

Genome Wide Association Study using Espresso Methods

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1 Goal

Genome wide association studies (GWAS) are increasingly reliable and thus able to explain biological phenomena. A polygenic risk score is easily computable, but only gives limited insight to the inner mechanisms which are involved. Analyzing all possible combinations by brute-force for large data sets is not feasible and we must find methods which circumvent this complexity and still produce reliable results.

We are using minimal cover algorithms (namely Espresso) to find a small selections of single nucleotide polymorphisms (SNPs) in a causal relationship for a given binary phenotype. With these selections, we would like to explain the phenotypes. Further, we investigate the genome-wide spanning relations between SNPs and potential groupings of the phenotype such as different subtypes of a disease.

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2 Quality control

We want to emphasize the importance of quality control (QC) for this approach as it generally relies on the assumption that the data has no inconsistencies at all.

Our data stems from a GWAS/PRS tutorial and the corresponding Git directory which we also use for quality control [1]. It has 109 subjects and 1'073'226 SNPs after the aforementioned QC.

For the selection of approaches the number of permitted unknowns is crucial, which is set to 2% per individual and SNP. In the scope this means that there might still exist above 20'000 unknowns per individual.

We found a second data set on imputed simulated blood lipid data on the 1k Genome Project [3]. It contains more than one million SNPs for 2504 individuals before QC. There are no missing genotype data. After QC, with 1% minor allele frequency threshold 75733 SNPs remain, no individuals have to be filtered out.

3 Espresso

Espresso is a tool that performs 2-level logic minimization. It uses heuristics to find a satisfying minimal cover. It takes a logic table as input and outputs a minimized circuit. The output is not guaranteed to be optimal.

"-" can be used as don't cares. It is possible to specify special input types such as *.type fr* to indicate that not in case of an underspecified truth table the underspecified cases are treated as don't cares.

4 Translation of genetic data into binary

Most genetic data is stored in two pairwise inherited strings this is true particular for human autosomal genes. The manifestation can be made up from one of the four nucleobases (A/C/G/T) or an indentation of any length or a deletion (non-existence). The information for any particular SNP might also be partially missing. For any given position there commonly exist two different manifestations, one is labelled as the no-risk allele and the other allele is referred to as risk allele. There is no consensus for every SNP on what allele should be considered the risk allele, generally the variant with lower sampling rates is considered the risk allele. Two different studies might find different risk alleles but they would still agree on the same manifestation which is in correlation with the disease.

We abstract the manifestations to a count of the occurrences of the risk allele. This count we decode into two digits long binary number. To avoid the unnecessary big Hamming distance of 2 between the counts of 2 (10_2) and 1 (01_2), while the smaller distance between 2 and 0 (00_2) would only have a Hamming distance of 1, we encode 2 as 11_2 . For the effect of this choice consider Figure 5.

Phenotypes for each individual should already exist in an easily binary translatable form. We focus on binary phenotypes, if this approach shows promising results it is also possible to extend the approach to continuous data or different potentially related diseases with pleiotropy.

5 Evaluation of results

We use a range of different criteria to approximate the quality of our method. Each criteria has its own advantages and flaws which should always be taken into consideration. No single criteria is sufficient for showing a working method rather does it give an indication.

1. Previous identification of SNPs by other researchers
2. Out of data prediction accuracy of the phenotype
3. PRS
4. Products
5. Literals
6. Time and complexity of the solution

We might also discuss potential issues or perks of a specific method which go beyond this list.

5.1 Previous identification of SNPs by other researchers

If there already exists a genetic analysis on the specific phenotype we are researching, we can compare those findings. In other cases, it might even be the case that datasets or phenotypes are simulated. Knowing the underlying structure, we expect our results to match the simulations SNPs, if our method works.

5.2 Out-of-data accuracy

We apply a k-fold process to our data where we select 90% of the individuals and the rest serves as out-of-sample data. We can then test the individuals not included whether they satisfy the minimal cover or no and determine whether this deviates from their phenotype. We expect $2p^2 - 2p + 1$ to be the share of correctly guessed by random guessing where p is the probability of having the phenotype of the "disease". In practice, an ever higher limit should be set at the max of p or $1-p$ as simply always guessing the same outcome would reach this accuracy.

5.3 PRS

PRS or polygenic risk score can be easily determined. It is a single value for each SNP indicating the modelled risk of having the disease when having the risk allele. The distribution of the PRSs is likely to be normal with barycentre around 0. PRS can be simply added up to get a risk score.

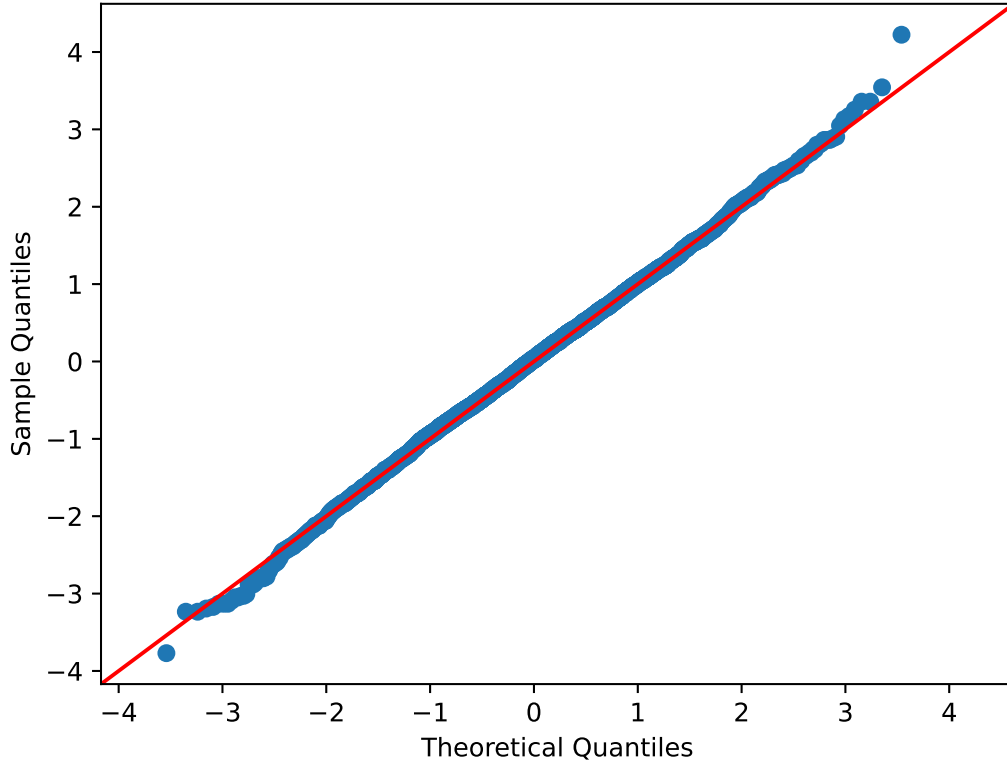


Figure 1: QQ-plot of combined PRS, clearly distributed normally

It is possible to select a couple of SNPs including their direction (risk/non-risk allele) and calculate a score. If the score is highly positive it indicates predictive power while scores near 0 indicate low predictive power.

For the purpose of analyzing the combined predictive power of SNPs the PRS is not a particularly effective metric as it only states the individual predictive power, which may be obfuscated by balancing out effects. Generally, a high PRS indicates that SNP with high predictive power have been found, but it is not necessary that they are balanced and therefore low scores should a priori not indicate a failure of the method.

5.4 Products

The amount of products we get in output from Espresso can be used as an indication of how many subgroups there are needed to find a minimal cover.

Generally, we assume less products in the result to be preferable as this can be seen as simpler relationship, which we deem to be more probable to fit the real cause.

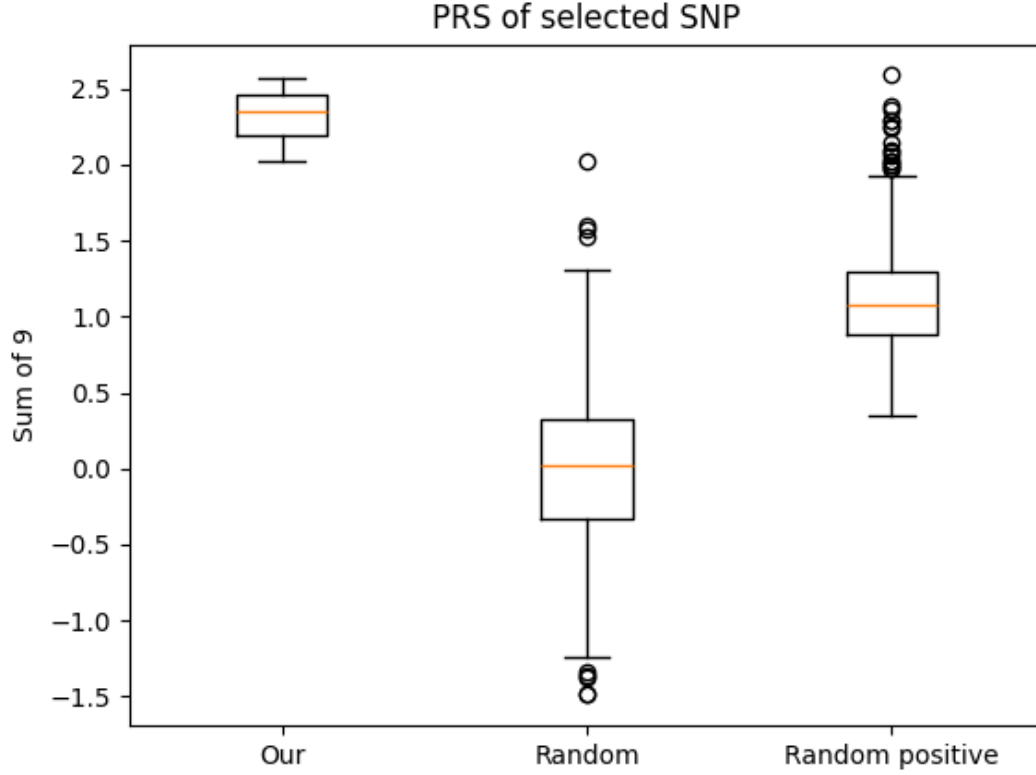


Figure 2: Boxplot of PRS of different selections with same size, our selections stem from the pyramid scheme, they deviate significantly from a random selection.

It should be analyzed what impact each of the products have if there are multiple products, eventually it might prove necessary to discard some products which are specifying on one small subset. This is to avoid overfitting by stratifying to fit the given data perfectly but not the out-of-sample data.

If multiple products with considerable sizes (for in- and out-of- sample data), deviate in many SNPs, it can be speculated that two different groups being affected by different subtypes of diseases were classed into one single phenotype.

5.5 Literals

The amount of literals like for products indicate how complex the found solution is, again we prefer the simplest solution as we deem it to fit the real causes with a higher chance.

5.6 Time & Complexity

Since the complexity of our approach is high, it is inevitable to consider it as part of the evaluation. An easy measure of the complexity is the time it took us to run the program. Change of dataset, parameters or computational capabilities all have an influence on the time.

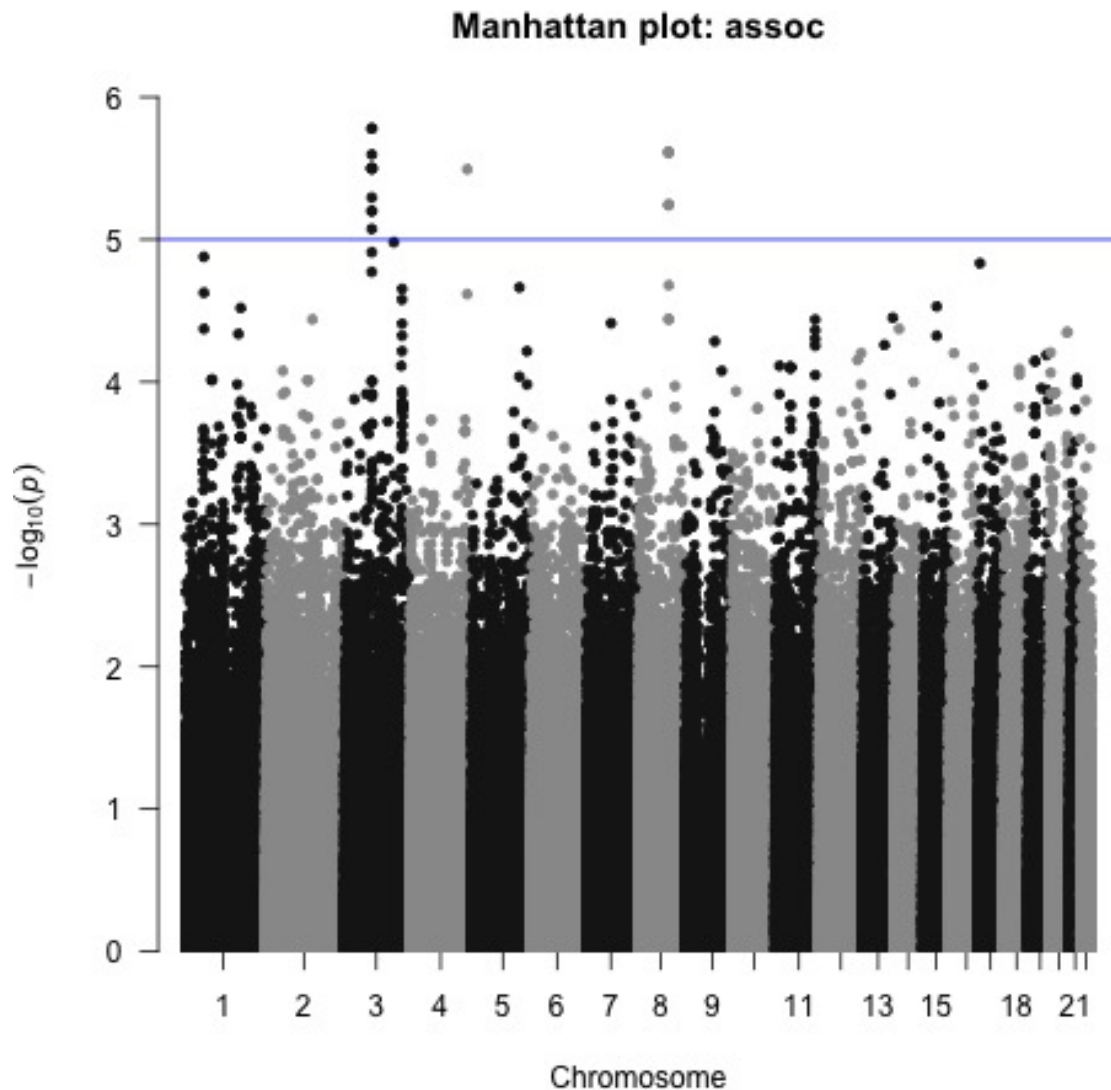


Figure 3: Manhattan plot of our toy data

6 Tried methods

6.1 Primitive approaches

6.1.1 Entire

Taking the entire dataset and let it be solved by Espresso, is impossible as the time requirements grow exponentially, as we know the problem is not even in P.

For small datasets, it might be possible and useful, though wrong genotyping or incorrect phenotypes will remain hidden.

6.1.2 Omitting parts of the dataset

Taking partial parts of the dataset and never discovering other parts is possible though it is highly doubtful how a good solution should be found consistently. For discovering the dataset and experimenting this we did run some experiments. In the context of this experiment when we refer to n we mean the amount of SNPs.

2. The out-of-data prediction accuracy predicted around the 50% mark, according to the formula $2p^2 - 2p + 1$ for cases where $p \neq 0.5$, assuming that the prediction also predicts with the same probability p , else it would simply be p or $1 - p$ for $p < 0.5$.
4. High n yield a smaller product, we explain deviations from this rule by the diverging paths taken in Espresso or in case of different dataset by the difference of possibilities.
5. Same as for products, just more pronounced as in practice this are higher numbers and therefore more possible outcomes(events).
6. This analysis is fast and easy to execute for small n , it scales exponentially with n .

The following table gives an overview of the acquired results with all 109 subjects considered. Each SNP is decoded as two digits, hence the input size is double the amount of selected SNPs. The results are averaged, estimated and depend on the selection of the SNPs and are used as a baseline assumption:

Input size (2n)	Products	Literals	Time
<120	-	-	< 1s
200	50	3-6	5s
400	40	2-4	10s
1000	30	2-3	40s
2000	25	2	8min

If there are less than 60 SNP, it is generally not possible to find an assignment as the truth table would be over-specified.

A random selection of SNP generally decreases the identified products required for a minimal cover, this trend also affects the literals but is less pronounced. Additionally, it was discovered that missing data is not distributed equally among the data and therefore in sequential analysis even for a relative high n it might not be possible to find a cover due to over-specification. For random selections this constitutes less of a concern as we are guaranteed to have at most 2% missingness.

The random method will be used exclusively in all future analysis.

Different encoding methods (see 4) also influence the result. The adjusted method is selected for the rest of this document.

6.1.3 Iterative approaches

We can try to start with some small selection and iteratively build up until the entire data set is included. We start with the smallest possible set such that the truth table is not over-specified and add one SNP, we evaluate this table, find a minimal cover and from the number



Figure 4: Comparison between random and sequential selection methods

of literals in the minimal cover, we decide whether this SNP stays in the selection depending on some criteria. Then we do same for every SNP.

The time required to go through the entire dataset is immense and from some point onwards there are likely no gains. This approach does not consider all possible relationships between different SNPs, in fact in only remarks the ones with a strong PRS anyways or the starting set, making this approach not equilibrated.

6.2 Pyramid scheme

In the pyramid scheme, the algorithm runs sequentially through the entire dataset by separating all SNPs into groups of size k , finding a minimal cover for every group. All the SNPs making part of a found minimal cover are then subsequently taken to the next level. This is repeated until in one level all the identified SNPs from the previous level are less than k SNPs, from where a minimal cover for the entire universe of less than k SNPs can be found.

The selection of the group can be done in many ways, we decided to analyze the random selection approach as well as sequential, which should correspond to the sequences in the DNA as already explained in Chapter 6.1.2, and clearly preferable as we can see in Figure 4.

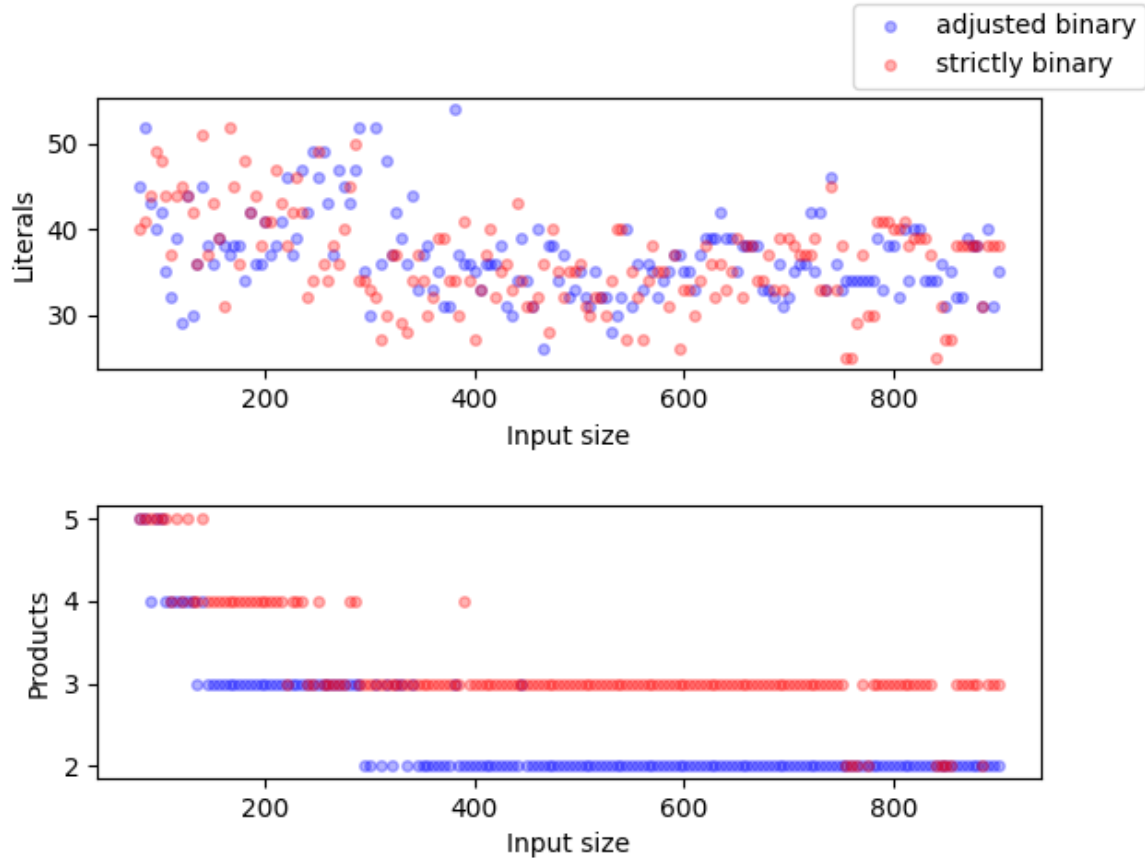


Figure 5: Comparison between different binary encoding methods

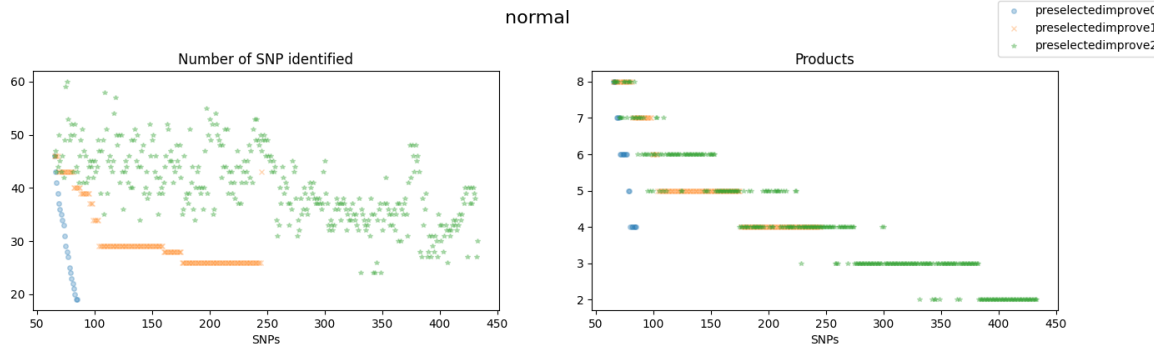


Figure 6: Iterative approaches with SNP indicating the number of selected SNPs, the limit was set on literals. 0: strictly decreasing, 1: monotonically decreasing, 2: no equivalences (increasing or decreasing)

6.3 Phenotype shuffling

We shuffle the phenotypes of all the individuals, this is achieved through random selection of k persons which have phenotype 2 respectively 1. This preserves p of having the phenotype. This altered dataset can be run to test different methods. When this is done we expect the resulting minimal cover to be random, meaning that the method we compare to where structure exist should need less literals or products as there is an underlying structure dictating the outcome, where this is obviously not true for shuffled phenotypes. We can also control with the frequencies of the selected SNPs with shuffled phenotypes whether there exists an overlap with the SNPs of our normal method.

It was found that not all schemes support phenotype shuffling. For example the grouping scheme relies on the fact that there is some underlying determining for genotypes per phenotype. It therefore might occur that since the involved individuals from one level have an over-specified truth table due to the phenotypes basically being random as the different sub-groups of individuals on each level are independent.

6.4 Grouping scheme

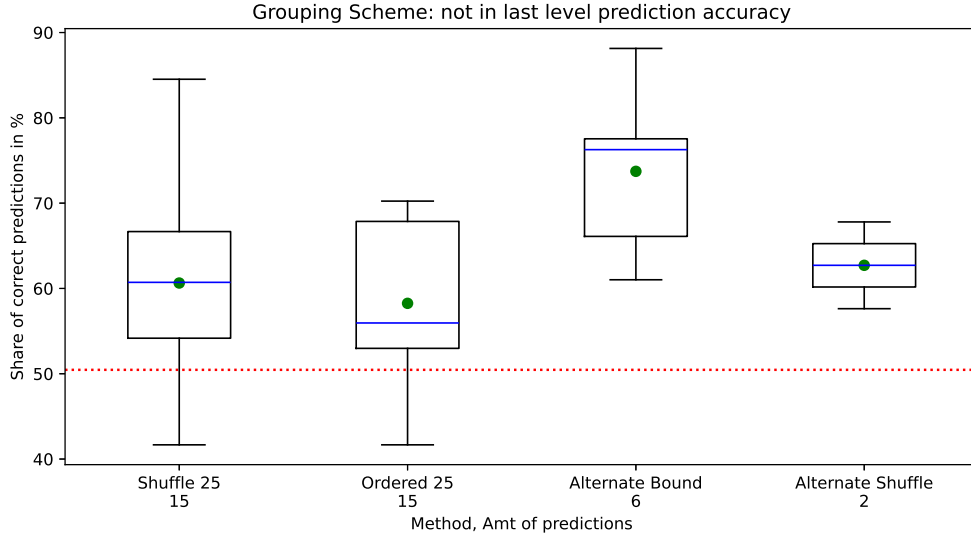


Figure 7: Prediction accuracies of the grouping scheme for the out of sample which means not in last level. Every category originally had 15 sample, because of over-specification some are eliminated, the updated count is displayed below the category name.

This method divides the individuals into different equal-sized groups, in each level one group is chosen and the dataset of only those individuals is considered on this level similar to the Pyramid scheme.

On each level, only a part of the individuals is considered, this means, that the minimal cover is optimized only for those people. On the next level, different individuals get selected, we can assume their phenotype is determined by the same underlying structure, therefore the

previous minimal covers should suffice to find a minimal cover. If this is not the case, we have an over-specified truth table, this might be due to there not being an underlying structure or the missing detection of it or due to errors in genotyping. We tested two methods for passing SNPs down a level: shuffling in every level or preserving original state. Preserving states did generally perform worse in terms of products and literals but also for out-of-sample accuracy. On the other hand, it might occur that good combinations from the previous level are split when shuffling in every level.

We found 25% of the in-sample data to be fitting for the approach. With alternating groups such as when splitting the individuals into two groups yields lower prediction accuracies, while optimizing for the in-sample data. Additionally, alternating often fails due to over-specification especially if the order from the previous level is not respected (shuffle SNPs after each level).

Later the code was changed to refer to the previous level’s selection of individuals if the final analysis is over-specified. This should resolve most cases where over-specification was posing a problem.

6.5 Introducing artificial diffusion based on specific criteria

We would like to test how multiple underlying products affect the data. Unfortunately, the available test data only requires one product. Although if we change the phenotype of all persons based on some specific genotype variation, we hope to see this exact configuration in the results furthermore we expect two products of which one is composed of our chosen diffusion criteria and to be closely related to a valid minimal cover for the original data. Further alterations or even combining of phenotypes might prove to be useful not only for evaluating the results but also for analyzing phenotypes with considerable pleiotropy or suspected subtypes.

We could not replicate the expectations in any of the 5 run experiments with the grouping scheme with group sizes of 25 with the pairwise selection of SNP number 28 as present for the second individual or phenotype as the diffused phenotype. We will repeat the experiment with different SNPs.

7 Evaluation of the methods

From the out-of-sample prediction accuracy, we determine that some approaches perform better than random, though we would hope for higher accuracy.

We also consistently see some of the SNPs reappearing in different covers, which might signal the importance of that specific SNP. For shuffled phenotypes, this phenomena was not observed, hence there is no selection which is generally good for identifying persons. This result is expected and supports the claim of significance of the found SNPs.

The histogram seems less crowded than the Manhattan plot of the PRS of Figure 3. Also most of the SNP also have a high PRS value indicating correlation with the phenotype. Repeated results are a sign of confidence, but it also needs to be mentioned, that we are far away from having equal results in all iterations. This also indicates that potentially multiple global optima exist or it hasn’t even been found yet. Except for the pyramid scheme there do not exist clear ”favorite” SNPs but rather a small collection of SNPs appearing more than once.

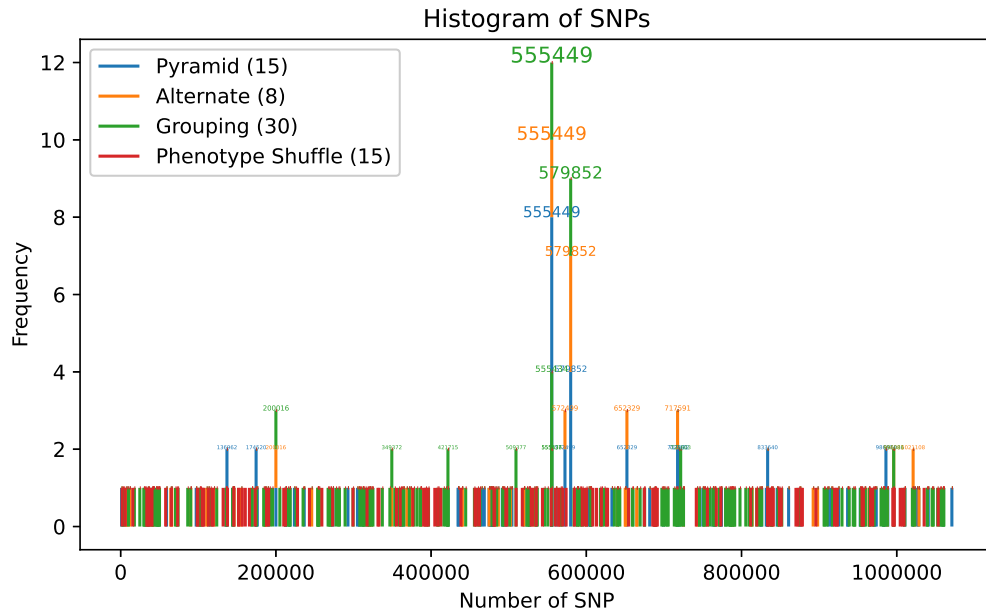


Figure 8: Histogram of multiple k-folds using one out of pyramid scheme, grouping scheme or phenotype shuffling schemes. The grouping scheme is superimposed on top of the k-fold. No overlap is detected, clear trends with the pyramid scheme are visible, while for shuffled phenotypes no order is apparent.

Another question is whether the global optima or any found cover for this purposes actually has biological credibility in explaining the surveyed phenotype.

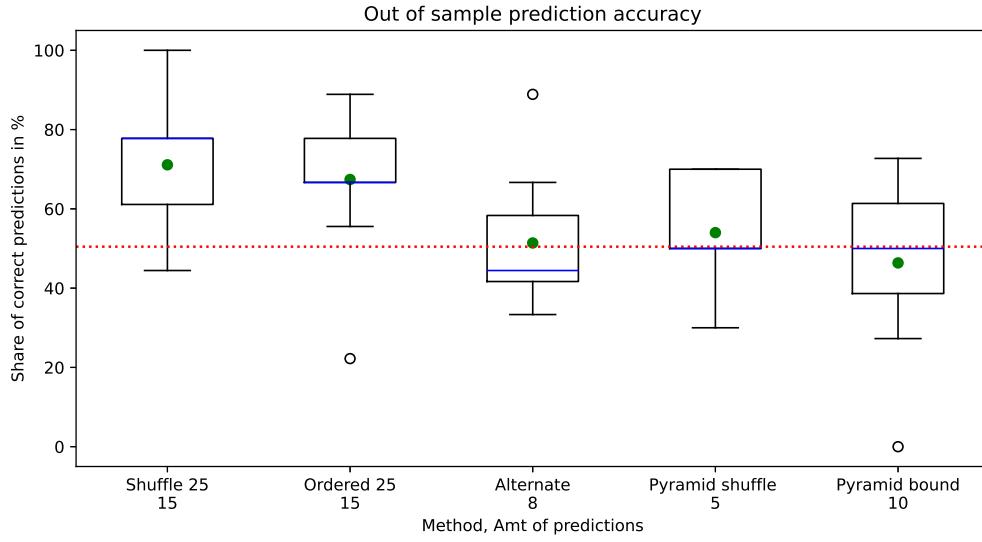


Figure 9: Prediction accuracies of predicted outcomes in data out of sample data, never included in the data before. The red lines indicates the expected guessing accuracy for this data with a random approach. The data is theoretically continuous, here we observe the out-of-sample size to be too small (about 10) , explaining the occurrences of overlapping quartiles and medians.

References

- [1] *Marees AT et al.* A tutorial on conducting genome-wide association studies: Quality control and statistical analysis. <https://pmc.ncbi.nlm.nih.gov/articles/PMC6001694/>
- [2] *Hajiaghazorgi, M.* GWAS dataset with simulated binary phenotypes for 1000 Genome Project. March 1, 2023. <https://doi.org/10.5281/zenodo.7683384>
- [3] *The 1000 Genomes Project Consortium.* A global reference for human genetic variation. Nature 526, p. 68–74. 2015.<https://doi.org/10.1038/nature15393>