Differential confounding of rare and common variants in spatially structured populations

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Well-powered genome-wide association studies, now made possible through advances in technology and large-scale collaborative projects, promise to characterize the contribution of rare variants to complex traits and disease. However, while population structure is a known confounder of association studies, it remains unknown whether methods developed to control stratification are equally effective for rare variants. Here, we demonstrate that rare variants can show a stratification that is systematically different from, and typically stronger than, common variants, and this is not necessarily corrected by existing methods. We show that the same process leads to inflation for load-based tests and can obscure signals at truly associated variants. Furthermore, we show that populations can display spatial structure in rare variants, even when Wright’s fixation index \( F_{ST} \) is low, but that allele frequency-dependent metrics of allele sharing can reveal localized stratification. These results underscore the importance of collecting and integrating spatial information in the genetic analysis of complex traits.

Quantifying the contribution of rare variants to the heritability of different traits is an important and open question in complex trait genetics1. Although there is no universally accepted definition of what constitutes a ‘rare’ variant, by convention, a ‘polymorphism’ has a minor allele frequency (MAF) of \( \geq 1\% \) (ref. 2). Below this frequency, the power of the current generation of genome-wide association studies (GWAS) is negligible for modest effect sizes3. Therefore, although a small number of associations with rare variants have been reported, for example, with type 1 diabetes4 and cholesterol levels5,6, it has not been possible to test the hypothesis that rare variants account for a substantial proportion of the missing heritability for most complex traits. Recently, however, four factors have combined to make the direct investigation of rare variants possible. First, the increasing size of GWAS samples and meta-samples, now approaching cohort sizes of 100,000 through large-scale international collaborations, boosts power. Second, the ascertainment of many rare variants through the 1000 Genomes Project7 has enabled imputation of millions of rare and low-frequency variants and led to the development of a new generation of low-cost genotyping platforms that interrogate rare variants directly. Third, the decline in the cost of sequencing technology has enabled large-scale sequencing studies to be performed, which, in principle, allow the detection of all variants in a sample. Finally, the recent development of new statistical tests for association studies for rare variants8–13 (reviewed in ref. 14) potentially provides power to detect genes or pathways harboring multiple rare variants for which individually there would be low power to detect association.

The large sample sizes required for such studies typically require combining information across multiple geographic locations within and across countries. Population structure, which can lead to spurious correlations between allele frequencies and nongenetic risk factors, has long been known to be a major potential confounding factor for association studies15–17. The effects of stratification have been studied extensively18–20, and testing and correcting for structure is now standard practice in GWAS through methods such as genomic control (GC)21,22, principal-component analysis (PCA)23 and mixed models24. However, analyses of these methods have typically concentrated on common variants, and there has been little investigation of the effect that structure might have specifically on rare variants. Informally, rare variants, because they have typically arisen recently, may tend to have different geographic distributions than more common variants that are typically older.

We therefore set out to investigate (i) under what conditions population structure leads to differential test statistic inflation for variants of different frequencies, (ii) whether methods effective for controlling stratification of common variants are also appropriate for rare variants, (iii) whether different ways of analyzing rare variants (single-marker versus aggregating tests) are equally affected by structure and (iv) how best to measure population structure in empirical data in a manner that is informative about differential stratification. We used a simple lattice model to approximate population structure across a geographic region and investigated the interaction between the spatial distribution of nongenetic risk and inflation of standard association tests under the null model of no genetic risk (see Online Methods). We compared a situation where nongenetic risk was smoothly distributed (for example, a latitudinal effect) with the situation where the same overall risk was concentrated into one or more small, sharply defined regions (for example, localized environmental pollution).

**RESULTS**

As is well documented, population structure leads to systematic underestimation of \( P \) values through inflation of association test statistics under the null. We analyzed the effect of different kinds of risk
Figure 1 Differential inflation of rare and common variants. (a,b) Quantile-quantile plots of association test $P$ values broken down by allele frequency for a broad, smoothly varying (Gaussian) nongenetic risk factor (a) and a small, sharply defined region of constant nongenetic risk (b). (c,d) Inflation plots showing the amount by which the observed $-\log_{10} P$ value exceeds the expected value across allele frequencies for Gaussian risk (c) and small, sharp risk (d). Different lines represent different levels of significance, with $-\log_{10} P$ value equal to 1, 2, 3 or 4. Insets, the spatial distribution of risk, with color indicating by how many s.d. the phenotypic mean is shifted in each grid square. The populations simulated here are uniformly distributed over the grid, with two individuals in each square and a migration rate ($M$) of 0.01.

In the simulations, the first few principal components always included the axes of the grid and so could correct for any nongenetic risk that could be expressed as a linear function of these axes. However, the small, sharp region of risk would require a highly nonlinear function to be expressed in these terms, which cannot be achieved simply by including the top components. Ultimately, including a large enough number of principal components will remove virtually all stratification (here, the inclusion of between 20 and 100 principal components was sufficient; Supplementary Fig. 3), but it is not possible to know how many are required, and the inclusion of many components will lead to substantial reduction in power to detect true associations.

Where variants are sufficiently rare that they are unlikely to be observed in more than a few samples, adequate power to detect true association can only be obtained by combining information across multiple variants within a gene, although this can be approached in many ways. To assess the effects of stratification of such aggregating tests, we considered one of the simplest ‘load-based’ tests, which tests association with the number of rare variants carried in a region, typically a gene. For smoothly varying Gaussian risk, test statistic inflation was largely independent of the number of variants considered (Fig. 3c). For sharply defined risk, test statistic inflation was reduced as more variants were considered but still increased sharply for low $P$ values (Fig. 3d). Given that some versions of these

Figure 2 Spatial distribution of rare and common variants. (a–c) Examples from simulations of the spatial distribution of rare (a), low-frequency (b) and common (c) variants. In each case, grid squares where the allele is present are colored. (d,e) The distribution of the correlation coefficient between genotypes and nongenetic risk for rare, low-frequency and common variants. These are kernel density estimates of the distribution of the correlation between genotypic value (0 or 1) and associated environmental risk for individuals from the simulations described in Figure 1 with Gaussian risk (d) or small, sharply defined risk (e). Insets in e, successive enlargements of the boxed areas in the tail of the distribution. All parameters are the same as in Figure 1.

on this inflation (Fig. 1). When the risk had a wide and smooth distribution, rare variants showed less inflation than common variants (Fig. 1a,c). In contrast, when the risk had a small, sharp spatial distribution, rare variants showed more inflation than common variants, particularly for small $P$ values (Fig. 1b,d). The magnitude of inflation increased as the $P$ value decreased in both scenarios, and the greatest inflation was found for variants with frequency approximately equal to the fraction of the area with elevated risk (Fig. 1c,d). As the size or smoothness of the area of risk increased, the inflation was spread over a wider range of $P$ values (Supplementary Figs. 1 and 2).

Such differential behavior can be understood to be a result of the interaction between the spatial distribution of risk and the spatial distribution of variants. Small $P$ values occur when a variant shows strong correlation with the nongenetic risk. Rare variants, as a result of being recent, tend to show greater geographic clustering than common ones (Fig. 2a–c). When nongenetic risk was varied on a large scale, rare variants could not be highly correlated with it (Fig. 2d). In contrast, when nongenetic risk was varied on a small scale, although most variants were uncorrelated with the risk, rare variants had a tail of highly correlated variants that drive the inflation (Fig. 2e).

Several methods for correcting for population stratification in GWAS have been developed. The most popular are GC, PCA and linear mixed models. These corrections are known to be effective in the standard GWAS setting, and we found that they are all effective when nongenetic risk has a large and smooth distribution (Fig. 3a). However, none of these methods were effective for the small, sharp distribution of risk (Fig. 3b). GC failed in this case because most variants, even rare ones, have correlation with the nongenetic risk of close to 0 (Fig. 2e). PCA and mixed models failed because they effectively try to correct on the basis of linear functions of relatedness.
tests cannot easily accommodate relatedness information and that the problems of spatial structure increase as allele frequency decreases, these results suggest that similar care needs to be taken when interpreting enrichment of either single or multiple variants within cases or controls.

The results discussed so far relate to inflation under the null hypothesis of no genetic risk. However, another implication of differential structure is that causal rare variants may be geographically localized. Thus, even when there is no spatial structure to non-genetic risk, test statistic inflation will be observed. When there are many loci with rare variants contributing to the background genetic effect, inflation is typically stronger for common variants and will be corrected for by standard approaches. However, when there are only a few loci driving risk, inflation is greater for rare variants (Supplementary Fig. 4). Consequently, if genetic risk is driven by small numbers of rare variants, then true signals are more likely to be obscured by rare variants that show association, even though they are not physically linked to the causal variants.

**DISCUSSION**

We have shown that, under certain conditions, rare and common variants exhibit differential patterns of stratification. However, these results are qualitative, and we must also ask whether these conditions are likely to be met in practice. Although the data that would be required to investigate this effect directly are not yet available, we can nonetheless consider metrics that could be used to relate our simulations to real populations. Historically, approaches to summarizing population structure in genetic data have focused on simple statistics, such as Wright’s fixation index $F_{ST}$ which measures the proportion of overall genetic variation that results from between-population variation. Among human populations, $F_{ST}$ is typically estimated to be <0.1 (for example, 0.071 between the 1000 Genomes populations of Utah residents of Northern and Western European ancestry (CEU) and Yoruba from Ibadan (YRI) and typically <0.02 within Europe). When the simulated grid was divided into two equal subpopulations for the migration parameter used for Figure 1 ($M = 0.01$), $F_{ST}$ was approximately 0.1, which is comparable to a worldwide sample. Within a European sample, a more appropriate migration parameter might be $M = 10$, which gives $F_{ST} < 0.01$, a value that would be considered negligible. However, $F_{ST}$ estimates are driven by common variants and also depend on the relative sizes and number of the subpopulations (Supplementary Fig. 5). In analysis of allele sharing by distance as a function of distance, although common variants had effectively no excess allele sharing at short ranges, even with $M = 10$, rare variants still showed excess clustering (Fig. 4), and, although stratification was much reduced compared to a low migration rate, it was still greatest for rare variants (Supplementary Fig. 6). These results are consistent with empirical observations that show very low rates of sharing of rare alleles even between very closely related human populations. The fact that excess allele sharing increases as frequency decreases implies that, even for relatively unstructured populations, this effect will be observed below some sufficiently low variant frequency. These results highlight the need for methods for explicitly showing spatial structure, including the allele sharing plot (Fig. 4) and other spatial correlation measures, such as Moran’s I statistic.

There are three ways in which nongenetic risk might show sharply defined boundaries of the type for which we have shown differential inflation. First, localized environmental exposure could be highly patchy, for example, associated with urban areas. Second, there could be systematic measurement bias at a single recruitment center. Third and more subtly, there could be local variation in recruitment policy or rates of misclassification (the effect of which can be thought of as changing the background disease risk). Although we have simulated

![Figure 3](http://www.nature.com/naturegenetics/journal/v44/n3/fig/concept/1/supplementary/naturegenetics4403-f3.png)

**Figure 3** Comparison of methods for correcting for population structure. (a,b) Quantile-quantile plots of $-\log_{10} P$ values showing the uncorrected values and the values under different corrections. (c,d) Simulated rare variant load tests (see Online Methods). All parameters are the same as in Figure 1, except that the nongenetic risk is doubled, such that, for the Gaussian risk in a and c, the phenotypic mean is shifted by at most 0.8 s.d., whereas for the small, sharp risk in b and d, it is shifted by at most 2 s.d. All quantile-quantile plots are averaged over multiple simulations in order to show the average effect. Individual experiments may vary as a result of the sampling variance of the trait. In a and b, results were averaged over 100 simulations, each testing one trait at 10,000 loci in total (10 loci on each of 1,000 genealogies, representing independent genomic regions). In c and d, results were averaged over 10 simulations, each one testing 10,000 genealogies with either 1, 3 or 10 variants in each. Insets, the spatial distribution of risk, with color indicating by how many s.d. the phenotypic mean is shifted in each grid square. PCA, principal-component analysis using the first ten principal components; rare PCA, performed as with standard PCA but using only variants with MAF of <4%.

![Figure 4](http://www.nature.com/naturegenetics/journal/v44/n3/fig/concept/1/supplementary/naturegenetics4403-f4.png)

**Figure 4** Excess allele sharing. (a,b) A ratio measuring how much more likely two individuals at a given spatial distance are to share a derived allele compared to what would be expected in a homogenous population (see Online Methods). The parameters are the same as those used in Figure 1, apart from the migration rate ($M$), which is 0.01 (a) and 10 (b). In a, $F_{ST} = 0.1$, and in b, $F_{ST} < 0.01$. DAF, derived allele frequency.
quantitative trait data, case-control studies are subject to the same issues of population structure, and a case-control study that randomly misclassifies cases and controls will bias effect-size estimates. When this misclassification is restricted to a particular spatial area, for example, a single recruitment center in a large study, it will produce the effects described here. In fact, if we added additional disconnected small areas of risk of the same size as the first, the inflation in P value had the same distribution with respect to frequency (Supplementary Fig. 7), and this observation can be extended to the case where multiple collection centers are making biased measurements or random misclassifications. Because the extent and clustering of nongenetic risk will differ between phenotypes and study designs, it is not possible to predict any general influence of differential stratification. The principal problem with trying to account for known nongenetic risk factors (by including them as covariates within the analysis) is that, although information about broad-scale risk factors may be available, it is typical that the more localized a risk factor is, the less we are likely to know about it and the greater effect this lack of knowledge will have on rare variants.

Given that existing methods can fail to correct for rare variant stratification, what approaches can be taken to guard against its effects? One approach is to use methods that are robust with respect to stratification (although at a cost to power and ease of experimental design), such as family-based association, perhaps only for replication. Another is to adapt existing methods to work better with rare variants. For example, although PCA with rare variants did not effectively control inflation when we linearly corrected using the top components (Fig. 3b), in principle, more sophisticated methods for selecting nonlinear functions of components could correct appropriately. Alternatively, we might look to the development of new measures of relatedness that are more sensitive to recent ancestry and fine-scale structure. Whichever approach is taken, it is likely to require finely grained information about the geographic origin and recruitment path of each sample. The collection of such information must be an important consideration in the design of future studies.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

G.M. conceived and designed the study. I.M. ran simulations and collected results. G.M. and I.M. jointly wrote the simulation code and manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Manolio, T.A. et al. Finding the missing heritability of complex diseases. Nature 461, 747–753 (2009).
2. Bodmer, W. & Bonilla, C. Common and rare variants in multifactorial susceptibility to common diseases. Nat. Genet. 40, 695–701 (2008).
3. Spencer, C.C., Su, Z., Donnelly, P. & Marchini, J. Designing genome-wide association studies: sample size, power, imputation, and the choice of genotyping chip. PLoS Genet. 5, e1000477 (2009).
4. Nejentsev, S., Walker, N., Riches, D., Egholm, M. & Todd, J.A. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science 324, 387–389 (2009).
5. Cohen, J.C. et al. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science 305, 869–872 (2004).
6. Wang, J. et al. Common and rare ABCA1 variants affecting plasma HDL cholesterol. Nat. Genet. 38, 1933–1935 (2000).
7. 1000 Genomes Project Consortium. et al. A map of human genome variation from population-scale sequencing. Nature 467, 1061–1073 (2010).
8. Ionita-Laza, I., Budbaum, J.D., Laird, N.M. & Lange, C. A new testing strategy to identify rare variants with either risk or protective effect on disease. PLoS Genet. 7, e1001289 (2011).
9. Li, B. & Leal, S.M. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. Am. J. Hum. Genet. 83, 311–321 (2008).
10. Madsen, B.E. & Browning, S.R. A groupwise association test for rare mutations using a weighted sum statistic. PLoS Genet. 5, e1000384 (2009).
11. Morris, A.P. & Zeggini, E. An evaluation of statistical approaches to rare variant analysis in genetic association studies. Genet. Epidemiol. 34, 189–193 (2010).
12. Mukhopadhyay, I., Feingold, E., Weeks, D.E. & Thalamuthu, A. Association tests using kernel-based measures of multi-locus genotype similarity between individuals. Genet. Epidemiol. 34, 213–221 (2010).
13. Neale, B.M. et al. Testing for an unusual distribution of rare variants. PLoS Genet. 5, e1001322 (2009).
14. Bansal, V., Libiger, O., Torkamani, A. & Schork, N.J. Statistical analysis strategies for association studies involving rare variants. Nat. Rev. Genet. 11, 773–785 (2010).
15. Knowler, W.C., Williams, R.C., Pettitt, D.J. & Steinberg, A.G. Gm3;5,13,14 and type 2 diabetes mellitus: an association in American Indians with genetic admixture. Am. J. Hum. Genet. 43, 520–526 (1988).
16. Lander, E.S. & Schork, N.J. Genetic dissection of complex traits. Science 265, 2037–2048 (1994).
17. Pritchard, J.K. & Donnelly, P. Case-control studies of association in structured or admixed populations. Theor. Popul. Biol. 60, 227–237 (2001).
18. Cardon, L.R. & Palmer, L.J. Population stratification and spurious allelic association. Lancet 361, 988–994 (2003).
19. Clayton, D.G. et al. Population structure, differential bias and genomic control in a large-scale, case-control association study. Nat. Genet. 37, 1243–1246 (2005).
20. Mani, N., Cardon, L.R., Phillips, M.S. & Donnelly, P. The effects of human population structure on large genetic association studies. Nat. Genet. 36, 512–517 (2004).
21. Bacanu, S.A., Devlin, B. & Roeder, K. The power of genomic control. Am. J. Hum. Genet. 66, 1933–1944 (2000).
22. Devlin, B. & Roeder, K. Genomic control for association studies. Biometrics 55, 997–1004 (1999).
23. Price, A.L. et al. Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 38, 904–909 (2006).
24. Kang, H.M. et al. Efficient control of population structure in model organism association mapping. Genetics 178, 1709–1723 (2008).
25. Neils, M. et al. Genetic structure of Europeans: a view from the North-East. PLoS ONE 4, e5472 (2009).
26. Bustamante, C.D., Burchard, E.G. & De la Vega, F.M. Genomics for the world. Nature 475, 163–165 (2011).
27. Moran, P.A.P. Notes on continuous stochastic phenomena. Biometrika 37, 17–23 (1950).
28. Copeland, K.T., Checkoway, H., McMichael, A.J. & Holbrook, R.H. Bias due to misclassification in estimation of relative risk. Am. J. Epidemiol. 105, 488–495 (1977).
ONLINE METHODS

Simulations of association studies. We simulated genotypes and quantitative traits by starting with a number of individuals and their locations on the grid and working backward in time to generate random genealogical events. Each event was either a coalescence of two lineages or a migration of a single lineage from one square to another. The relative rates of coalescence and migration depended on the population-scaled migration rate $M$ and the number and distribution of lineages on the grid.

More precisely, supposing we had a $K × K$ grid and we wished to simulate a sample of $G = K^2$ individuals, with $C$ the number of individuals in each grid square. We simulated $L$ loci on each of $G$ genealogies for a total of $LG$ loci. Each genealogy represented an independent genomic region, with no recombination inside each region. We indexed the grid squares by $i,j$ and denoted the number of lineages in grid square $i,j$ at time $t$ by $n^t_{i,j}$. We let $s_{i,j}$ represent the number of grid squares adjacent to $i,j$ in a Manhattan sense, such that $s_{i,j} ∈ [2,3,4]$.

Then, we started at $t = 0$ and repeated the following steps until only one lineage remained.

1. At time $t$, the rate of coalescence within grid square $i,j$ and the total rate of coalescence were, respectively,

$$\lambda^{t}_{i,j} = \frac{n^t_{i,j}(n^t_{i,j} - 1)}{2} \text{ and } \lambda^t_{\ast} = \sum_{i,j} \frac{n^t_{i,j}(n^t_{i,j} - 1)}{2}$$

where we use ‘*’ to represent summation over indices. The rate of migration for each grid square was

$$\mu^t_{i,j} = \frac{Mn^t_{i,j}s_{i,j}}{2}$$

and the total rate of migration was $\mu_{\ast}^t$. The next event occurred at time $t + T$, where $T = \exp(\lambda^t_{\ast} + \mu^t_{\ast})$. This next event was chosen on the basis of the probability of coalescence

$$\frac{\lambda^t_{\ast}}{\lambda^t_{\ast} + \mu^t_{\ast}}$$

and the probability of migration.

$$\frac{\mu^t_{\ast}}{\lambda^t_{\ast} + \mu^t_{\ast}}$$

2. If the next event was coalescence, it occurred in grid square $i,j$ with probability $\lambda^{t}_{i,j} / \lambda^{t}_{\ast}$. In this grid square, we chose two lineages uniformly and joined them together. We then returned to step 1 with $t$ replaced by $t + T$.

3. If the next event was migration, it occurred in grid square $i,j$ with probability $\mu^t_{i,j} / \mu^t_{\ast}$. In this grid square, we chose one lineage uniformly and moved it uniformly to one of the adjacent grid squares. We returned to step 1 with $t$ replaced by $t + T$.

Once we had simulated a single instance of the genealogy, we generated genotypes at $L$ random loci by sampling $L$ nodes from the genealogy with replacement, selecting each node with probability proportional to the length of the branch above that node and setting each individual's genotype to 0 or 1 at each locus according to whether they were descended from that node or not, such that a genotype of 0 represented an ancestral allele and a genotype of 1 represented a derived allele.

We generated quantitative traits for each locus for each individual by sampling from a standard normal distribution. We shifted the mean of the distribution for each individual according to the nongenetic risk in the square that that individual came from. We let $φ: [1,C] → [1,K] × [1,K]$ be a function that mapped each individual to the grid square from which they originated. Then, for individual $k$, we simulated the trait value $Y_k = N(R_gk,1)$, where $R_g$ was the nongenetic risk in grid square $i,j$. In a real experiment, each individual would have one value of $Y_k$ that would be tested against every locus, but to reduce the uncertainty due to sampling error, in our simulations, we resampled the trait independently for each locus, except where that would be inappropriate—for example, when testing corrections—in which case we instead averaged the results over many experiments.

We performed association tests for each locus by fitting a simple linear model $Y_k = \mu Y + \beta_k X_k + \epsilon_k$, where the $\epsilon_k$ are distributed independently as $N(0,\sigma^2_k)$ for some $\sigma^2_k$ and computing the $P$ values of the beta estimates. We then repeated this for $l = 1...L$ and $g = 1...G$.

The results shown in Figure 1 used the following parameters: $K = 20$ and $C = 2$, such that $G = 800$, $M = 0.01$, $G = 100,000$ and $L = 1,000$. This gave us $1 × 10^8$ points in each quantile-quantile plot. The maximum nonnongenetic risk for the Gaussian risk was 0.4 s.d. and 1 s.d. for the small, sharp risk. We computed the statistic

$$Q = \frac{\text{var}(R_g(k))}{\text{var}(Y_k)}$$

which is the proportion of the phenotypic variance explained by the nongenetic risk and which was equal to 1.4% for the Gaussian risk and 2.2% for the small, sharp risk.

Correcting for stratification. In order to investigate the effect of corrections for population structure, we sampled genotypes for multiple loci and genealogies as described above. However, for this purpose, we sampled only one realization of the quantitative trait $Y_k = N(R_g(k),1)$ to use for every $lg$. We computed single-marker test statistics as described above and then performed the following corrections.

1. Genomic control. We took the $P$ values for each locus $p^l_s$ and computed $X^l_s$ statistics

$$X^l_s = F^{-1}(1 - p^l_s),$$

where $F^{-1}$ is the cumulative distribution function of the $X^2$ distribution with one degree of freedom. We then computed adjusted test statistics

$$\hat{X}^l_s = X^l_s \frac{\lambda}{\lambda + (s_f (0.5)}$$

where the genomic inflation constant

$$\lambda = \frac{(X^l_s)^2}{F^{-1}(0.5)}$$

and $s_f$ represents the median, and computed adjusted $P$ values $\hat{p}^l_s = 1 - F^{-1}(\hat{X}^l_s)^2$.

2. Principal-component analysis. We computed the principal components of the $LG × C$ genotype matrix

$$X = \{X_k^l\}_l p^l...p^L$$

and then fit the linear model

$$Y_k = \mu + \beta X_k + \epsilon_k, \text{ where } \epsilon_k \sim \text{MVN}(0,\sigma^2_k),$$

where $\epsilon_k \sim \text{MVN}(0,\sigma^2_k)$ for some $\sigma^2_k$. We tested the significance of the beta estimates as before. We also tried a PCA variation in which we calculated the principal components only from rare markers with MAF of <5%.

3. Mixed-model analysis. The linear mixed model has the form $Y = X^T \beta + \epsilon_k$, where $\epsilon_k \sim \text{MVN}(0,\sigma^2_k)$ and $\beta$ is the fixed effect for each $k$. This fits the same model as the EMMA package23 but with a more efficient numerical algorithm.

Because we were sampling a single trait for all loci, there was some variance in the amount of inflation and the effectiveness of the corrections, mainly

$$\lambda = \frac{(X^l_s)^2}{F^{-1}(0.5)}$$
because of the sampling variance of the phenotype. To give an accurate idea of the overall effect, we performed these simulations 100 times and show the pointwise average of the quantile-quantile plots.

**Load-based test.** We implemented a test\(^{11}\) for association of a quantitative trait with rare variant load (sometimes described as a ‘collapsing’ or ‘burden’ test).

We simulated variants

\[ X = \{ X_k \} \]

as described above but included only rare variants (MAF < 4%). We imagined that each genealogy represented an independent genomic region (say, a gene) and that each of the \( L \) variants on that genealogy represented a rare variant that segregated in the population. We then computed the rare variant load (or burden) \( B_k \) for each individual for each region by counting the number of derived alleles at each locus, such that \( B_k = \sum X_k \).

We simulated traits as described above and tested association with the rare variant load by fitting the model

\[ B_k = \mu_k + \beta_k X_k + e_k \]

with allele frequencies

\[ f_r = \frac{X_r}{C} \]

and the spatial distance between individuals \( i \) and \( j \) to be given by \( D_{ij} \). Then, we computed the excess allele sharing at distance \( d \) as

\[
Q_d = \frac{1}{R} \sum_{r=1}^{R} \sum_{i=1}^{C} X \left( \hat{X}_i = 1 \right) \sum_{j=1}^{N} X \left( \hat{X}_j = 1 \right) \chi \left\{ D_{ij} = d \right\}
\]

where \( \chi(A) \) is the indicator function of the event \( A \), and recalling that \( \hat{X}_k = \{0, 1\} \) with values of 0 and 1 represents the ancestral and derived alleles, respectively. So, for a given distance for each derived allele, we counted the number that were shared at a given distance and divided by the total number of individuals at that distance to get the allele sharing probability. We then divided by the allele frequency, which is the allele-sharing probability in an infinite homogenous population, to get the excess allele-sharing probability. The \( \log_{10} Q_d \) for rare, low-frequency and common variants are shown in **Figure 3c,d**, with simulation parameters described in the figure legend.