Phenotype-aware decoupling of related subjects using KDPS

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**Introduction**

In genetic research, the importance of considering the relatedness among subjects cannot be overstated. Geneticists have developed a suite of sophisticated methodologies to decode the complex tapestry of heredity, including the use of genome-wide association studies (Uffelmann et al. 2021), linkage and pedigree-based analyses (Glazner and Thompson 2012, Hu et al. 2014, Feng, Leckman and Zhang 2004), quantitative trait locus (QTL) mapping (Zeng 1994), and transmission disequilibrium tests (TDT) (Allison 1997, Ruiz-Narváez and Campos 2004, Sun et al. 1999). These tools are invaluable for unraveling genetic connections that account for family structure and relatedness, allowing for a more precise understanding of genetic inheritance patterns and their influence on diseases and traits. However, the landscape of genetic methodologies is diverse, and despite the advances that accommodate relatedness, a significant number of genetic analyses, particularly those used in population genetics such as positive selection scans (Akbari et al. 2018, Booker, Jackson and Keightley 2017) and admixture mapping (Ali-Khan and Daar 2010), still necessitate the study of unrelated individuals. This requirement also extends to case-control studies and polygenic risk score analysis (Choi, Mak and O’Reilly 2020), where the absence of relatedness is critical to mitigate confounding biases.

Cohort-based studies serve as a cornerstone for unveiling the associations between genetic variants and complex traits. However, one of the most significant challenges is maintaining adequate sample sizes, especially when investigating rare phenotypes or those with low prevalence within the target cohort. This limitation becomes particularly pronounced in the study of conditions such as Huntington's disease, which has an estimated prevalence of 5-10 cases per 100,000 individuals in most Western countries (Medina et al. 2022). Consequently, within a standard mid-sized cohort of 50,000 individuals, one might expect to find only 25 to 50 cases, a number insufficient for traditional genetic association analyses. Moreover, when one considers even rarer phenotypes or diseases with a strong geographical and ethnic prevalence, such as the cardiac condition Brugada syndrome, which occurs in approximately 1-5 per 10,000 individuals in Europe but has a higher incidence in Southeast Asia, the challenge becomes even more daunting (Li et al. 2020, Vutthikraivit et al. 2018). The rarity of such phenotypes necessitates the aggregation of multiple cohorts or alternative methodological innovations to ensure robust statistical power, a prerequisite for the identification of the genetic underpinnings of these elusive traits.

The current prevalent approach to managing related subjects, particularly as executed by tools like KING (Manichaikul et al. 2010) and Plink2 (Chang et al. 2015), does not take phenotypes into account. The current method employs a greedy algorithm that indiscriminately removes individuals most related to others, resorting to random selection among equally related subjects. This non-selective pruning poses a significant problem when dealing with phenotypes that are rare or uncommon within the cohort, as it could inadvertently eliminate subjects with valuable phenotypic traits, thus compromising the sample size and the potential for meaningful analysis. Recognizing this gap, our study introduces the Kinship Decouple and Phenotype Selection (KDPS) method. KDPS separates related individuals by considering kinship matrix or identity by descent (IBS) scores, while simultaneously prioritizing the retention of subjects based on phenotypes of interest, as determined by user-defined weights. This innovative approach aims to retain those subjects with desired phenotypes, ensuring a robust sample for subsequent analysis even after the necessary exclusion of related individuals.

**Materials and methods**

Loading relatedness and phenotype data

The process of integrating kinship matrices (Manichaikul et al. 2010) or identity by descent (IBS) score (Su et al. 2012) matrices along with phenotype data into the analysis begins with the loading of these files into KDPS. This initial step is crucial for ensuring that only subjects present in both the kinship and phenotype datasets are included in further analysis (Figure 1A). The phenotypes considered can be of two types: categorical, with two or more categories, or numerical. To facilitate focused analysis, users are required to specify which phenotypes are of primary interest. For categorical phenotypes, an ordered list indicating priority is necessary, whereas for numerical phenotypes, users must designate whether subjects with higher or lower values are to be prioritized. In scenarios where multiple phenotypes are under consideration, a prioritization score must be established. This score is designed to rank subjects based on a combination of traits (e.g., gender and body height), thereby allowing for nuanced prioritization within the study. Additionally, a fuzziness score is set, allowing for a degree of tolerance towards the variability in the number of connections each subject has within the network. This score plays a key role in determining which subjects are systematically excluded based on their phenotype prioritizations.

Pruning complex relatedness network

The kinship pruning process initiates by identifying subjects that are directly related to only one other subject within the study, termed related pairs (Figure 1A). These pairs are then segregated according to the predefined phenotype prioritization criteria, significantly streamlining the relatedness matrix. The method then advances to tackle more intricate relatedness networks, employing a dual strategy based on the fuzziness score. With a fuzziness score of zero, the program adopts a stringent approach, prioritizing the removal of subjects interconnected beyond single relationships, focusing initially on what are termed as super-subjects. These are individuals linked to multiple subjects within the network, whereas these linked subjects are not related with any other subjects in the study. This targeted pruning is particularly effective in cohort studies, where such super-subjects can constitute a considerable portion of the network. Conversely, with a fuzziness score above zero, a greedy algorithm is employed, sequentially eliminating subjects based on their relatedness exceeding a threshold defined by the maximum number of connections (m) subtracted by the fuzziness score (f). This iterative removal continues until only related pairs remain, which are then subjected to a final work-up step. The culmination of this process is a curated list of subjects to be excluded, thereby refining the cohort to a set of unrelated individuals.

Benchmarking the method performance using simulations

To rigorously evaluate the efficacy of the KDPS method across various scenarios reflective of real-world cohorts, a comprehensive simulation study was conducted. Utilizing the kinship matrix derived from the UK Biobank dataset (Bycroft et al. 2018) and a corresponding set of categorical and numerical phenotypes, a complex related network (n ≈ 100,000) was simulated. The performance of KDPS was then assessed under a diverse array of parameters, as detailed in Table 1.

**Results**

Simulation test results

The computational duration of the KDPS method is observed to escalate logarithmically in relation to an increase in the fuzziness score, adhering to an approximate O(log(n)) complexity. Specifically, with a dataset comprising 50,000 subjects, the execution time of KDPS spans approximately 1.5 minutes at a fuzziness score of zero, contrasting starkly with over 10 minutes when the fuzziness score is elevated to 10 (Figure 1B). Additionally, the processing time required by KDPS exhibits a logarithmic dependency on the aggregate level of relatedness among subjects, as evidenced in simulations where the fuzziness score is maintained at zero (Figure 1C). Notably, in simulations emulating 100,000 relationships, akin to the expected interconnections within the UK Biobank cohort, KDPS completes its run within an approximate timeframe of 15 minutes. Given the magnitude of data reflective of the upper bounds in current cohort studies, a computation period of around 15 minutes is deemed practical. However, it is pertinent to acknowledge that actual computation times may diverge, influenced by the intricacy of the relatedness network and additional factors such as the fuzziness score.

Despite the incremental computational demand imposed by higher fuzziness scores, which in turn affects the retention ratio (the proportion of subjects retained post-kinship separation relative to the total initial count), this impact is minimally pronounced. For instance, in a simulation involving 50,000 subjects, an increase in fuzziness score from zero to ten marginally reduces the retention ratio from 0.51 to 0.49 (Figure 1D). Moreover, comparative analyses underscore the superiority of KDPS over traditional phenotype-agnostic approaches, such as those implemented in PLINK2, particularly in augmenting the prevalence of subjects possessing the phenotype of interest post-kinship decoupling (Figure 1E). In simulations where the baseline prevalence of the phenotype of interest was set at 0.2, KDPS significantly enhances this prevalence by more than 10%, contrary to the phenotype-naïve approach that shows negligible variation in prevalence after decoupling.

In essence, the simulation exercises illuminate KDPS's computational efficiency and its capacity to substantially conserve subjects with desired traits, presenting a notable advancement over preceding methodologies.

Results for the real-world datasets

To comprehensively evaluate the efficacy of the KDPS within practical applications, we conducted a series of tests across multiple cohort studies. The outcomes, detailed in Table 2, highlight the capability of KDPS to preserve a significantly higher number of subjects possessing phenotypes of interest when juxtaposed against conventional phenotype-agnostic methodologies. Moreover, KDPS demonstrated remarkable efficiency, successfully completing the decoupling processes for all tested cohort studies within a stipulated timeframe of X minutes.

**Discussion**

In this study, we introduced the Kinship Decouple and Phenotype Selection method, a novel approach designed to address the challenges of phenotype-aware kinship decoupling in genetic and epidemiological research. KDPS represents a significant advancement in the field, as it is specifically engineered to consider phenotype data during the subject selection process, thereby prioritizing individuals with phenotypes of interest. This capability is particularly crucial in studies focusing on uncommon or rare phenotypes, where the scarcity of suitable subjects often poses significant hurdles to obtaining statistically meaningful sample sizes.

The computational performance of KDPS is another aspect where it shines, demonstrating the ability to efficiently process large cohort studies within a practical timeframe. This efficiency is vital for enabling researchers to undertake kinship decoupling tasks without the prohibitive time costs associated with many existing methods, thereby facilitating more rapid progression from data processing to analysis and interpretation.

Despite its strengths, KDPS is not without limitations. One potential challenge arises when dealing with datasets substantially larger than those akin to the UK Biobank. As the size of datasets expands, potentially to ten times the magnitude of the UK Biobank, the non-linear computational demands of KDPS may result in significantly extended processing times. To address this scalability issue, future developments might include reimplementing KDPS in a lower-level programming language, such as C++, which could offer enhanced performance efficiencies.

Furthermore, KDPS, similar to other subject selection methodologies, is susceptible to introducing collider bias (Tönnies, Kahl and Kuss 2022). Collider bias occurs when the selection of subjects based on certain criteria inadvertently skews the analysis, potentially leading to misleading associations between study variables. This bias is a critical consideration in genetic and epidemiological studies, where the integrity of findings is paramount. To mitigate the impact of collider bias, researchers are advised to employ strategies such as conducting analyses with randomly selected subjects in parallel with KDPS-selected cohorts and performing sensitivity analyses. These steps can help elucidate the statistical power gains attributable to KDPS while ensuring that results are robust and free from unduly bias.

In conclusion, KDPS stands out as a powerful tool for phenotype-aware kinship decoupling, offering substantial improvements in both the inclusion of relevant subjects and computational efficiency. However, awareness of its limitations and the implementation of strategies to counteract potential biases are essential for maximizing the utility and accuracy of research findings utilizing this method.

**Data and code availability**

The latest release of KDPS and documentation can be found at <https://github.com/Broccolito/kdps>. The KDPS R package can be installed via GitHub release. Scripts for the benchmarking are available at <https://github.com/Broccolito/kdps_dev>.

**Acknowledgements**

The authors extend their sincere gratitude to Dr. Erin Richard and Mijia Ma from the University of California, San Diego, for their invaluable support and insightful comments regarding both the methodology and the manuscript.

**Supplementary data**

Supplementary data are available at *Bioinformatics* online.

**Conflict of interest**

The authors of the manuscript declare no conflict of interest.

**Funding**

This study is partially funded by NIH R00HL122515

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| **Parameter** | **Description** | **Values** |
| Number of Relationships | Defined as the number of kinship relationships to consider in the analysis. | 100, 500, 1000, 5000, 10000, 50000, 100000 |
| Fuzziness | The degree of fuzziness allowed in the model. | 0, 1, 2, 5, 10 |
| Phenotypic Naïve | A Boolean parameter representing whether subjects are prioritized based on their phenotypic information. | FALSE, TRUE |
| Phenotype | Tested with three phenotype configurations (pheno1: binary; pheno2: categorical with 3 categories, pheno3: numerical). | pheno1, pheno2, pheno3 |

Table 1. Simulation parameters used to run KDPS.

A diagram of a graph

Description automatically generated with medium confidence

Figure 1. Algorithm flowchart and performance benchmarking of KDPS.

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