Taking population stratification into account by local permutations in rare-variant association studies on small samples

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1 **Abstract**

2 Many methods for rare variant association studies require permutations to assess the significance of
3 tests. Standard permutations assume that all individuals are exchangeable and do not take population
4 stratification (PS), a known confounding factor in genetic studies, into account. We propose a novel
5 strategy, *LocPerm*, in which individuals are permuted only with their closest ancestry-based
6 neighbors. We performed a simulation study, focusing on small samples, to evaluate and compare
7 *LocPerm* with standard permutations and classical adjustment on first principal components. Under
8 the null hypothesis, *LocPerm* was the only method providing an acceptable type I error, regardless of
9 sample size and level of stratification. The power of *LocPerm* was similar to that of standard
10 permutation in the absence of PS, and remained stable in different PS scenarios. We conclude that
11 *LocPerm* is a method of choice for taking PS and/or small sample size into account in rare variant
12 association studies.

13

14 **Introduction**

15 Population stratification (PS) is a classic confounding factor in genetic association studies of common
 16 variants (1). It also affects association studies involving rare variants in the context of next-generation
 17 sequencing (NGS) analyses (2–4). Principal component analysis (PCA) is the most widely used
 18 approach to correction for stratification. The use of principal components (PCs) computed from
 19 common variants as covariates in a regression framework to test for association has been widely
 20 investigated (1,5,6). This strategy provides a satisfactory correction in a number of settings, but is
 21 subject to several limitations, particularly in cases of complex population structure (4,7). In addition,
 22 the regression framework implicitly assumes an asymptotic distribution of the test statistics, which is
 23 rarely achieved when sample size is small (8), and few studies of PC-based correction in this context
 24 have been published (9).

25 Permutation methods (particularly the derivation of an empiric distribution by the random permutation
 26 of phenotype labels) are classically used in strategies for deriving p -values from a test statistic with a
 27 probability distribution that is unknown or from which it is difficult to sample (10). However, this
 28 approach assumes that all individuals are equally interchangeable under the null hypothesis, an
 29 assumption that is not valid in the presence of PS (11). When ancestry is known, it is reasonable to
 30 ensure that permutations result exclusively in the exchange of individuals of the same ancestry, but
 31 this information is rarely available in practice. We investigated the impact of PS on association studies
 32 based on NGS data in the context of limited sample sizes, a situation frequently observed in rare
 33 disorders. We propose a new method, *LocPerm*, based on population-adapted permutation and taking
 34 into account the genetic distance between individuals. We describe a detailed analysis of its properties
 35 with respect to PC adjustment and standard permutation.

36

37 **Materials and methods**

38 We propose a new approach, *LocPerm*, in which permutation is restricted such that each individual
 39 can be exchanged only with one of its nearest neighbors in terms of a PC-based genetic distance
 40 (Supplementary note). Here, we focused on a binary phenotype and the cohort allelic sum test (CAST)
 41 approach (12) implemented in a logistic regression framework, using the likelihood ratio test (LRT)
 42 statistic. The *LocPerm* *p*-value can be calculated by either the usual *full empiric* (*FE*) approach (in
 43 which the *p*-value is equal to the number of permutation samples with a test statistic as extreme as that
 44 observed, divided by the total number of permutation samples), or a *semi-empiric* (*SE*) approach. In
 45 the SE approach, a limited number of permuted statistics are used to estimate the mean (μ) and
 46 standard deviation (σ) of the test statistic under the null hypothesis, and the *p*-value is calculated from
 47 the $N(\mu, \sigma^2)$ distribution (Supplementary note).

48 We performed a simulation study based on real NGS data, to assess the type I error and power of the
 49 *LocPerm* procedure in the context of small sample sizes. We compared *LocPerm* to the asymptotic
 50 CAST approach with (CAST-3PC) and without (CAST) inclusion of the three principal components
 51 (PCs) in the regression model, and to standard permutations applied to CAST. We extracted 1,523
 52 individuals — 745 of Southern European ancestry, 651 of Central European ancestry and 127 of
 53 Northern European ancestry (**eFigure1**) — from our in-house HGID (Human Genetic of Infectious
 54 Diseases) whole-exome sequencing dataset and the public 1000 Genomes Phase 3 whole-genome
 55 sequencing dataset (Supplementary note). Under the null hypothesis, cases and controls were
 56 randomly drawn from the source population according to three PS scenarios (absence of PS,
 57 intermediate and extreme stratification, supplementary note). For power analysis, we selected one gene
 58 with a cumulative frequency of rare variants of 6.2%, for which we simulated a binary phenotype in
 59 the source population, assuming a relative risk of the disease of 4 for individuals carrying at least one
 60 rare variant. We then conducted a sensitivity analysis to investigate the effect on the type I error of the
 61 number of neighbors in the *LocPerm* procedure.

62 Results

63 The results of the simulation study under the null hypothesis (H_0) for the three stratification scenarios
 64 and various sample sizes are shown in **Table 1** (for $\alpha=0.01$) and **eTable 1** (for $\alpha=0.05$). In the absence
 65 of PS, methods based on test statistics following an asymptotic distribution (CAST and CAST-3PC)
 66 had inflated type I errors for small sample sizes. Stronger inflation was observed for CAST-3PC than
 67 for CAST (e.g. type I error=0.0124 vs. 0.0114 at $\alpha=0.01$ for samples of 30 cases and 180 controls).
 68 Methods based on permutation (standard and *LocPerm*) gave correct type I errors. In the presence of
 69 PS, the strongest type I error inflation was observed for CAST. The addition of the first three PCs to
 70 the model took PS into account only partially. Inflated type I errors were also observed for standard
 71 permutations in the presence of PS. Type I error inflation increased with the degree of PS and with
 72 sample size for CAST and standard permutation, whereas small sample size appeared to be the main
 73 source of inflation for CAST-3PC. The *LocPerm* procedures (*FE* and *SE*) provided type I errors close
 74 to the expected α threshold across all sample sizes and PS scenarios, despite being slightly
 75 conservative in the presence of extreme stratification, particularly for *LocPerm-SE*.

76 We further investigated the sensitivity of the *LocPerm* procedure to the number of neighbors under H_0
 77 (Figure 2). With an α threshold of 0.01, the type I error of the *LocPerm* procedure remained stable
 78 over a wide range of numbers of neighbors (from 20 to 170 for a total sample of 210 individuals), and
 79 the use of 30 neighbors appeared to be a reasonable choice. The results of the simulation study under
 80 the alternative hypothesis are shown in **Figure 1** for methods providing a non-inflated type I error rate
 81 (i.e. standard permutation in the absence of stratification and *LocPerm-SE* and *LocPerm-FE* with and
 82 without stratification). In the absence of stratification, a similar power was achieved for standard
 83 permutation, *LocPerm-FE* and *LocPerm-SE* (43%, 42% and 42% at $\alpha=0.01$ for standard permutation,
 84 *LocPerm-FE* and *LocPerm-SE*, respectively). In the presence of extreme stratification, the power of
 85 *LocPerm-FE* was well conserved, whereas that of *LocPerm-SE* decreased slightly, consistent with its
 86 conservative type I error rate in this scenario.

87 Discussion

88 The inclusion of the first few PCs in the association model is a popular strategy for taking population
 89 structure into account. However, it is suitable only for methods implemented in a regression
 90 framework and requires large sample sizes. We found that, in small samples, inclusion of the first
 91 three PCs in CAST failed to control the type I error in the presence of PS. In situations in which
 92 permutations were required, the *LocPerm* procedure proposed here took PS into account effectively,
 93 with no significant power loss relative to other methods in the absence of PS. The SE approximation
 94 performed well in all scenarios, being only slightly conservative in the context of extreme PS and
 95 reducing the computational cost by a factor 10 relative to the *FE* approach. We did not include
 96 adaptive permutation (13), in which the number of permutation samples decreases as the observed p -
 97 value increases, in our comparison. However, we would expect the *SE* approximation to be faster than
 98 adaptive permutation because it requires only 500 permutation samples, whatever the observed p -
 99 value.

100 A permutation approach handling PS was proposed in a previous study (14). The odds of disease
 101 conditional on covariates were estimated under a null model of no genetic association, and individual
 102 phenotypes were resampled, using these disease probabilities as individual weights, to obtain
 103 permuted data with a similar PS. However, subsequent studies showed that this procedure was less
 104 efficient than regular PC correction for dealing with fine-scale population structure (15). We show
 105 here that *LocPerm*, which uses the first 10 PCs weighted by their eigenvalues to compute a genetic
 106 distance matrix, handles complex and extreme PS more effectively than the standard PC-based
 107 correction approach, particularly in the context of small sample size. We focused here on binary traits
 108 and the CAST approach, but it should be straightforward to extend the *LocPerm* approach to
 109 quantitative traits and other rare variant association tests, particularly for adaptive burden tests
 110 requiring permutations.

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119 **Conflict of interest**

120 All authors declare no conflict of interest related to this work.

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169 **Figure legends**

170 **Figure 1:** Power at 1% significance level for different PS scenarios, permutation procedures and
 171 number of cases and controls in the sample

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173 **Figure 2:** Influence of the number of neighbors for the generation of local permutation (x axis) on
 174 type I error (y axis) for the scenario with 30 cases and 180 controls.

175 The situation with 210 neighbors corresponds to standard permutation.

176 **Table 1. Type I error rates of the different approaches and stratification scenarios at a nominal alpha level of 1%. Type I error rates above the upper**
177 **bound of the 95% prediction interval in bold.**

Stratification	N cases	Ncontrols	N genes*	CAST	CAST-3PC	Std. Perm	LocPerm FE	LocPerm SE
Absence	30	60	136932	1.09	1.32	0.97	0.96	1.06
	30	120	186513	1.2	1.37	0.99	0.97	0.98
	30	180	210287	1.14	1.24	0.96	0.98	0.94
	60	60	167621	1.06	1.25	1	1	1.02
	60	120	200883	1.08	1.2	0.98	0.99	1.02
	60	180	217518	1.14	1.26	0.96	0.99	1.03
	120	120	217319	1.05	1.16	1	1.02	1.04
	120	180	227650	1.02	1.11	0.97	1	1
	381	1142	265365	1.02	1.04	1	0.98	0.97
Intermediate	30	60	135896	1.52	1.43	1.3	0.99	0.99
	30	120	187282	1.62	1.41	1.33	1.01	0.93
	30	180	210510	1.48	1.28	1.29	0.94	0.84
	60	60	166912	1.58	1.17	1.45	0.97	0.92
	60	120	200578	1.74	1.24	1.58	0.98	0.94
	60	180	217720	1.87	1.23	1.61	0.92	0.89
	120	120	218007	2	1.12	1.91	0.98	0.9
	120	180	228317	2.17	1.08	2.05	0.93	0.85
	381	1142	265365	2.09	1.13	2.04	1.03	0.9
Extreme	30	60	135054	1.57	1.53	1.37	0.87	0.74
	30	120	187267	1.76	1.63	1.47	0.92	0.78
	30	180	210373	1.68	1.54	1.47	0.98	0.81
	60	60	167433	1.74	1.2	1.64	0.81	0.63
	60	120	201217	1.97	1.35	1.81	0.9	0.75
	60	180	218030	2.21	1.41	1.9	0.87	0.77

120	120	218063	2.46	1.09	2.32	0.88	0.71
120	180	228643	2.73	1.2	2.59	0.94	0.79
381	1142	265365	3.59	1.4	3.52	0.91	0.74

178 * N protein coding genes with at least 10 carriers of rare variants over 15 replicates