Phenotype-aware decoupling of related subjects

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**Abstract**

Relatedness within genomic cohorts is a potential source of bias for many genetic analyses. Existing tools to break relatedness are phenotype naïve, indiscriminately remove subjects to break relationship, risking the loss of valuable data, especially in studies targeting uncommon and rare phenotypes. To address this limitation, we developed the Kinship Decouple and Phenotype Selection (KDPS) tool, with a novel algorithm designed to enhance the precision of subject selection in genetic and epidemiological research by incorporating phenotype prioritization. KDPS separates related individuals by considering kinship or identity by descent scores, while simultaneously prioritizing subjects based on phenotypes of interest. This approach enables the retention of valuable subjects for analysis, even in the face of necessary exclusions due to relatedness. Furthermore, KDPS accommodates a wide range of phenotypes, including binary, ordinal, and quantitative types, and allows for customization to either prioritize specific phenotypes or maximization of sample size. In simulations based on the UK Biobank dataset and real-world datasets, KDPS demonstrated significant improvements in retention of subjects with prioritized phenotypes and computational efficiency compared to previously published software. The ability of this method to process biobank-scale studies within practical timeframes marks a considerable advancement over existing techniques. KDPS offers tailored solutions for complex analytical challenges and broad applicability in genetics and epidemiology research. To our knowledge, KDPS is the first tool to perform phenotype-aware decoupling, paving the way for more powerful genetic and epidemiological analyses.

**Introduction**

Geneticists utilize a broad suite of sophisticated methodologies to decode the complex architecture of genotype-phenotype relationship. Despite recent advances in statistical methods to accommodate relatedness, a significant number of genetic analysis methods require studies of unrelated individuals, including: selection scans (Akbari et al. 2018, Booker, Jackson and Keightley 2017), admixture mapping (Ali-Khan and Daar 2010), gene by environment interaction analysis (Ottman 1996, Virolainen et al. 2023), and analyses across stratum (Winkler et al. 2017). Inclusion of related subjects in these scenarios violates model assumptions of independence and results in inflation of significance of test statistics.

Over the past two decades, population-based genetic studies have become the central methodology for elucidating the relationships between genetic variation and complex human traits. The expansion of dataset sizes introduces potential bias of cryptic relatedness (Voight and Pritchard 2005), especially in expansive initiatives like the UK Biobank (Bycroft et al. 2018) and All of US research program. Cryptic relatedness occurs when two or more genetically related individuals are included in a study and the genetic relationship is unrecognized. Investigators need to check for cryptic relatedness in their study population and remove subjects to break relatedness if their analytical approach cannot accommodate relatedness, albeit at the cost of excluding individuals with relevant phenotypes. This strategy, while reducing the inclusivity of certain phenotypic representations, is crucial to ensure the retention of a robust case sample size. The rationale behind this lies in the need to investigate not only prevalent conditions such as diabetes (Ong et al. 2023) or obesity (Hruby and Hu 2015) but also the gene-environment interactions associated with these traits. The lack of sufficient cases often results in underpowered analyses in gene-environment interaction studies and many other analytic techniques. Furthermore, the imperative to optimize the number of case subjects becomes even more critical when examining the genetics of rarer phenotypes, where every individual case contributes significantly to the understanding of the genetic architecture. For conditions with lower incidence rates, such as colorectal cancer (Rawla, Sunkara and Barsouk 2019), neuroblastoma (Yan et al. 2020), psychiatric disorders like schizophrenia (Charlson et al. 2018) and autism (Talantseva et al. 2023), as well as autoimmune diseases like Lupus (Tian et al. 2023), algorithms designed to maximize case sample size while excluding related individuals can drastically improve statistical power to uncover the genetics of the traits and complex trait correlations.

Several tools and approaches are available to manage break related subjects in a study, including KING (Manichaikul et al. 2010), PLINK2 (Chang et al. 2015), Friends and family (de Jager et al. 2017), SampleSeq2 (Edwards and Li 2012) and FastIndep (Abraham and Diaz 2014). However, none of which take phenotypes into account. For instance, the most widely used decoupling method is executed in PLINK2, employs a greedy algorithm that indiscriminately removes individuals most related to others, resorting to random selection among equally related subjects. Naïve non-selective pruning is particularly problematic in scenarios in which the phenotype of interest is rare or uncommon in the cohort. Eliminating subjects with valuable phenotypic traits results in reduced sample size and the statistical power. We introduce the Kinship Decouple and Phenotype Selection (KDPS) method to address this limitation. KDPS separates related individuals by considering genetic relatedness metrics and can prioritize retention of subjects based on phenotypes of interest. This innovative approach maximizes the number of subjects with desired phenotypes and/or exposure of interest.

**Materials and methods**

Loading relatedness and phenotype data

KDPS requires an kinship relatedness (Manichaikul et al. 2010) or identity by descent (IBS) score (Su et al. 2012) and phenotypic data files. These are used to ensure that only subjects present in both the kinship and phenotype datasets are included (Figure 1A). KDPS accepts two categories of phenotypes: categorical, with two or more categories, or numerical. Users are required to specify the primary phenotypes of interest. For categorical phenotypes, an ordered list indicating priority is required. For numerical phenotypes, users must designate whether higher or lower values are prioritized. More complex scenarios with multiple phenotypes and exposures of interest can be accommodated via composite scores, which facilitate prioritization based on a combination of traits (e.g., sex and body height), thereby allowing for nuanced selection within the study sample. KDPS can be run with phenotype prioritization or phenotype naïve. Two additional user parameters are required: relatedness cutoff values (Kinship or IBS) and a fuzziness score. The relatedness cutoff value is used to sets the degree relatedness threshold and thereby tolerance towards the variability in the number of connections each subject has within the network. A fuzziness score (f) sets the criteria for selection by assigning subjects who are related with m individuals and subjects who are related to m – f (f < m) individuals with the same kinship weight and prioritize the pruning of their relatedness network based on their corresponding phenotype weights. The score allows user granularity in determining which subjects are systematically excluded based on their phenotype prioritizations, e.g., prioritizing subjects with phenotype of interest versus maximizing set of unrelated subjects.

Pruning complex relatedness network

The first step of the relatedness pruning process is to identify sets of subjects that are related to each other. First, subjects that are not related with any other subjects in the study are designated for curated unrelated list and removed from the kinship matrix. Next, pairs of subjects that are each related only to each other (related pairs) are identified. These pairs are then split according to the predefined phenotype prioritization criteria(Figure 1A). The algorithm next proceeds stepwise increasing the relatedness group size to tackle more complex relatedness networks, employing a dual strategy based on the fuzziness score. With a fuzziness score of zero, the program adopts a simplified approach by prioritizing the removal of super-subjects. Super-subjects act as hubs, linking to multiple subjects within the network that are otherwise not related to each other or 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 greater than zero, a greedy algorithm is employed, sequentially eliminating subjects with the lowest phenotypic weight who are related to more than m - f subjects, where m is the number of related pairs of the subject in the cohort who is related to the most people, and f represents the fuzziness score. 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

We evaluated the performance of the KDPS method via simulation and real-world scenarios. A complex related network (n~100 000 pairs) was simulated based on on UK Biobank kinship structure based on and a simulated set of categorical and numerical phenotypes,, as detailed in Table 1. Furthermore, KDPS was applied to real-world phenotypes in the UK Biobank dataset, using four binary phenotypes: schizophrenia, acute myocardial infarction, multiple sclerosis and alcohol drinking (ever consumed alcohol). A fuzziness score of zero was used and results were compared between the approach where phenotypic information was considered during the subject prioritization process (phenotype-aware) and in which phenotypic information was not considered (phenotype-naïve). All testing was performed using a single thread job (Intel(R) Xeon(R) CPU E5-4650 v3 @ 2.10GHz) and 4 Gb of memory

**Results**

Simulation test results

The computational time required by KDPS exhibits a logarithmic dependency on the aggregate level of relatedness among subjects, as demonstrated in simulations with increasing number of relatedness, where fuzziness score was set at zero (Figure 1C). Similarly, the computational time increases logarithmically in relation to increasing fuzziness score, adhering to an approximate O(log(n)) complexity. Specifically, with a dataset comprising 50 000 subjects with over 10 000 relationship pairs the execution time of KDPS spans approximately 1.5 minutes at a fuzziness score of zero, increasing to over 10 minutes when the fuzziness score is set to 10 (Figure 1B). Notably, in simulations with computational time of the KDPS on ‘UK Biobank’ scale complex related network (~100 000 related pairs) was under 15 minutes. Real world computation times may diverge, influenced by the intricacy of the relatedness network and additional factors such as the fuzziness score.

In addition to increased computational demand, the choice of fuzziness score also subtly influences the retention ratio, defined as the percentage of subjects remaining after kinship decoupling compared to the original total. 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, we compared performance of phenotype selection and retention rates using KDPS and standard phenotype-agnostic approaches (e.g., PLINK2). Using the simulated datasets, KDPS demonstrated an advantage in augmenting the prevalence of subjects possessing the phenotype of interest after kinship decoupling (Figure 1E). In simulations where the baseline prevalence of the phenotype of interest was set at 20%, KDPS significantly enhances this retention of subjects with phenotype of interest to approximately 30%, while the phenotype-naïve approach that retained 20% of samples with phenotype of interest.

Results for the real-world datasets

We next evaluated the efficacy of the KDPS in real-world scenarios, using UK Biobank cohort and varied set of outcomes and exposures, detailed in Table 2. KDPS phenotype aware selection preserved a significantly higher proportion of subjects possessing phenotypes of interest when juxtaposed against conventional phenotype-naïve methodologies. When prioritizing case subjects, KDPS resulted in 11.8% increase in the number of case subjects with schizophrenia, 11.1% increase in subjects with acute myocardial infarction, 12.1% increase in subjects with multiple sclerosis and 8.7% increase in subjects who have self-reported to have never consumed alcohol. KDPS successfully completed the decoupling and phenotype selection processes for all tested phenotypes in the UK Biobank within 35 minutes.

**Discussion**

In this report, we introduce KDPS, a novel tool and algorithm to address the lack of phenotype-aware kinship decoupling tools in genetic and epidemiological investigations. KDPS substantially improves over existing phenotype naïve selection methods by allowing incorporation of phenotypic information in subject selection. KDPS can be extended to allow complex and tailored sample selections via the use of composite trait scores (combination of traits and/or exposures of interest). KDPS has maximum utility in scenarios when the analytical method cannot accommodate relatedness and maximizing trait sample count is crucial to achieving the necessary statistical power.

Strengths of KDPS include efficiently of algorithm to process biobank-scale studies within a practical timeframe. KDPS accommodates a broad set of phenotypes for prioritization, including numeric (binary, ordinal and quantitative measures) and categorical phenotype definitions. Simulation and real-world applied analyses illustrate KDPS's computational efficiency and its capacity to substantially conserve subjects with traits.

There are important considerations and limitations of KDPS. One potential challenge arises when dealing with datasets substantially more extensive and/or complex relatedness than UK Biobank (>100K related pairs), such as national biobanks and studies leveraging medical systems (e.g., AllofUS, UCLA ATLAS, BioVU, *etc*.). Computation times may significantly increase with sample size, amount and complexity of relatedness, this may not represent a major burden as sample selection is typically performed only once per study. Moreover, future improvements such as reimplementing KDPS in a lower-level programming languages or novel algorithms, may improve performance. Finally, users are cautioned that subject selection may to introduction of collider bias (Tönnies, Kahl and Kuss 2022). In brief, collider bias occurs when the selection of subjects is based on criteria that is associated with both the exposure and outcome of interest, potentially leading to spurious or artificial association between the exposure and outcome variables. Mitigation of collider bias should optimally be done at the design stage. Researchers are also advised to consider strategies such as conducting sensitivity analyses to check of collider bias, *e.g.*, repeating analyses in randomly selected subjects and compare to phenotype-selected results.

In conclusion, KDPS is a fast, computationally efficient, and powerful tool for phenotype-aware kinship decoupling, offering substantial improvements in both the inclusion of relevant subjects and computational efficiency. The integration of KDPS paves the way for phenotype-informed selection of unrelated subjects, offering broad applicability in genetics and epidemiology research.

**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>.

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**Supplementary data**

Supplementary data are available at *Bioinformatics* online.

**Conflict of interest**

The authors of the manuscript declare no conflict of interest.

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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, 5 000, 10 000, 50 000, 100 000 |
| 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 three categories, pheno3: numerical). | pheno1, pheno2, pheno3 |

Table 1. Simulation parameters used to run KDPS.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Phenotype** | **Schizophrenia** | **Acute Myocardial Infarction** | **Multiple**  **Sclerosis** | **Alcohol Drinking (Never)** |
|  | UK Biobank ID | 130 874 | 131 298 | 131 042 | 20 117 |
| **Full Sample** | Sample Size | 502 420 | 502 420 | 502 420 | 502 420 |
| Case/Exposure | 1 356 | 22 635 | 2 500 | 22 382 |
| Control/Ref | 501 064 | 479 785 | 499 920 | 480 038 |
| Prevalence | 2.70E-03 | 4.51E-02 | 4.98E-03 | 4.45E-02 |
| **Phenotypic Naïve Selection** | Sample Size | 427 803 | 427 797 | 427 795 | 427 793 |
| Case/Exposure | 1 184 | 19 662 | 2 175 | 19815 |
| Control/Ref | 426 619 | 408 135 | 425 620 | 407 978 |
| Prevalence | 2.77E-03 | 4.60E-02 | 5.08E-03 | 4.63E-02 |
| **Phenotypic Aware Selection** | Sample Size | 427 817 | 427 819 | 427 817 | 427 822 |
| Case/Exposure | 1 324 | 21 836 | 2 439 | 21 547 |
| Control/Ref | 426 493 | 405 983 | 425 378 | 406 275 |
| Prevalence | 3.09E-03 | 5.10E-02 | 5.70E-03 | 5.04E-02 |
| % Additional Case/Exposure Subjects Included | 11.8% | 11.1% | 12.1% | 8.7% |

Table 2. Testing results of removing related subjects in UK Biobank regarding four phenotypes of interest.

A diagram of a graph

Description automatically generated with medium confidence

Figure 1. Algorithm flowchart and performance benchmark of KDPS.

**References**

Abraham, Kuruvilla Joseph, and Diaz, Clara, “Identifying Large Sets of Unrelated Individuals and Unrelated Markers,” *Source Code for Biology and Medicine*, 9/1 (2014), 6

Akbari, Ali, Vitti, Joseph J., Iranmehr, Arya, Bakhtiari, Mehrdad, Sabeti, Pardis C., Mirarab, Siavash, et al., “Identifying the Favored Mutation in a Positive Selective Sweep,” *Nature Methods*, 15/4 (2018), 279–82

Ali-Khan, Sarah E., and Daar, Abdallah S., “Admixture Mapping: From Paradigms of Race and Ethnicity to Population History,” *The HUGO Journal*, 4/1–4 (2010), 23–34

Booker, Tom R., Jackson, Benjamin C., and Keightley, Peter D., “Detecting Positive Selection in the Genome,” *BMC Biology*, 15/1 (2017), 98

Bycroft, Clare, Freeman, Colin, Petkova, Desislava, Band, Gavin, Elliott, Lloyd T., Sharp, Kevin, et al., “The UK Biobank Resource with Deep Phenotyping and Genomic Data,” *Nature*, 562/7726 (2018), 203–9

Chang, Christopher C., Chow, Carson C., Tellier, Laurent Cam, Vattikuti, Shashaank, Purcell, Shaun M., and Lee, James J., “Second-Generation PLINK: Rising to the Challenge of Larger and Richer Datasets,” *GigaScience*, 4 (2015), 7

Charlson, Fiona J., Ferrari, Alize J., Santomauro, Damian F., Diminic, Sandra, Stockings, Emily, Scott, James G., et al., “Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016,” *Schizophrenia Bulletin*, 44/6 (2018), 1195–1203

Edwards, Todd L., and Li, Chun, “Optimized Selection of Unrelated Subjects for Whole-Genome Sequencing Studies of Rare High-Penetrance Alleles,” *Genetic Epidemiology*, 36/5 (2012), 472–79

Feng, Rui, Leckman, James F., and Zhang, Heping, “Linkage Analysis of Ordinal Traits for Pedigree Data,” *Proceedings of the National Academy of Sciences of the United States of America*, 101/48 (2004), 16739–44

Glazner, Chris, and Thompson, Elizabeth Alison, “Improving Pedigree-Based Linkage Analysis by Estimating Coancestry among Families,” *Statistical Applications in Genetics and Molecular Biology*, 11/2 (2012) <http://dx.doi.org/10.2202/1544-6115.1718>

Hruby, Adela, and Hu, Frank B., “The Epidemiology of Obesity: A Big Picture,” *PharmacoEconomics*, 33/7 (2015), 673–89

Hu, Hao, Roach, Jared C., Coon, Hilary, Guthery, Stephen L., Voelkerding, Karl V., Margraf, Rebecca L., et al., “A Unified Test of Linkage Analysis and Rare-Variant Association for Analysis of Pedigree Sequence Data,” *Nature Biotechnology*, 32/7 (2014), 663–69

de Jager, Deon, Swarts, Petrus, Harper, Cindy, and Bloomer, Paulette, “Friends and Family: A Software Program for Identification of Unrelated Individuals from Molecular Marker Data,” *Molecular Ecology Resources*, 17/6 (2017) <https://pubmed.ncbi.nlm.nih.gov/28503747/> [accessed 28 February 2024]

Manichaikul, Ani, Mychaleckyj, Josyf C., Rich, Stephen S., Daly, Kathy, Sale, Michèle, and Chen, Wei-Min, “Robust Relationship Inference in Genome-Wide Association Studies,” *Bioinformatics* , 26/22 (2010), 2867–73

Ong, Kanyin Liane, Stafford, Lauryn K., McLaughlin, Susan A., Boyko, Edward J., Vollset, Stein Emil, Smith, Amanda E., et al., “Global, Regional, and National Burden of Diabetes from 1990 to 2021, with Projections of Prevalence to 2050: A Systematic Analysis for the Global Burden of Disease Study 2021,” *The Lancet*, 402/10397 (2023), 203–34

Ottman, R., “Gene-Environment Interaction: Definitions and Study Designs,” *Preventive Medicine*, 25/6 (1996), 764–70

Rawla, Prashanth, Sunkara, Tagore, and Barsouk, Adam, “Epidemiology of Colorectal Cancer: Incidence, Mortality, Survival, and Risk Factors,” *Przeglad Gastroenterologiczny*, 14/2 (2019), 89–103

Spielman, R. S., McGinnis, R. E., and Ewens, W. J., “Transmission Test for Linkage Disequilibrium: The Insulin Gene Region and Insulin-Dependent Diabetes Mellitus (IDDM),” *The American Journal of Human Genetics*, 52/3 (1993), 506–16

Su, Shu-Yi, Kasberger, Jay, Baranzini, Sergio, Byerley, William, Liao, Wilson, Oksenberg, Jorge, et al., “Detection of Identity by Descent Using Next-Generation Whole Genome Sequencing Data,” *BMC Bioinformatics*, 13 (2012), 121

Talantseva, Oksana I., Romanova, Raisa S., Shurdova, Ekaterina M., Dolgorukova, Tatiana A., Sologub, Polina S., Titova, Olga S., et al., “The Global Prevalence of Autism Spectrum Disorder: A Three-Level Meta-Analysis,” *Frontiers in Psychiatry / Frontiers Research Foundation*, 14 (2023), 1071181

Taliun, Daniel, Harris, Daniel N., Kessler, Michael D., Carlson, Jedidiah, Szpiech, Zachary A., Torres, Raul, et al., “Sequencing of 53,831 Diverse Genomes from the NHLBI TOPMed Program,” *Nature*, 590/7845 (2021), 290–99

Tian, Jingru, Zhang, Dingyao, Yao, Xu, Huang, Yaqing, and Lu, Qianjin, “Global Epidemiology of Systemic Lupus Erythematosus: A Comprehensive Systematic Analysis and Modelling Study,” *Annals of the Rheumatic Diseases*, 82/3 (2023), 351–56

Tönnies, Thaddäus, Kahl, Sabine, and Kuss, Oliver, “Collider Bias in Observational Studies,” *Deutsches Arzteblatt International*, 119/7 (2022), 107–22

Uffelmann, Emil, Huang, Qin Qin, Munung, Nchangwi Syntia, de Vries, Jantina, Okada, Yukinori, Martin, Alicia R., et al., “Genome-Wide Association Studies,” *Nature Reviews Methods Primers*, 1/1 (2021), 1–21

Virolainen, Samuel J., VonHandorf, Andrew, Viel, Kenyatta C. M. F., Weirauch, Matthew T., and Kottyan, Leah C., “Gene-Environment Interactions and Their Impact on Human Health,” *Genes and Immunity*, 24/1 (2023), 1–11

Voight, Benjamin F., and Pritchard, Jonathan K., “Confounding from Cryptic Relatedness in Case-Control Association Studies,” *PLoS Genetics*, 1/3 (2005), e32

Winkler, Thomas W., Justice, Anne E., Cupples, L. Adrienne, Kronenberg, Florian, Kutalik, Zoltán, Heid, Iris M., et al., “Approaches to Detect Genetic Effects That Differ between Two Strata in Genome-Wide Meta-Analyses: Recommendations Based on a Systematic Evaluation,” *PloS One*, 12/7 (2017), e0181038

Yan, Ping, Qi, Feng, Bian, Lanzheng, Xu, Yajuan, Zhou, Jing, Hu, Jiajie, et al., “Comparison of Incidence and Outcomes of Neuroblastoma in Children, Adolescents, and Adults in the United States: A Surveillance, Epidemiology, and End Results (SEER) Program Population Study,” *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, 26 (2020), e927218

Zeng, Z. B., “Precision Mapping of Quantitative Trait Loci,” *Genetics*, 136/4 (1994), 1457–68