that can predict the geographical origins of the responders (Elhaik et al. 2014),

PaM can also be used to guide precision medicine approaches to treatment, for instance, in characterizing a subgroup of responders or mutation carriers (Baughn et al. 2018) and designing follow up trials focusing on this group.

2 Methods

2.1 Simulated population datasets

We generated 24 datasets that comprised of 980-1000 individuals each in ADMIXTURE's Q file format (individuals x proportion of admixture components) (Alexander, Novembre, and Lange 2009). Here and throughout this work, we adopted the admixture model of Elhaik et al. (2014) of nine admixture components representing: North East Asia, the Mediterranean, South Africa, South West Asia, Native America, Oceania, South East Asia, Northern Europe, and Sub-Saharan Africa. Each dataset consisted of a file with nine admixture components generated randomly for individuals and their matching pairs and normalized so that each row would sum to 1. Dataset 1 consisted of 500 identical pairs (Fig S2). The genetic heterogeneity between the pairs of Datasets 2-8 was increased in a controlled manner by modifying the admixture components of one individual from each pair of Dataset 1 through a perturbation of X [0...20%] subtracted from the odd numbered admixture components and added to the even numbered admixture components. The perturbation percentage was applied alternately (negative to the first component, positive to second component etc.) to prevent normalization to undo the perturbation.

To assess pairing in less ideal datasets, the remaining datasets were created by removing random individuals from the original datasets. Datasets 9-16 were created by removing one individual from each cohort (remove-1) and Datasets 17-24 were created by removing 20 individuals from each cohort (remove-20), leaving datasets of 999 and 980 individuals, respectively.

2.2 Worldwide population dataset

We used the Genographic dataset that comprises of ~128,000 markers genotyped in 633 unrelated worldwide individuals of known geographic origins who have four grandparents from their population affiliation and geographic region of origin (Elhaik et al. 2014). We created 13 two-way mixed individuals by hybridizing 13 Indians with 13 British to yield a final cohort of 646 individuals. The hybridization was done by merging an even amount of random SNPs from random Indian and British individuals and calculating the admixture components of these genomes (Elhaik et al. 2014). The admixture components of the Genographic and simulated individuals (Elhaik et al. 2014, Figure 1) were provided as input to PaM. We also analysed 40 Bedouin and 40 Pakistani (25 Brahui and 15 Burusho) individuals (Patterson et al. 2012) and calculated their nine admixture components as in Elhaik et al. (2014).

2.3 Unmixed and mixed population datasets

We used the Lazaridis et al. (2014) dataset that comprises of ~600,841 markers genotyped in 2,345 unrelated worldwide individuals. From each population that had at least four individuals, we selected two pairs of individuals who showed the highest identity-by-state (IBS) similarity to

Pair Matcher (PaM)

each other as inferred by PLINK (`-cluster -matrix`) (Purcell et al. 2007). From the 42 populations (168 individuals) identified in that manner, 100 random individuals were paired to a random member of their population, creating the unmixed dataset ($n=200$). A mixed dataset was next created by randomly selecting three individuals and using the consecutive thirds of their genomes to create a 3x hybrid individual. A matching pair was created in a similar way using different individuals from the same populations. This process was repeated 100 times ($n=200$). Similarly, we created x5 and x7 datasets of the same size. Finally, we assembled three combined datasets that consist of: unmixed + 3x mixed ($n=400$), the latter dataset + 5x mixed ($n=600$), and the latter dataset + 7x mixed ($n=800$).

2.4 Comparing PaM_{simple} and PaM_{full}

To optimise matches, PaM analyses the age (optional), gender (optional), and admixture components for each individual in the studied cohort. These three parameters are obtained from PLINK's fam file (using columns 4 [age] and 6 [gender]) and ADMIXTURE's Q file. The Genetic Distance (GD) between the paired individual is defined as $\sqrt{\sum_k (\hat{t}_k - \hat{j}_k)^2}$, where i and j are the individuals with k admixture components. PaM calculates the $n \times n$ GD matrix for each possible pairing, where n is the number of individuals in the Q file. Each element of the matrix, specified by row i and column j , corresponds to a pair (i, j) . The matrix is symmetric with respect to the diagonal, which contains all zeros. A corresponding $n \times n$ score matrix is calculated as follows: pairs that are age (within five years by default) and gender matched get one point. Nine additional points are awarded for every matching admixture component if $|\hat{t}_k - \hat{j}_k| \leq 1\%$ for the pair (i, j) . An ideally matched pair has a score of 10 (age/gender and nine admixture components). An optimal pairing solution for Dataset 1 that consists of 500 pairs would be a GD of 0 between all pairs, a total score of 5,000 (top score of 10 for 500 pairs), and no unpaired individuals (Fig S3).

PaM operates in two modes: PaM_{simple} and PaM_{full}. The PaM_{simple} algorithm starts by selecting matrix row 1 (individual 1) and finding the column j which yields the minimum GD for pair $(1, j)$. This matrix element corresponds to the first pair with row index 1 and column index j . Row 1 and column j and their symmetric element $(j, 1)$ are removed from the GD matrix. Row 2 is next selected (provided it has not been removed in the previous step) and the column which yields the minimum GD is selected to form the second pairing. The corresponding rows and columns are then removed from the matrix. The optimisation proceeds until all possible pairings are created and all unpaired individuals are stored. If the test cohort is an odd number then at least one unpaired individual is expected. The paired and unpaired individuals are reported in separate text files. To filter pairings with a score lower than a specified acceptable value, a user-controlled threshold was implemented.

The threshold is related to the expected genetic homogeneity of the pairs. A high threshold would result in homogeneous pairs and a large number of unpaired individuals. A threshold of 7 indicates that the pair's age and gender matched as well as 6 of their admixture components.

When matrix rows have multiple identical minima, there is a potential dilemma since the specific row minimum selection could affect subsequent pairings (due to the row/column removal upon pair selection), and the final pairing solution may not be optimal. We explored different selection schemes through exhaustive testing using single and random selection of

the row minima, as well as a more complex method of minimising the sum of the remaining row minima, however, the end results were very similar. Therefore, PaM uses a single minimum selection for each row, and this selection is the minimum with the lowest column index j .

PaM_{full} extends PaM_{simple} by carrying out a more exhaustive pairing search. PaM_{full} sorts the test cohort data iteratively in ascending order using the admixture components. The pairing procedure starts at a random row index (multiple times). The model starts by sorting the cohort data by the first admixture component then commences the search starting with a random row, i , index. The best pairing solution is stored. The cohort data is next sorted by the second admixture component, and the best pairing solution is found. If this solution minimizes the total GD of the final solution compared to the previous iteration then, the 'sorted admixture component 2' solution is stored. The model proceeds by successively sorting the remaining admixture components to find the best pairing solution. Poor pairs are handled in a similar manner to PaM_{simple}. However, when the data are re-ordered, all previously discarded individuals are included in the new solution search.

2.5 Comparing PaM with demographic matches

PaM matches for the simulated datasets (2.1) were compared with a priori matches based on age (within 5 years), gender, and "race" defined as "African," "Asian," "Latino," or "White." Following Elhaik et al. (2014, Figure 1), ancestry was inferred from the admixture components as follows: "African" ancestry was assigned if the sum of Sub-Saharan Africa and South Africa admixture components was larger than 50%; "Asian" ancestry was assigned when the North East Asian component was larger than 10%; and "Latino" ancestry was assigned when the Native American component was larger than 50%. All the remaining individuals were considered "White." Since self-reported "race" differs between studies, we considered three models: i) an individual is either African, Asian, Latino, or White. ii) an individual is considered either an African or non-African; iii) an individual is considered a mixture of Africans, Asians, Latinos, and Whites. The assignment accuracy of all matches was measured based on the correct pairing of individuals and their known pairs with some individuals expected to be unpaired due to the removal of their exact match.

2.6 Comparing PaM with PCA matches

PaM matches for the Genographic dataset (2.2) were compared with PCA-based ones. PCA's top two eigenvectors were calculated using SNPRelate (Zheng et al. 2012). These eigenvectors were clustered using the k -means method `kmeans` in R. Similarly to Luca et al. (2008), pairs were determined by a random assignment within each cluster. To compare the quality of the results, the pairing solutions for PaM and PCA were evaluated using IBS clusters as an impartial genetic distance independent of admixture or PCA and geographic distances calculated with the Haversine formula (Gellert et al. 1989). Due to the data's high heterogeneity, PaM was used with threshold of 5.

2.7 Assessing PaM's performances on small datasets

To evaluate PaM's performances on small datasets (2.2), we constructed four datasets consisting of 20, 40, 60, and 80 individuals. Each dataset consisted of an even number of Bedouins and Pakistanis. We applied PaM_{simple} without threshold to each dataset. To examine their effect, we also applied PaM with various thresholds to the largest dataset.

2.8 Comparing PaM with various clustering tools on unmixing and mixed individuals

PaM matches were compared with those of several clustering tools: PCA and multidimensional scaling (MDS), both available from PLINK, whose pairs were calculated as in 2.6; and genetic relationship matrix (GRM) (Yang et al. 2011) (version 1.91.2beta) and TreeMix (Pickrell and Pritchard 2012) (version 1.13), whose pairs were identified using a greedy approach that paired individuals with the highest covariance. All the tools were assessed by their ability to match individuals in each dataset and reproduce the results in the combined datasets. The tools were applied to the complete and LD pruned (PLINK command `--indep 50 5 2`) datasets and to the SNPs that overlapped PaM's gene pools, which consist of ancestry informative markers (AIMs). PaM was utilized with three thresholds.

3 Results

3.1 Assessing PaM's performances

We first evaluated the performances of PaM_{simple} without a threshold (no limit placed on the acceptable score of pairs) and with a threshold of 7 (necessitating the matching of age/gender and at least 6 admixture components) across all simulated datasets. As expected, when applying PaM without a threshold, the GD increased with increasing perturbation or heterogeneity while the score decreased, and the number of misassigned pairs increased (Fig 1, Table S1). However, despite the perturbation and removal of individuals, most of the original pairs (80-100%) were correctly identified, particularly in Datasets 1-16. The increase in the number of misassigned pairs is related to how PaM_{simple} searches for an optimum solution. PaM_{simple} selects a case index *i* (row *i* of the GD matrix) and finds the best match for this index by locating the minimum GD in the row corresponding to the best possible match. This, however, does not constitute an ideal match considering all other individuals, some of whom are best left unpaired. Since PaM_{simple} does not leave any individual unpaired (for an even cohort), a poor pairing may create a "snowball" effect triggering other poor pairings, resulting in an overall increased GD and reduced score for the final pairing solution.

We addressed this problem by applying a threshold of 7 on the match score. Under these settings, the GD curve decreased sharply at a perturbation level of ~5%; identifying genetically homogeneous pairs, despite the increased perturbation, and discarding genetically mismatched pairs. The score decreased due to the growing number of unpaired individuals, which represents the conservative choice of what is considered an acceptable pair. The trade-off for the low GD of acceptable pairs is that more individuals are left unpaired due to their low pairing score and are omitted from the total GD score (Fig 1, Table S1). The advantage of applying a threshold is that it reduces the number of

misassigned pairs by allowing only pairs with a high match score (genetically homogeneous). This prevents the model from selecting pairs that satisfy the low GD minimum but do not have a favourable match score, thus avoiding the "snowball" effect. Since the matrix is symmetric and each row has all possible pairing for each individual, individuals with a match score lower than the threshold are considered too genetically heterogeneous and placed on the unpaired list (Table S2).

For Datasets 1-8, the best solution was obtained with a threshold at a perturbation level of 11%, where the GD was ~0 for all matched pairs, nearly half the individuals had an acceptable score, and the number of misassigned pairs was 0. We note that for heavier perturbations, not all the misassignments are false positives since the perturbation created, by chance, more suitable pairing matches than the predefined ones. Considering the low GD between all pairs, the majority of matches were near-optimal ones even after removing individuals from the dataset. Interestingly, we observed a repeated single misassignment in most of the remove-20 datasets (Table S2). Examination of this unexpected misassignment showed it to be a pairing with a very low GD and a match score of 7, making it an acceptable assignment though not between the original partners, which could potentially be suboptimal.

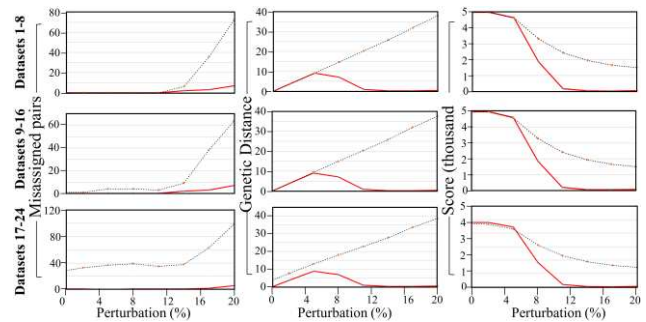


Fig 1. PaM_{simple} performances on simulated datasets. Rows show the results of eight perturbed datasets (full dataset [left], remove-1 [center], and remove-20 [right]). PaM_{simple} was applied without a threshold (dashed) and with a threshold of 7 (solid red). Columns show the number of individuals assigned to a different pair than their original counterpart per dataset (x-axis), total GD between all matched pairs, and total score (maximum of 10 per pair with each dataset having 500-480 pairs).

There are two ways to address the vexing issue of 'rogue' misassignments. The first is to set a higher threshold, and the second is to use PaM_{full}, which carries out a more exhaustive pairing search by iteratively sorting (in ascending order) the cohort data by the admixture components. The pairing procedure for the cohort commences at a random row index (multiple times). This approach does not produce rogue misassignments and hence finds an optimum or near-optimum pairing solutions. The numerical results for the three datasets using PaM_{full} are shown in Tables S3 and S4. PaM_{full} results are similar to those of PaM_{simple}, except that they do not allow the accidental misassignments (Table S4, perturbation <11%) observed with PaM_{simple} (Table S2).

As before, the misassignments detected beyond the 11% threshold are due to the high similarity in admixture components in the post-perturbation stage and are not truly false positives. The cost of using PaM_{full} is increased computation time, almost an order of magnitude greater than PaM_{simple}'s run time. Due to its superior performances, the remaining analyses were done with PaM_{simple}.

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3.2 Comparing the performances of PaM and alternative methods on simulated datasets

We next compared the assignment accuracy of PaM_{simple} and alternative solutions in terms of misassigned pairs with the GD and Score illustrating the quality of the matches (Fig 2). PaM correctly identified nearly all pairs. The GDs for the random assignment, where the age and gender matched but “race” was randomly determined, were much larger than the competing solutions. Correspondingly, the random assignment’s score is mostly lower than the alternative solutions. Nearly none of the pairs randomly assigned were with their original counterparts.

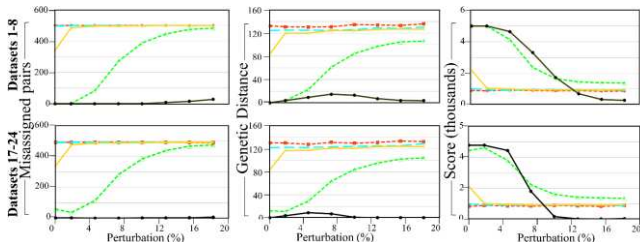


Fig 2. PaM_{simple} (threshold of 7) performances on 16 simulated datasets against five competing methods. Columns show the number of misassigned individuals, total GD, and pair score for Random assignment (red), Race model 1 (cyan), Race model 2 (yellow), Race model 3 (green), and PaM (black). Results for Datasets 9-16 were identical to those of Datasets 1-8 and are not shown.

The first two self-reported “race” models (African, Asian, Latino, or White; African/non-African) perform only slightly better than the random assignment in terms of GD and the score. The results of the third model (mixtures of African, Asian, Latino, or White) are considerably better than the previous models or random assignments. This is to be expected, since this model can be considered a reduced form of PaM’s nine-admixture components model. Our results indicate that pairs obtained through standard demographics criteria (age, gender, and self-defined “race”) are as poor as those obtained at random. We note, that since the simulated datasets comprised of the same admixture components used by PaM, the performances observed in simulation may not reflect the algorithm’s accuracy for real populations.

3.3 Comparing PaM’s performances with PCA’s

We next compared the performances of PaM with a PCA-based approach on the Geographic dataset consisting of worldwide individuals alongside 13 simulated Indian-British individuals. PaM was applied to the admixture components of all individuals and PCA was applied to the SNP data.

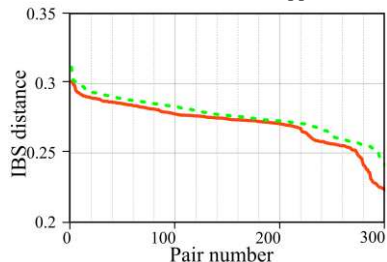


Fig 3. IBS distance between PaM (solid) and PCA (dashed) inferred pairs.

We evaluated the homogeneity of the pairs inferred by PaM and PCA using both geographic and genetic distances. All of PaM’s inferred pairs had

higher genetic similarity (i.e., smaller IBS distances) than PCA’s inferred pairs (Fig 3). We identified twelve IBS clusters (Fig S4) and divided all inferred pairs to “matches” if individuals were in the same cluster and “mismatches” if otherwise. PCA pairs had 270 “matches” and 46 “mismatches” with mean distances of 1,042km and 6,124km, respectively, and 10 unpaired individuals (Fig 4). PaM pairs had 284 “matches” and 17 “mismatches” with mean distances of 484km and 557km, respectively, and 40 unpaired individuals. Compared to PCA, individuals matched by PaM were significantly geographically closer regardless of the category (Kolmogorov-Smirnov goodness-of-fit test, $p\text{-value}_{(\text{matches})}=2.74 \times 10^{-6}$, $p\text{-value}_{(\text{mismatches})}=3.58 \times 10^{-4}$, $p\text{-value}_{(\text{all})}=4.85 \times 10^{-10}$). In one “match” case, PaM paired an individual from Papua New Guinea with a Peruvian, which yielded a geographic distance of over 13,000 km. However, Skogland et al. (2015) showed that some native American populations can trace their origins to Papua New Guinea, suggesting that PaM’s assignment was appropriate. The 13 mixed Indo-British individuals formed a part of the Tartar/Tajikistan IBS cluster (Fig S4). PCA paired the Indo-British individuals with people from Tajikistan (4), Iran (2), Tatar (1), Russia (1), Ingush (1) and India (1). It correctly made one Indo-British pair and left out one individual. By contrast, PaM formed 6 Indo-British pairings, leaving the 13th individual unpaired (although the Indo-British were part of the same IBS cluster consisting of Tartars and Tajikistanians). Overall PaM produced pairs that are genetically (Fig 3) and geographically (Fig 4) significantly more homogeneous than PCA. These results highlight the accuracy of PaM and its ability to handle admixed individuals.

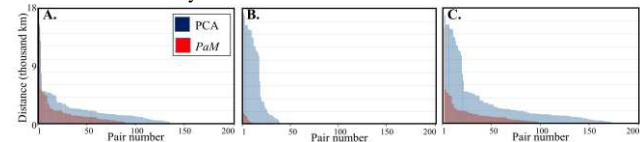


Fig 4. The geographical distance between individual pairs inferred by PaM and PCA. Geographic distances are calculated between pairs where both individuals are within the IBS-defined clusters (A), where individuals are in different clusters (B), and for all individuals regardless of cluster assignment (C).

3.4 Evaluating PaM’s performances on small datasets

Applied to datasets ranging from 20 to 80 individuals of Bedouin and Pakistani descent (Fig S5), PaM (no threshold) perfectly paired all individuals with members of their populations each time. Applying PaM with higher thresholds to the largest dataset created slightly fewer pairs but more genetically homogeneous ones (Table S5).

3.5 Comparing the performances of PaM and various clustering tools for unmixed and mixed individuals

Clustering accuracy is typically demonstrated by showing that well-curated individuals are predicted to geographic regions, whereas mixed individuals are more challenging to analyse under various population and data settings. Here, we evaluated the pairing accuracy of five tools that implement different clustering strategies in datasets that consist of unmixed and mixed individuals and combinations of those datasets. PaM significantly outperformed all tools in each test (Fig 5, Table S6), except in comparison to MDS in the 3x Mixed dataset, with an average accuracy

of $87\pm 9\%$ compared to PCA ($68\pm 16\%$), MDS ($72\pm 14\%$), GRM ($29\pm 16\%$), and TreeMix ($7\pm 18\%$). The accuracy for PCA and MDS varied with the number of loadings used. The pairing with both 10 ($76\pm 15\%$ [PCA] and $79\pm 17\%$ [MDS]) and 20 ($71\pm 9\%$ [PCA] and $78\pm 9\%$ [MDS]) loadings was similar and higher than with two loadings ($58\pm 10\%$ [PCA] and $57\pm 10\%$ [MDS]). MDS outperformed PCA in nearly every test. TreeMix performed the worst. When admixed individuals were provided TreeMix reports were highly inaccurate (see a simplistic example in Fig. S6). The combined datasets (unmixed + mixed), designed to test reproducibility, proved challenging with PaM exhibiting the smallest drop in average accuracy (-5%), compared to PCA (-12%), MDS (-7%), and GRM (-9%). All tools performed better on the gene pool SNP set (59%) than on the LD-pruned (53%) and genome-wide datasets (57%).

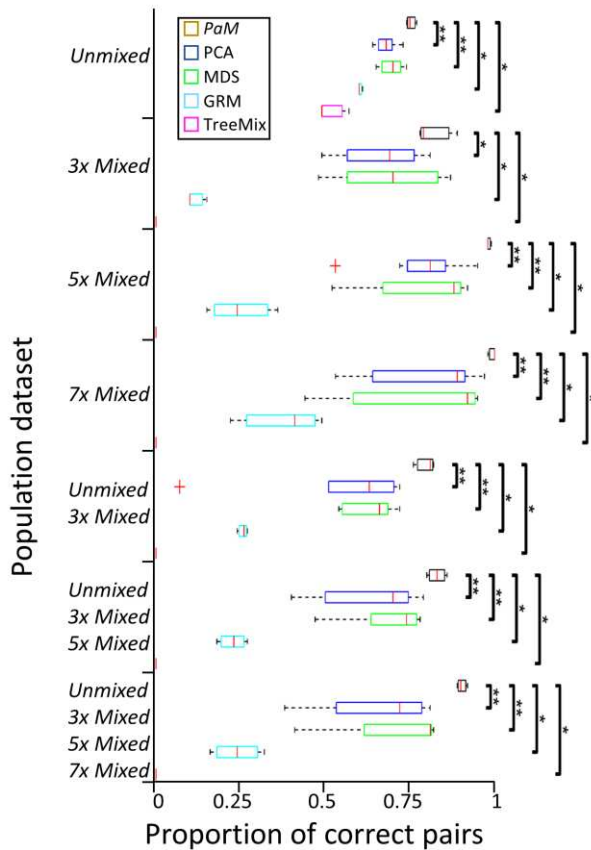


Fig. 5. Pairing accuracy for various tools across multiple datasets. Boxplots summarize the pairing accuracy of all the trials in each population dataset (Table S6), e.g., the PCA for unmixed individuals include the three analyses (PCA2/10/20) for each of the three datasets. The order of the tools' results per population dataset is shown in the legend. Significance was estimated for PaM using Wilcoxon rank-sum test (p -value ≤ 0.05 , $**\leq 0.01$).

3.6 Running time

Running on a single core Intel i5 computer, PaM_{simple} finds the near optimum pairings in ~ 15 minutes for a test cohort of 1,000 individuals, whereas PaM_{full} finds the optimised pairings in ~ 3 hours. If accessed online, results are typically emailed within 20 minutes.

3.7 Software availability

PaM is freely available as a downloadable R package from <https://github.com/elhaik/PAM> (16 Mb). In addition, a web-service has been created that allows users to upload genetic and demographic data for their test cohort in PLINK format and receive the optimised pairings solution by email (<http://elhaik-matcher.sheffield.ac.uk/ElhaikLab/>).

4 Discussion

Clinical trials are required to determine drug efficacy on multiple cohorts of sizes ranging from 40 to 10,000, where participants are split into treatment and control groups. The outcomes of these trials determine whether a drug should be tested in a larger cohort and, if successful, approved for use (Roy 2012). To evaluate the therapeutic effects of the tested drug, treatment and control pairs have to be genetically homogeneous to minimize the variation in the response that is due to different genetic backgrounds. Therefore, pairing of cohort individuals is typically done at random after controlling for demographic criteria (e.g., age, sex, and self-reported "race") a priori to the trial. However, randomisation does not resolve population stratification, particularly in very small cohorts or multiple strata with few individuals (Ganju and Zhou 2011) and the results may not be replicated in a follow up larger trial, which may disqualify an effective drug. Correcting for population stratification a posteriori to the trial is also problematic due to the difficulty in modelling ancestry and admixture and the reliance on self-defined "race," a highly unreliable predictor (De Bono 1996; Fustinoni and Biller 2000). A similar challenge exists in case-control genetic investigations intend to find a loci associated with a phenotype of interest. Unfortunately, even after decades of genetic research, the use of self-defined racial categorization is still highly prevalent in clinical setting. Though most of the genetic variation in humans is between continental populations (12%) (Elhaik 2012) who exhibit biological variety, like different drug responses, racial terminology is an ineffective mean to classify mixed people, even those believed to be unmixed due to ignorance of their demographic history (e.g., Marshall et al. 2016; Das et al. 2017). Applying various tools to unmixed and mixed datasets provided a unique view of their clustering accuracy. We demonstrated that using standard demographic criteria, such as self-reported "race" yields random results, suggesting that ancestry should be identified genetically (e.g., Baughn et al. 2018). We further showed that PCA pairs geographically and genetically suboptimal and that it is incapable of modelling mixed populations (Figs. 4 and 5), representing the vast majority of the population in countries like the USA. That PCA and PCA-like tool are still being used in GWAS and even considered the "gold standard" by some and that PCA loadings from past GWAS are being used in GWAS meta-analyses is puzzling provided PCA's known weaknesses. The uneven sampling, for instance, which exist in any dataset biases PCA predictions (McVean 2009; Elhaik et al. 2014). There is no consensus on the number of PCs to analyze: although Price et al. (2006) used a default of ten PCs and Patterson et al. (2006) advised using the Tracy–Widom statistic to determine the number of components, in practicality, authors use an arbitrary number of PCs or adopt ad hoc strategies to aid in their decision (e.g., Solovieff et al. 2010). This may be due to the high sensitivity of the Tracy–Widom statistics to linkage disequilibrium, which inflates the number of PCs (Patterson, Price, and Reich 2006) and the expectation that the PCs would

Pair Matcher (PaM)

reflect genetic similarities that are difficult to observe in higher PCs. PCA is also sensitive to the choice of markers (Table S6). The GRM estimates the genetic relationship between two individuals and is one of the core functions of the GWAS package GCTA (Yang et al. 2011). It calculates the average ratio of the covariance over the expected heterogeneity across all genes. In other words, it represents how much two individuals covary relative to what is expected on average for an average SNP. This measure is susceptible to LD and cannot be expected to handle mixed individuals. Indeed, its best performances were for the unmixed individuals (Table S6). Its prioritization over PCA (Yang et al. 2011) is, thereby, inconsistent with its low performances compared to PCA with two PCs. Remarkably, the less popular MDS outperformed PCA in almost every trial. This may be explained by the tendency of MDS to preserve pairwise distances between the points, which is in line with how the data were generated and evaluated. By contrast, PCA attempts to preserve the covariance of the data, which may be less sensitive to population structure. PCA's requirement that the data will follow a multivariate normal distribution may also pose a challenge that does not exist in MDS. Our analysis of TreeMix results was based on the covariance matrix, which limitations were already discussed, rather than on the tree's topology. This is because TreeMix's furthest assumption that the history of the sampled populations is approximately tree-like (Pickrell and Pritchard 2012) is not met in the mixed and combined datasets. Nonetheless, the limitations of the covariance matrix observed here (Table S6) and TreeMix's limitation in capturing complex admixture events (Lipson et al. 2013) are reflected in the poor performances of TreeMix (Fig. S6). Interestingly, TreeMix also attempts to model migration, that is, explain shared genetic similarity that cannot be properly modeled in a tree configuration. However, its predictions for humans (Pickrell and Pritchard 2012) appear inconsistent, incomplete, and fit only partially with known population history. Phylogenetic models that represent admixture were proposed (Lipson et al. 2013); however, it is unclear how they can be applied to pair individuals. The dissonance between the commonality of these tools and their accuracy raises concerns that their popularity may be due to other factors than rigorousness and that these tools contribute to the reproducibility problem in clinical and medical studies.

As expected, all the methods performed better on the AIMs dataset than on the complete or LD-pruned ones as AIMs amplify the ancestral differences between individuals, whereas non-AIMs act to even the population differences. These findings imply that correcting for population structure will be more difficult in exome studies. In such studies investigators should utilize the few AIMs captured on their platform or genotype their samples on a dedicated population microarray (e.g., Elhaik et al. 2017). Evaluated on simulated and real datasets, PaM outperformed all alternative classifiers. Among its advantages are high accuracy, sample-independent approach that allows reproducibility (Fig. 5), and the use of a finite set of AIMs that improve the depiction of population structure (Table S6). PaM's admixture model has several more advantages. The admixture components are calculated relative to the putative ancestral populations so their meaning remains the same between different analyses. The admixture components allow intuitive and accurate insight into the ancestry (Fig. S6) and geographical origins (Elhaik et al. 2014) of individuals. The genetic characterization of individuals can be used to identify subgroups of responders in drug trial, which can promote

personalised medicine solutions tailored to population groups. To avoid suboptimal pairings when all pairs are assigned, we introduced a threshold for the minimum acceptable genetic similarity between tested pairs, which significantly reduced spurious assignments. The score and GD provided in the output allow further prioritization of the pairs. Though PaM seeks the best matching pair for each individual and is agnostic to the size of the dataset and admixture scheme, we caution from applying PaM to a poorly constructed admixture schemes that fail to capture the global genetic biodiversity. Finally, due to its short computational time, we recommend using PaM_{simple} over PaM_{full}, which performs a nearly exhaustive search.

In summary, we develop PaM – a software tool that employs demographic and genetic criteria to find optimised or near-optimised pairings solution for test cohorts consisting of unmixed and mixed individuals. PaM can be accessed online or be installed on the local computer.

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