SUPPLEMENTAL MATERIAL

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Association of the marker SNP

The strength of association of the marker SNP is measured by its coefficient of determination (i.e., QT variance explained by the SNP), R^2_{mrk}. We first express this amount using the frequency and effect-size of the variants. The covariance between the marker SNP and QT level is decomposed into contributions by each causal variant (see equation S8 below for derivation):

$$\mathrm{Cov}[x,q] = \sum_{i=1}^{l} d_i \mathrm{Cov}[x,y_i] + \sum_{j=1}^{m} e_j \mathrm{Cov}[x,z_j], \qquad \text{(S1)}$$

where Cov[·,·] represents the covariance. The covariance between the genotypes of the marker SNP and a causal variant becomes

$$\mathrm{Cov}[x,y_i] = 2p_{B_i}(1-p_A),$$
$$\mathrm{Cov}[x,z_j] = 2p_{c_j}p_A.$$

By substituting the genotype covariance to equation (S1), we obtain

$$\mathrm{Cov}[x,q] = 2(1-p_A)\sum_{i=1}^{l} d_i p_{B_i} + 2p_A \sum_{j=1}^{m} e_j p_{c_j}. \qquad \text{(S2)}$$

The ratio of the QT variance explained by the marker to the residual variance is

$$\begin{aligned}
\frac{R^2_{mrk}}{1-R^2_{mrk}} &= \frac{\mathrm{Cov}[x,q]^2}{\mathrm{Var}[x]\,E[\mathrm{Var}[q\mid x]]} \\
&= \frac{\left(2(1-p_A)\sum_{i=1}^{l} d_i p_{B_i} + 2p_A \sum_{j=1}^{m} e_j p_{c_j}\right)^2}{2p_A(1-p_A)\left\{1+2\left(\sum_{i=1}^{l} d_i^2 p_{B_i} + \sum_{j=1}^{m} e_j^2 p_{c_j}\right)\right\}} \\
&= \frac{2\left[\left(\sqrt{\frac{1-p_A}{p_A}}\sum_{i=1}^{l} d_i p_{B_i}\right) + \left(\sqrt{\frac{p_A}{1-p_A}}\sum_{j=1}^{m} e_j p_{c_j}\right)\right]^2}{1+2\left(\sum_{i=1}^{l} d_i^2 p_{B_i} + \sum_{j=1}^{m} e_j^2 p_{c_j}\right)}, \qquad \text{(S3)}
\end{aligned}$$

where, equations (M4) and (S2) were substituted for the second equality, and Var[·] represents

the variance. Using equation (S3), we can relate R^2_{mrk} with the frequency and effect-size of the variants. If the sign of the effect-sizes $(d_1,\ldots,d_l,e_1,\ldots,e_m)$ differ among the causal variants, the terms in the numerator of the last formula cancel each other and weaken the association of the marker. Thus, for a marker actually identified in a GWA study, it is reasonable to assume the effect-sizes mostly have the same sign.

For marker(s) identified in a GWA study, the coefficient of determination would be in a certain range constrained by the sample size of the study. We focused on testing heteroscedasticity and skewness of an SNP showing association at a borderline genome-wide significance level. Precisely, we assumed the coefficient of determination to be R^2_{mrk}=0.00592; such an association is detectable with 50% power under the significance level of 5×10^{-8} when tested in 5000 individuals (see next paragraph). Alternatively, to assess how test statistics change according to the coefficient of determination, we examined a doubled value of R^2_{mrk}=0.0118 for comparison.

The above-mentioned coefficient of determination of the marker, R^2_{mrk}, was calculated using power assessment for linear regression analysis conducted in N samples. The probability (power) to detect the association of a marker at a significance level of α equals

$$\int_v^\infty F(u;1,N-2,NR^2_{mrk}/(1-R^2_{mrk}))\,du,$$

where $F(u;\ 1,\ N–2,\ \theta^2)$ is the probability density function for an F distribution with 1, N–2 degrees of freedom and non-centrality parameter θ^2 (Section 28.28 in (Stuart et al. 1999), and Example 8.4 in (Knight 2000)). Here, the constant v is the upper critical value corresponding to α of a central F distribution with the same degrees of freedom.

Derivation of key equations

Among haplotype classes with a specific marker allele (*A* or *a*), we assumed the conditional probability distribution of alleles to be independent among causal variants (see Methods). Then, under Hardy-Weinberg equilibrium, probability distribution of causal variant genotypes becomes independent when conditioned on a marker genotype x_0:

$$p(y_1,...,y_l,z_1,...,z_m \mid x = x_0) = \prod_{i=1}^{l} p(y_i \mid x = x_0) \cdot \prod_{j=1}^{m} p(z_j \mid x = x_0). \qquad \text{(S4)}$$

The expectation of the QT value is

$$\begin{aligned} E[q] &= \int_{-\infty}^{\infty} dq \sum_{x,y_1,...,y_l,z_1,...,z_m} p(x,y_1,...,y_l,z_1,...,z_m) \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\left(q - \sum_{i=1}^{l} d_i y_i - \sum_{j=1}^{m} e_j z_j \right)^2}{2} \right) q \\ &= \sum_{x,y_1,...,y_l,z_1,...,z_m} \left(p(x,y_1,...,y_l,z_1,...,z_m) \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) \right) \\ &= \sum_{i=1}^{l} \left(d_i \sum_{x,y_1,...,y_l,z_1,...,z_m} p(x,y_1,...,y_l,z_1,...,z_m) y_i \right) + \sum_{j=1}^{m} \left(e_j \sum_{x,y_1,...,y_l,z_1,...,z_m} p(x,y_1,...,y_l,z_1,...,z_m) z_j \right) \\ &= \sum_{i=1}^{l} d_i E[y_i] + \sum_{j=1}^{m} e_j E[z_j], \qquad \text{(S5)} \end{aligned}$$

where q is integrated for the second equality, and summations are exchanged for the third equality.

The variance of QT value within individuals of a specific genotype x_0 at the marker becomes

$$
\begin{aligned}
&\mathrm{Var}[q \mid x = x_0] \\
&= \int_{-\infty}^{\infty} dq \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\left(q - \sum_{i=1}^{l} d_i y_i - \sum_{j=1}^{m} e_j z_j \right)^2}{2} \right) (q - E[q \mid x = x_0])^2 \\
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \left\{ 1 + \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - E[q \mid x = x_0] \right\}^2 \right\} \\
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \left\{ 1 + \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - \left(\sum_{i=1}^{l} d_i E[y_i \mid x = x_0] + \sum_{j=1}^{m} e_j E[z_j \mid x = x_0] \right) \right\}^2 \right\} \\
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \left\{ 1 + \left\{ \sum_{i=1}^{l} d_i (y_i - E[y_i \mid x = x_0]) + \sum_{j=1}^{m} e_j (z_j - E[z_j \mid x = x_0]) \right\}^2 \right\} \\
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \left\{ 1 + \left(\sum_{i=1}^{l} d_i^2 (y_i - E[y_i \mid x = x_0])^2 \right) \right. \\
&\quad + \left(\sum_{j=1}^{m} e_j^2 (z_j - E[z_j \mid x = x_0])^2 \right) \\
&\quad + \left(\sum_{i \neq i'} d_i d_{i'} (y_i - E[y_i \mid x = x_0])(y_{i'} - E[y_{i'} \mid x = x_0]) \right) \\
&\quad + \left(\sum_{j \neq j'} e_j e_{j'} (z_j - E[z_j \mid x = x_0])(z_{j'} - E[z_{j'} \mid x = x_0]) \right) \\
&\quad \left. + \left(\sum_{i,j} d_i e_j (y_i - E[y_i \mid x = x_0])(z_j - E[z_j \mid x = x_0]) \right) \right\} \\
&= 1 + \left(\sum_{i=1}^{l} d_i^2 \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0)(y_i - E[y_i \mid x = x_0])^2 \right) \\
&\quad + \left(\sum_{j=1}^{l} e_j^2 \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0)(z_j - E[z_j \mid x = x_0])^2 \right) \\
&\quad + \left(\sum_{i \neq i'} d_i d_{i'} \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0)(y_i - E[y_i \mid x = x_0])(y_{i'} - E[y_{i'} \mid x = x_0]) \right) \\
&\quad + \left(\sum_{j \neq j'} e_j e_{j'} \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0)(z_j - E[z_j \mid x = x_0])(z_{j'} - E[z_{j'} \mid x = x_0]) \right) \\
&\quad + \left(\sum_{i,j} d_i e_j \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0)(y_i - E[y_i \mid x = x_0])(z_j - E[z_j \mid x = x_0]) \right) \\
&= 1 + \sum_{i=1}^{l} d_i^2 \mathrm{Var}[y_i \mid x = x_0] + \sum_{j=1}^{m} e_j^2 \mathrm{Var}[z_j \mid x = x_0] + 0 + 0 + 0, \qquad \text{(S6)}
\end{aligned}
$$

where q is integrated for the second equality, equation (M1) is applied for the third equality, summations are exchanged for the sixth equality, and equation (S4) is applied for the last

equality.

The third central moment of QT within individuals of a specific genotype x_0 at the marker becomes

$$
\begin{aligned}
&\mu_3[q \mid x = x_0] \\
&= \int_{-\infty}^{\infty} dq \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\left(q - \sum_{i=1}^{l} d_i y_i - \sum_{j=1}^{m} e_j z_j \right)^2}{2} \right) (q - E[q \mid x = x_0])^3 \\
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \left[3 \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - E[q \mid x = x_0] \right\} \right. \\
&\qquad \left. + \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - E[q \mid x = x_0] \right\}^3 \right] \\
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \left[3 \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - \left(\sum_{i=1}^{l} d_i E[y_i \mid x = x_0] + \sum_{j=1}^{m} e_j E[z_j \mid x = x_0] \right) \right\} \right. \\
&\qquad \left. + \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - \left(\sum_{i=1}^{l} d_i E[y_i \mid x = x_0] + \sum_{j=1}^{m} e_j E[z_j \mid x = x_0] \right) \right\}^3 \right] \\
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x = x_0) \left[3 \left\{ \sum_{i=1}^{l} d_i (y_i - E[y_i \mid x = x_0]) + \sum_{j=1}^{m} e_j (z_j - E[z_j \mid x = x_0]) \right\} \right. \\
&\qquad \left. + \left\{ \sum_{i=1}^{l} d_i (y_i - E[y_i \mid x = x_0]) + \sum_{j=1}^{m} e_j (z_j - E[z_j \mid x = x_0]) \right\}^3 \right]
\end{aligned}
$$

$$
\begin{aligned}
&= \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)\Bigg\{3\left(\sum_{i=1}^{l} d_i (y_i - E[y_i \mid x=x_0])\right) \\
&\quad + 3\left(\sum_{j=1}^{m} e_j (z_j - E[z_j \mid x=x_0])\right) \\
&\quad + \left(\sum_{i=1}^{l} {d_i}^3 (y_i - E[y_i \mid x=x_0])^3\right) \\
&\quad + \left(\sum_{j=1}^{m} {e_j}^3 (z_j - E[z_j \mid x=x_0])^3\right) \\
&\quad + \left(\sum_{\substack{i,i',i'': \\ \text{not all equal}}} d_i d_{i'} d_{i''} (y_i - E[y_i \mid x=x_0])(y_{i'} - E[y_{i'} \mid x=x_0])(y_{i''} - E[y_{i''} \mid x=x_0])\right) \\
&\quad + 3\left(\sum_{i,i',j} d_i d_{i'} e_j (y_i - E[y_i \mid x=x_0])(y_{i'} - E[y_{i'} \mid x=x_0])(z_j - E[z_j \mid x=x_0])\right) \\
&\quad + 3\left(\sum_{i,j,j'} d_i e_j e_{j'} (y_i - E[y_i \mid x=x_0])(z_j - E[z_j \mid x=x_0])(z_{j'} - E[z_{j'} \mid x=x_0])\right) \\
&\quad + \left(\sum_{\substack{j,j',j'': \\ \text{not all equal}}} e_j e_{j'} e_{j''} (z_j - E[z_j \mid x=x_0])(z_{j'} - E[z_{j'} \mid x=x_0])(z_{j''} - E[z_{j''} \mid x=x_0])\right)\Bigg\}
\end{aligned}
$$

$$
\begin{aligned}
&= 3\left(\sum_{i=1}^{l} d_i \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(y_i - E[y_i \mid x=x_0])\right) \\
&\quad + 3\left(\sum_{j=1}^{m} e_j \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(z_j - E[z_j \mid x=x_0])\right) \\
&\quad + \left(\sum_{i=1}^{l} {d_i}^3 \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(y_i - E[y_i \mid x=x_0])^3\right) \\
&\quad + \left(\sum_{j=1}^{m} {e_j}^3 \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(z_j - E[z_j \mid x=x_0])^3\right) \\
&\quad + \left(\sum_{\substack{i,i',i'': \\ \text{not all equal}}} d_i d_{i'} d_{i''} \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(y_i - E[y_i \mid x=x_0])(y_{i'} - E[y_{i'} \mid x=x_0])(y_{i''} - E[y_{i''} \mid x=x_0])\right) \\
&\quad + 3\left(\sum_{i,i',j} d_i d_{i'} e_j \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(y_i - E[y_i \mid x=x_0])(y_{i'} - E[y_{i'} \mid x=x_0])(z_j - E[z_j \mid x=x_0])\right) \\
&\quad + 3\left(\sum_{i,j',j''} d_i e_j e_{j'} \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(y_i - E[y_i \mid x=x_0])(z_j - E[z_j \mid x=x_0])(z_{j'} - E[z_{j'} \mid x=x_0])\right) \\
&\quad + \left(\sum_{\substack{j,j',j'': \\ \text{not all equal}}} e_j e_{j'} e_{j''} \sum_{y_1,\ldots,y_l,z_1,\ldots,z_m} p(y_1,\ldots,y_l,z_1,\ldots,z_m \mid x=x_0)(z_j - E[z_j \mid x=x_0])(z_{j'} - E[z_{j'} \mid x=x_0])(z_{j''} - E[z_{j''} \mid x=x_0])\right) \\
&= 0 + 0 + \sum_{i=1}^{l} {d_i}^3 \mu_3[y_i \mid x=x_0] + \sum_{j=1}^{m} {e_j}^3 \mu_3[z_j \mid x=x_0] + 0 + 0 + 0 + 0, \qquad \text{(S7)}
\end{aligned}
$$

where q is integrated for the second equality, equation (M1) is applied for the third equality, summations are exchanged for the sixth equality, and equation (S4) is applied for the last equality.

The covariance between the marker genotype x and QT value q becomes

$$
\begin{aligned}
&\mathrm{Cov}[x,q] \\
&= \int_{-\infty}^{\infty} dq \sum_{x,y_1,\ldots,y_l,z_1,\ldots,z_m} p(x,y_1,\ldots,y_l,z_1,\ldots,z_m) \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\left(q - \sum_{i=1}^{l} d_i y_i - \sum_{j=1}^{m} e_j z_j \right)^2}{2} \right) (x - E[x])(q - E[q]) \\
&= \sum_{x,y_1,\ldots,y_l,z_1,\ldots,z_m} \left(p(x,y_1,\ldots,y_l,z_1,\ldots,z_m)(x - E[x]) \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - E[q] \right\} \right) \\
&= \sum_{x,y_1,\ldots,y_l,z_1,\ldots,z_m} \left(p(x,y_1,\ldots,y_l,z_1,\ldots,z_m)(x - E[x]) \left\{ \left(\sum_{i=1}^{l} d_i y_i + \sum_{j=1}^{m} e_j z_j \right) - \left(\sum_{i=1}^{l} d_i E[y_i] + \sum_{j=1}^{m} e_j E[z_j] \right) \right\} \right) \\
&= \sum_{x,y_1,\ldots,y_l,z_1,\ldots,z_m} \left(p(x,y_1,\ldots,y_l,z_1,\ldots,z_m)(x - E[x]) \left\{ \left(\sum_{i=1}^{l} d_i (y_i - E[y_i]) \right) + \left(\sum_{j=1}^{m} e_j (z_j - E[z_j]) \right) \right\} \right) \\
&= \sum_{i=1}^{l} \left(d_i \sum_{x,y_1,\ldots,y_l,z_1,\ldots,z_m} p(x,y_1,\ldots,y_l,z_1,\ldots,z_m)(x - E[x])(y_i - E[y_i]) \right) \\
&\quad + \sum_{j=1}^{m} \left(e_j \sum_{x,y_1,\ldots,y_l,z_1,\ldots,z_m} p(x,y_1,\ldots,y_l,z_1,\ldots,z_m)(x - E[x])(z_j - E[z_j]) \right) \\
&= \sum_{i=1}^{l} d_i \mathrm{Cov}[x,y_i] + \sum_{j=1}^{m} e_j \mathrm{Cov}[x,z_j], \qquad \text{(S8)}
\end{aligned}
$$

where q is integrated for the second equality, (S5) is applied for the third equality, and summations are exchanged for the fifth equality.

References

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Stuart A, J Ord, S Arnold. 1999. *Kendall's advanced theory of statistics*. Arnold publishers, London.

Supplemental Table 1. Type I error rate of tests for synthetic association under population stratification.

Model of population stratification	Frequency of *A* allele, p_A		Mean QT						Proportion of subpopulation		Variance of QT by genotype			Type I error rate		
			Genotype *aa*		Genotype *Aa*		Genotype *AA*									
	POP1	POP2	POP1	POP2	POP1	POP2	POP1	POP2	POP1	POP2	*aa*	*Aa*	*AA*	Heteroscedasticity	Skewness	Combined
Borderline genome-wide significant association (R^2_{mrk}=0.00592)																
No population stratification	0.05	NA	0.0000	NA	0.2504	NA	0.5008	NA	1	0	1.000	1.000	1.000	0.050	0.002	0.015
Effect size in POP2 is larger by 25%	0.05	0.05	0.0000	0.0000	0.2504	0.3130	0.5008	0.6260	0.9	0.1	1.000	1.000	1.001	0.046	0.001	0.016
Effect size in POP2 is larger by 25%	0.05	0.05	0.0000	0.0000	0.2504	0.3130	0.5008	0.6260	0.5	0.5	1.000	1.001	1.004	0.041	0.004	0.018
Effect size in POP2 is larger by 50%	0.05	0.05	0.0000	0.0000	0.2504	0.3756	0.5008	0.7512	0.9	0.1	1.000	1.001	1.006	0.054	0.004	0.019
Effect size in POP2 is larger by 50%	0.05	0.05	0.0000	0.0000	0.2504	0.3756	0.5008	0.7512	0.5	0.5	1.000	1.004	1.016	0.061	0.004	0.018
Effect size in POP2 is larger by 100%	0.05	0.05	0.0000	0.0000	0.2504	0.5008	0.5008	1.0016	0.9	0.1	1.000	1.006	1.023	0.056	0.008	0.026
Effect size in POP2 is larger by 100%	0.05	0.05	0.0000	0.0000	0.2504	0.5008	0.5008	1.0016	0.5	0.5	1.000	1.016	1.063	0.047	0.002	0.012
Allele frequencies differ by 0.05	0.05	0.10	0.0000	0.0000	0.2504	0.2504	0.5008	0.5008	0.9	0.1	1.000	1.000	1.000	0.051	0.005	0.019
Allele frequencies differ by 0.05	0.05	0.10	0.0000	0.0000	0.2504	0.2504	0.5008	0.5008	0.5	0.5	1.000	1.000	1.000	0.052	0.011	0.026
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.05	0.10	0.0000	0.0626	0.2504	0.3130	0.5008	0.5634	0.9	0.1	1.000	1.001	1.001	0.055	0.006	0.025
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.05	0.10	0.0000	0.0626	0.2504	0.3130	0.5008	0.5634	0.5	0.5	1.001	1.001	1.001	0.050	0.008	0.023
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.05	0.10	0.0000	0.1252	0.2504	0.3756	0.5008	0.6260	0.9	0.1	1.001	1.002	1.003	0.034	0.002	0.016
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.05	0.10	0.0000	0.1252	0.2504	0.3756	0.5008	0.6260	0.5	0.5	1.004	1.004	1.003	0.047	0.011	0.022
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.05	0.10	0.0000	0.2504	0.2504	0.5008	0.5008	0.7512	0.9	0.1	1.005	1.009	1.013	0.032	0.001	0.007
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.05	0.10	0.0000	0.2504	0.2504	0.5008	0.5008	0.7512	0.5	0.5	1.016	1.014	1.010	0.040	0.007	0.018
No population stratification	0.50	NA	0.0000	NA	0.1091	NA	0.2182	NA	1	0	1.000	1.000	1.000	0.047	0.045	0.054
Effect size in POP2 is larger by 25%	0.50	0.50	0.0000	0.0000	0.1091	0.1364	0.2182	0.2728	0.9	0.1	1.000	1.000	1.000	0.049	0.042	0.038
Effect size in POP2 is larger by 25%	0.50	0.50	0.0000	0.0000	0.1091	0.1364	0.2182	0.2728	0.5	0.5	1.000	1.000	1.001	0.050	0.043	0.040
Effect size in POP2 is larger by 50%	0.50	0.50	0.0000	0.0000	0.1091	0.1637	0.2182	0.3273	0.9	0.1	1.000	1.000	1.001	0.044	0.043	0.041
Effect size in POP2 is larger by 50%	0.50	0.50	0.0000	0.0000	0.1091	0.1637	0.2182	0.3273	0.5	0.5	1.000	1.001	1.003	0.048	0.038	0.051
Effect size in POP2 is larger by 100%	0.50	0.50	0.0000	0.0000	0.1091	0.2182	0.2182	0.4364	0.9	0.1	1.000	1.001	1.004	0.055	0.039	0.046
Effect size in POP2 is larger by 100%	0.50	0.50	0.0000	0.0000	0.1091	0.2182	0.2182	0.4364	0.5	0.5	1.000	1.003	1.012	0.048	0.051	0.046
Allele frequencies differ by 0.05	0.50	0.55	0.0000	0.0000	0.1091	0.2504	0.2182	0.5008	0.9	0.1	1.000	1.002	1.008	0.052	0.036	0.042
Allele frequencies differ by 0.05	0.50	0.55	0.0000	0.0000	0.1091	0.2504	0.2182	0.5008	0.5	0.5	1.000	1.005	1.020	0.051	0.034	0.046
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.50	0.55	0.0000	0.0273	0.1091	0.1364	0.2182	0.2455	0.9	0.1	1.000	1.000	1.000	0.060	0.044	0.047
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.50	0.55	0.0000	0.0273	0.1091	0.1364	0.2182	0.2455	0.5	0.5	1.000	1.000	1.000	0.042	0.032	0.038
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.50	0.55	0.0000	0.0546	0.1091	0.1637	0.2182	0.2728	0.9	0.1	1.000	1.000	1.000	0.046	0.051	0.051
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.50	0.55	0.0000	0.0546	0.1091	0.1637	0.2182	0.2728	0.5	0.5	1.001	1.001	1.001	0.046	0.043	0.053
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.50	0.55	0.0000	0.1091	0.1091	0.2182	0.2182	0.3273	0.9	0.1	1.001	1.001	1.001	0.048	0.043	0.052
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.50	0.55	0.0000	0.1091	0.1091	0.2182	0.2182	0.3273	0.5	0.5	1.003	1.003	1.003	0.050	0.035	0.038
No population stratification	0.90	NA	0.0000	NA	0.1819	NA	0.3638	NA	1	0	1.000	1.000	1.000	0.042	0.014	0.028
Effect size in POP2 is larger by 25%	0.90	0.90	0.0000	0.0000	0.1819	0.2274	0.3638	0.4548	0.9	0.1	1.000	1.000	1.001	0.048	0.012	0.023
Effect size in POP2 is larger by 25%	0.90	0.90	0.0000	0.0000	0.1819	0.2274	0.3638	0.4548	0.5	0.5	1.000	1.001	1.002	0.047	0.007	0.018
Effect size in POP2 is larger by 50%	0.90	0.90	0.0000	0.0000	0.1819	0.2729	0.3638	0.5457	0.9	0.1	1.000	1.001	1.003	0.045	0.013	0.013
Effect size in POP2 is larger by 50%	0.90	0.90	0.0000	0.0000	0.1819	0.2729	0.3638	0.5457	0.5	0.5	1.000	1.002	1.008	0.041	0.017	0.022
Effect size in POP2 is larger by 100%	0.90	0.90	0.0000	0.0000	0.1819	0.3638	0.3638	0.7276	0.9	0.1	1.000	1.003	1.012	0.050	0.016	0.030
Effect size in POP2 is larger by 100%	0.90	0.90	0.0000	0.0000	0.1819	0.3638	0.3638	0.7276	0.5	0.5	1.000	1.008	1.033	0.059	0.011	0.033
Allele frequencies differ by 0.05	0.90	0.95	0.0000	0.0000	0.1819	0.2504	0.3638	0.5008	0.9	0.1	1.000	1.000	1.002	0.046	0.010	0.020
Allele frequencies differ by 0.05	0.90	0.95	0.0000	0.0000	0.1819	0.2504	0.3638	0.5008	0.5	0.5	1.000	1.001	1.005	0.044	0.009	0.023
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.90	0.95	0.0000	0.0455	0.1819	0.2274	0.3638	0.4093	0.9	0.1	1.000	1.000	1.000	0.050	0.012	0.020
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.90	0.95	0.0000	0.0455	0.1819	0.2274	0.3638	0.4093	0.5	0.5	1.000	1.000	1.001	0.045	0.011	0.035
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.90	0.95	0.0000	0.0910	0.1819	0.2729	0.3638	0.4548	0.9	0.1	1.000	1.000	1.001	0.061	0.012	0.030
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.90	0.95	0.0000	0.0910	0.1819	0.2729	0.3638	0.4548	0.5	0.5	1.001	1.002	1.002	0.039	0.011	0.019
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.90	0.95	0.0000	0.1819	0.1819	0.3638	0.3638	0.5457	0.9	0.1	1.001	1.002	1.003	0.037	0.009	0.020
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.90	0.95	0.0000	0.1819	0.1819	0.3638	0.3638	0.5457	0.5	0.5	1.005	1.007	1.008	0.041	0.013	0.019

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Model of population stratification	Frequency of *A* allele, p_A		Mean QT						Proportion of subpopulation		Variance of QT by genotype			Type I error rate		
			Genotype *aa*		Genotype *Aa*		Genotype *AA*									
	POP1	POP2	POP1	POP2	POP1	POP2	POP1	POP2	POP1	POP2	*aa*	*Aa*	*AA*	Heteroscedasticity	Skewness	Combined
Strong association (R^2_{mrk}=0.05)																
No population stratification	0.05	NA	0.0000	NA	0.7443	NA	1.4886	NA	1	0	1.000	1.000	1.000	0.033	0.007	0.018
Effect size in POP2 is larger by 25%	0.05	0.05	0.0000	0.0000	0.7443	0.9304	1.4886	1.8608	0.9	0.1	1.000	1.003	1.012	0.037	0.007	0.017
Effect size in POP2 is larger by 25%	0.05	0.05	0.0000	0.0000	0.7443	0.9304	1.4886	1.8608	0.5	0.5	1.000	1.009	1.035	0.037	0.021	0.020
Effect size in POP2 is larger by 50%	0.05	0.05	0.0000	0.0000	0.7443	1.1165	1.4886	2.2329	0.9	0.1	1.000	1.012	1.050	0.032	0.011	0.016
Effect size in POP2 is larger by 50%	0.05	0.05	0.0000	0.0000	0.7443	1.1165	1.4886	2.2329	0.5	0.5	1.000	1.035	1.138	0.035	0.050	0.039
Effect size in POP2 is larger by 100%	0.05	0.05	0.0000	0.0000	0.7443	1.4886	1.4886	2.9772	0.9	0.1	1.000	1.050	1.199	0.032	0.032	0.030
Effect size in POP2 is larger by 100%	0.05	0.05	0.0000	0.0000	0.7443	1.4886	1.4886	2.9772	0.5	0.5	1.000	1.138	1.554	0.035	0.371	0.190
Allele frequencies differ by 0.05	0.05	0.10	0.0000	0.0000	0.7443	0.7443	1.4886	1.4886	0.9	0.1	1.000	1.000	1.000	0.048	0.007	0.020
Allele frequencies differ by 0.05	0.05	0.10	0.0000	0.0000	0.7443	0.7443	1.4886	1.4886	0.5	0.5	1.000	1.000	1.000	0.051	0.021	0.027
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.05	0.10	0.0000	0.1861	0.7443	0.9304	1.4886	1.6747	0.9	0.1	1.003	1.005	1.007	0.037	0.014	0.019
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.05	0.10	0.0000	0.1861	0.7443	0.9304	1.4886	1.6747	0.5	0.5	1.009	1.008	1.006	0.038	0.020	0.033
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.05	0.10	0.0000	0.3722	0.7443	1.1165	1.4886	1.8608	0.9	0.1	1.011	1.020	1.030	0.049	0.016	0.030
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.05	0.10	0.0000	0.3722	0.7443	1.1165	1.4886	1.8608	0.5	0.5	1.035	1.031	1.022	0.055	0.032	0.040
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.05	0.10	0.0000	0.7443	0.7443	1.4886	1.4886	2.2329	0.9	0.1	1.046	1.080	1.118	0.032	0.011	0.020
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.05	0.10	0.0000	0.7443	0.7443	1.4886	1.4886	2.2329	0.5	0.5	1.138	1.125	1.089	0.060	0.031	0.040
No population stratification	0.50	NA	0.0000	NA	0.3244	NA	0.6488	NA	1	0	1.000	1.000	1.000	0.034	0.028	0.028
Effect size in POP2 is larger by 25%	0.50	0.50	0.0000	0.0000	0.3244	0.4055	0.6488	0.8110	0.9	0.1	1.000	1.001	1.002	0.038	0.031	0.036
Effect size in POP2 is larger by 25%	0.50	0.50	0.0000	0.0000	0.3244	0.4055	0.6488	0.8110	0.5	0.5	1.000	1.002	1.007	0.039	0.022	0.027
Effect size in POP2 is larger by 50%	0.50	0.50	0.0000	0.0000	0.3244	0.4866	0.6488	0.9732	0.9	0.1	1.000	1.002	1.009	0.052	0.031	0.031
Effect size in POP2 is larger by 50%	0.50	0.50	0.0000	0.0000	0.3244	0.4866	0.6488	0.9732	0.5	0.5	1.000	1.007	1.026	0.039	0.025	0.023
Effect size in POP2 is larger by 100%	0.50	0.50	0.0000	0.0000	0.3244	0.6488	0.6488	1.2976	0.9	0.1	1.000	1.009	1.038	0.074	0.024	0.054
Effect size in POP2 is larger by 100%	0.50	0.50	0.0000	0.0000	0.3244	0.6488	0.6488	1.2976	0.5	0.5	1.000	1.026	1.105	0.245	0.015	0.152
Allele frequencies differ by 0.05	0.50	0.55	0.0000	0.0000	0.3244	0.3244	0.6488	0.6488	0.9	0.1	1.000	1.000	1.000	0.043	0.030	0.037
Allele frequencies differ by 0.05	0.50	0.55	0.0000	0.0000	0.3244	0.3244	0.6488	0.6488	0.5	0.5	1.000	1.000	1.000	0.042	0.028	0.036
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.50	0.55	0.0000	0.0811	0.3244	0.4055	0.6488	0.7299	0.9	0.1	1.000	1.001	1.001	0.032	0.032	0.031
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.50	0.55	0.0000	0.0811	0.3244	0.4055	0.6488	0.7299	0.5	0.5	1.002	1.002	1.002	0.049	0.032	0.035
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.50	0.55	0.0000	0.1622	0.3244	0.4866	0.6488	0.8110	0.9	0.1	1.002	1.002	1.003	0.047	0.026	0.037
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.50	0.55	0.0000	0.1622	0.3244	0.4866	0.6488	0.8110	0.5	0.5	1.007	1.007	1.007	0.035	0.024	0.026
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.50	0.55	0.0000	0.3244	0.3244	0.6488	0.6488	0.9732	0.9	0.1	1.008	1.009	1.011	0.041	0.032	0.038
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.50	0.55	0.0000	0.3244	0.3244	0.6488	0.6488	0.9732	0.5	0.5	1.026	1.026	1.026	0.036	0.037	0.024
No population stratification	0.90	NA	0.0000	NA	0.5407	NA	1.0814	NA	1	0	1.000	1.000	1.000	0.035	0.008	0.018
Effect size in POP2 is larger by 25%	0.90	0.90	0.0000	0.0000	0.5407	0.6759	1.0814	1.3518	0.9	0.1	1.000	1.002	1.007	0.039	0.009	0.014
Effect size in POP2 is larger by 25%	0.90	0.90	0.0000	0.0000	0.5407	0.6759	1.0814	1.3518	0.5	0.5	1.000	1.005	1.018	0.049	0.008	0.011
Effect size in POP2 is larger by 50%	0.90	0.90	0.0000	0.0000	0.5407	0.8111	1.0814	1.6221	0.9	0.1	1.000	1.007	1.026	0.049	0.013	0.027
Effect size in POP2 is larger by 50%	0.90	0.90	0.0000	0.0000	0.5407	0.8111	1.0814	1.6221	0.5	0.5	1.000	1.018	1.073	0.148	0.007	0.083
Effect size in POP2 is larger by 100%	0.90	0.90	0.0000	0.0000	0.5407	1.0814	1.0814	2.1628	0.9	0.1	1.000	1.026	1.105	0.126	0.013	0.049
Effect size in POP2 is larger by 100%	0.90	0.90	0.0000	0.0000	0.5407	1.0814	1.0814	2.1628	0.5	0.5	1.000	1.073	1.292	0.915	0.006	0.808
Allele frequencies differ by 0.05	0.90	0.95	0.0000	0.0000	0.5407	0.5407	1.0814	1.0814	0.9	0.1	1.000	1.000	1.000	0.033	0.009	0.020
Allele frequencies differ by 0.05	0.90	0.95	0.0000	0.0000	0.5407	0.5407	1.0814	1.0814	0.5	0.5	1.000	1.000	1.000	0.035	0.017	0.016
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.90	0.95	0.0000	0.1352	0.5407	0.6759	1.0814	1.2166	0.9	0.1	1.000	1.001	1.002	0.044	0.011	0.020
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 25% of effect size in every genotype	0.90	0.95	0.0000	0.1352	0.5407	0.6759	1.0814	1.2166	0.5	0.5	1.003	1.004	1.005	0.047	0.013	0.021
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.90	0.95	0.0000	0.2704	0.5407	0.8111	1.0814	1.3518	0.9	0.1	1.002	1.004	1.007	0.039	0.009	0.021
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 50% of effect size in every genotype	0.90	0.95	0.0000	0.2704	0.5407	0.8111	1.0814	1.3518	0.5	0.5	1.012	1.017	1.018	0.035	0.008	0.012
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.90	0.95	0.0000	0.5407	0.5407	1.0814	1.0814	1.6221	0.9	0.1	1.008	1.015	1.029	0.039	0.006	0.017
Allele frequencies differ by 0.05; Mean QT in POP2 is larger by 100% of effect size in every genotype	0.90	0.95	0.0000	0.5407	0.5407	1.0814	1.0814	1.6221	0.5	0.5	1.047	1.066	1.073	0.058	0.014	0.024

Population stratification was modeled as a mixture of two subpopulations, POP1 and POP2, which differed by effect size, allele frequency, or mean QT. The propotion of POP1 was 0.9 or 0.5. The QT was normally distributed with unit variance for individuals having the same genotype in a subpopulation.

As the "null hypothesis" for synthetic association, we assumed indirect association, where a causal variant was completely correlated with the marker SNP. The marker allele *A* had frequency p_A = 0.05, 0.50 or 0.90 in POP1.

For each model of stratification, we performed 1000 simulation trials. In each trial, 5000 individuals were generated, and synthetic association was tested.

For each test, the type I error rate was assessed by the ratio of trials attaining nominal p-value <0.05. Numbers are highlighted red when the type I error rate was significantly inflated (≥0.065).

When the strength of association was at borderline level of genome-wide significance (approximately R^2_{mrk}=0.00592), there was no inflation in type I error rate under any model of stratificaiton. When the association was strong (R^2_{mrk}=0.05), type I error rate could inflate under strong population stratification, such as 50% difference in effect size.

Supplemental Table 2. Empirical distribution of the test statistics for synthetic association in expression data.

Gene_label	Illumina_Gene_ID	SNP_rsID	Chromosome	SNP_location B35	Distance SNP-probe midpoint (bp)	-log10(p-value for association)	Adjusted_R^2	P-value for tests of synthetic association		
								Heteroscedasticity	Skewness	Combined
TSGA10	GI_41281983-I	rs4340581	2	99,183,543	32,884	6.4	0.35	0.5907	0.7953	0.8247
MOCOS	GI_8923660-S	rs617505	18	32,051,949	50,616	6.2	0.34	0.0018	0.1613	0.0026
ZNF79	GI_24307936-S	rs2243898	9	127,299,544	12,379	6.3	0.34	0.8105	0.9360	0.9682
C7orf13	GI_14249145-S	rs4716648	7	155,948,932	17,999	6.1	0.34	0.4993	0.7055	0.7198
Hs.506072	Hs.506072-S	rs6451535	5	40,723,788	138,673	6.2	0.34	0.6737	0.9145	0.9145
DTNB	GI_37577100-A	rs1369704	2	25,535,241	23,436	6.2	0.34	0.9153	0.9135	0.9858
C8orf13	GI_32698772-S	rs2736340	8	11,381,382	64,837	6.2	0.34	0.7989	0.9034	0.9571
MCMDC1	GI_33469926-S	rs988098	6	119,281,925	8,141	6.2	0.34	0.6636	0.4872	0.6884
Hs.465789	Hs.465789-S	rs10411185	19	8,009,881	30,281	6.2	0.34	0.0848	0.8613	0.2641
MMRP19	GI_7705723-S	rs2956077	11	34,919,504	52,976	6.0	0.34	0.0993	0.4185	0.1737
LOC375399	GI_37540365-S	rs1048447	4	68,312,238	103,237	6.0	0.33	0.6728	0.5773	0.7557
LOC401135	GI_42657007-S	rs1048447	4	68,312,238	104,593	6.0	0.33	0.9910	0.5217	0.8581
PHEMX	GI_37595532-A	rs937614	11	2,283,496	12,377	5.9	0.32	0.2669	0.3797	0.3333
HIBCH	GI_37594468-A	rs291427	2	191,019,565	124,290	5.8	0.32	0.2946	0.4540	0.4028
IPP	GI_5174472-S	rs3811436	1	45,794,973	38,862	5.6	0.32	0.9956	0.7981	0.9773
MGC4083	GI_14210535-S	rs4796960	18	12,308,027	8,169	5.6	0.32	0.4726	0.6200	0.6527
TMPIT	GI_13994299-S	rs7794040	7	75,265,292	4,133	5.6	0.31	0.7520	0.5384	0.7709
FUT10	GI_40805105-S	rs2304748	8	33,489,486	140,780	5.5	0.30	0.9284	0.8384	0.9734
IRAK1BP1	GI_27498358-S	rs9359355	6	79,659,533	4,990	5.3	0.30	0.2028	0.7898	0.4535
MGC45416	GI_22748848-S	rs12504699	4	48,775,226	38,261	5.3	0.29	0.3222	0.6081	0.5153

Test p-values are shown for the SNP genotype and gene expression in lymphoblastoid cell lines of the HapMap CEU individuals (*N*=60).

From the reported significant associations (Stranger et al. 2007, *Science* 315:848, Table S2), those with $R^2 \leq 0.35$ and data available from a public database (http://www.ncbi.nlm.nih.gov/gtex/test/GTEX2/gtex.cgi) were extracted.

See Supplemental Figure 5 for explanation.

Supplemental Figure 1

LDL cholesterol association and LD structure at the *APOE* locus in a GWA study of 1210 Japanese individuals [subset of those reported in (Takeuchi et al. 2010)].

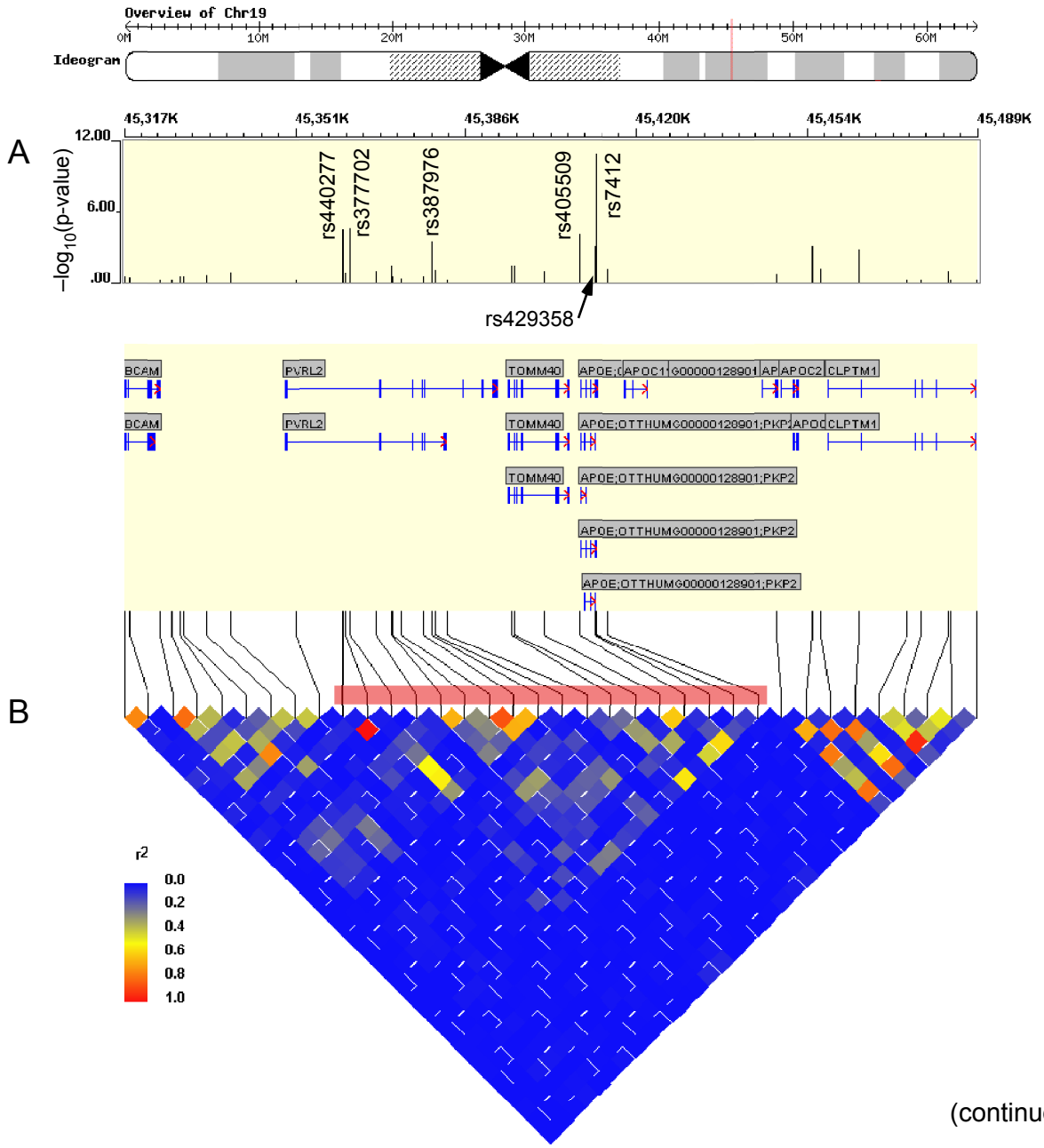
A. Association p-values are plotted for SNPs on a GWA study chip (illumina HumanHap550) and for functional variants rs429358 and rs7412, not included in the chip. Among the SNPs on the chip, four SNPs (rs440277, rs377702, rs387976 and rs405509) attained a locus-wise statistical significance level ($p < 0.0033 = 0.05/15$, corrected for 15 SNPs in the relevant LD block) for LDL cholesterol association. The causal variant rs7412 showed much stronger association ($p = 1.9 \times 10^{-11}$) than the four SNPs, while rs429358 showed modest association ($p = 0.0011$).

B. The LD block including the *APOE* gene extends from 45,360 kb to 45,420 kb on chromosome 19 (Build 37) as highlighted by the red rectangle.

C. LD coefficients between the relevant SNPs. As rs440277 is completely correlated ($r^2=1$) with rs377702, we only study the latter in the remaining analysis.

D. In a stepwise regression analysis only for the chip SNPs, rs377702 and rs405509 showed independent significant association. However, any of the chip SNPs lost association when adjusted for the two functional variants. The regression results were analyzed in a larger panel of 4840 individuals with complete observation from the Amagasaki study in (Takeuchi et al. 2010).

Plots were produced using the WGAViewer (Ge et al. 2008, *Genome Res.* 18:640, http://people.genome.duke.edu/~dg48/WGAViewer/).



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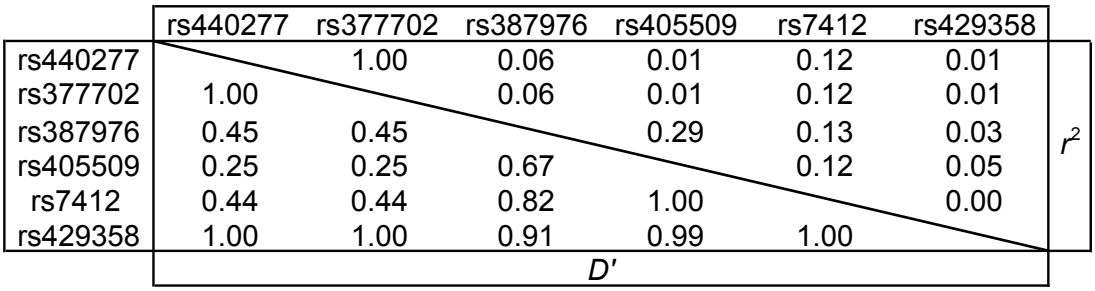
Supplemental Figure 1 (continued)

C

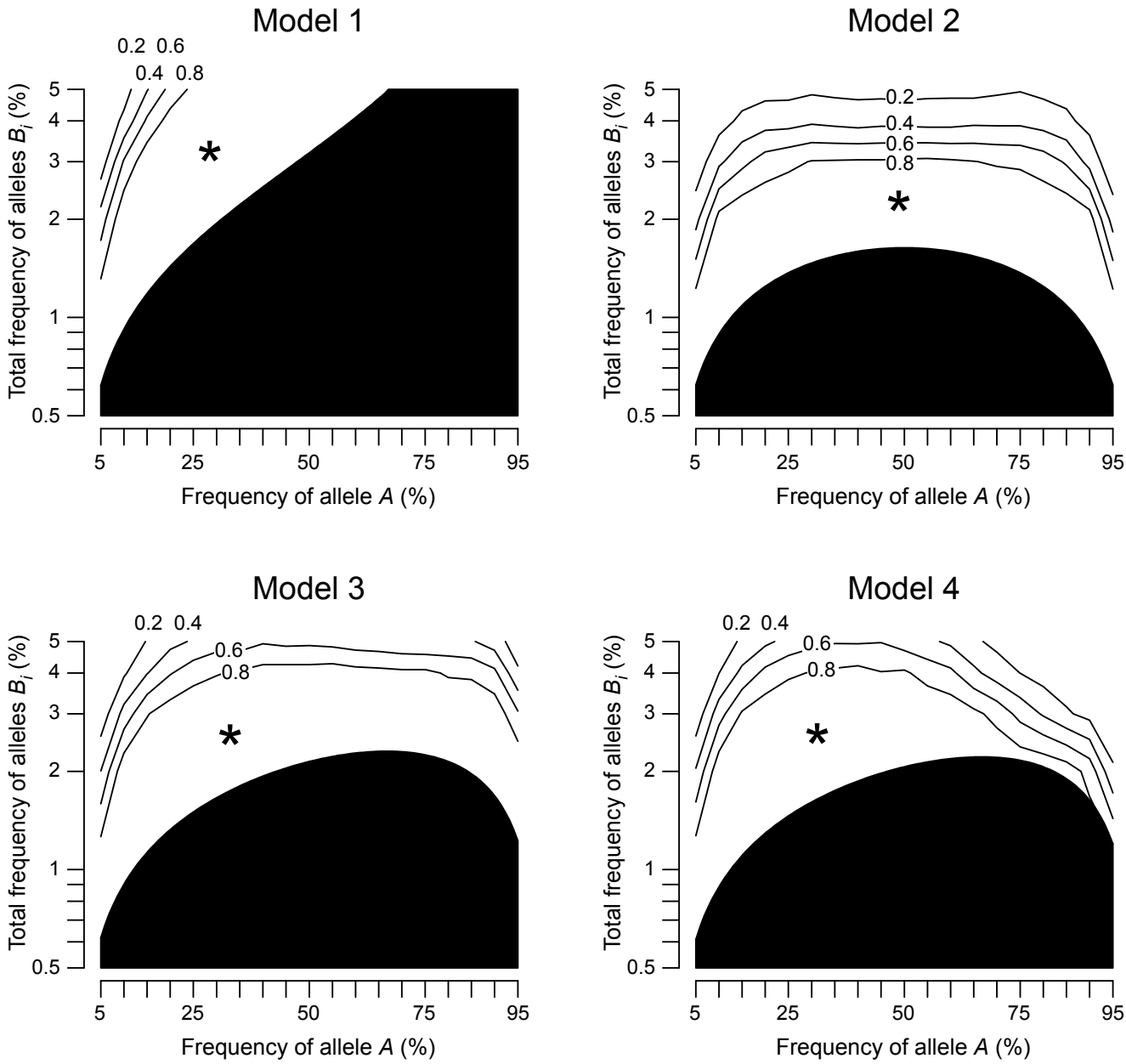


D

rs377702 (T vs C)		rs387976 (T vs G)		rs405509 (C vs A)		rs7412 (T vs C)		rs429358 (T vs C)		Whole model,	Comment
Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value	Beta	p-value	p-value	
-0.191	5.1E-07									5.1E-07	
		-0.126	3.0E-07							3.0E-07	
				-0.117	1.0E-07					1.0E-07	
-0.148	1.8E-04	-0.100	1.0E-04							1.8E-09	
-0.175	4.2E-06			-0.108	8.4E-07					1.8E-11	Best model for chip SNPs
		-0.080	5.3E-03	-0.080	1.7E-03					1.5E-08	
-0.158	6.6E-05	-0.047	1.1E-01	-0.087	6.3E-04					3.0E-11	
						-0.634	2.3E-42	-0.179	1.7E-07	4.2E-49	
-0.041	2.9E-01					-0.620	1.1E-37	-0.178	2.1E-07	2.9E-48	rs377702 not significant when adjusted for functional variants
		-0.004	8.6E-01			-0.632	3.4E-38	-0.179	2.4E-07	5.0E-48	rs387976 not significant when adjusted for functional variants
				0.009	6.9E-01	-0.641	9.2E-39	-0.182	2.1E-07	4.7E-48	rs405509 not significant when adjusted for functional variants

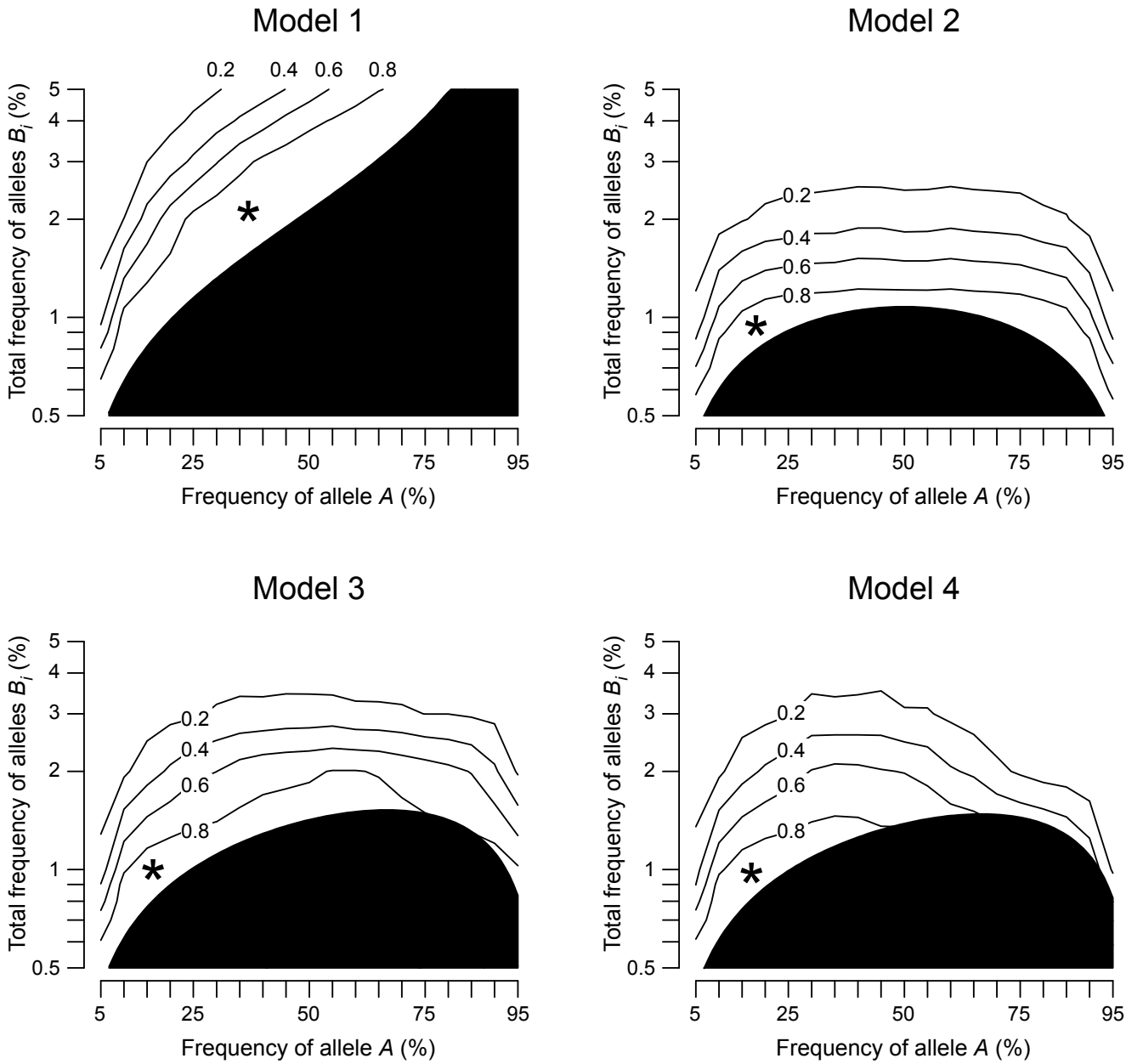
Supplemental Figure 2

Power for detecting synthetic association by the combined test of heteroscedasticity and skewness. Here, the power was computed from simulation under four representative genetic models, assuming the strength of marker association (R^2_{mrk}) of 0.0118, instead of 0.00592 used in Figure 5. The format of the figure is same as Figure 3.



Supplemental Figure 3

Power for detecting synthetic association by testing skewness in 2500 individuals, instead of 5000 used in Figure 5. The power was computed from simulation under four representative genetic models, assuming the strength of marker association (R^2_{mrk}) of 0.00592. The format of the figure is same as Figure 3.



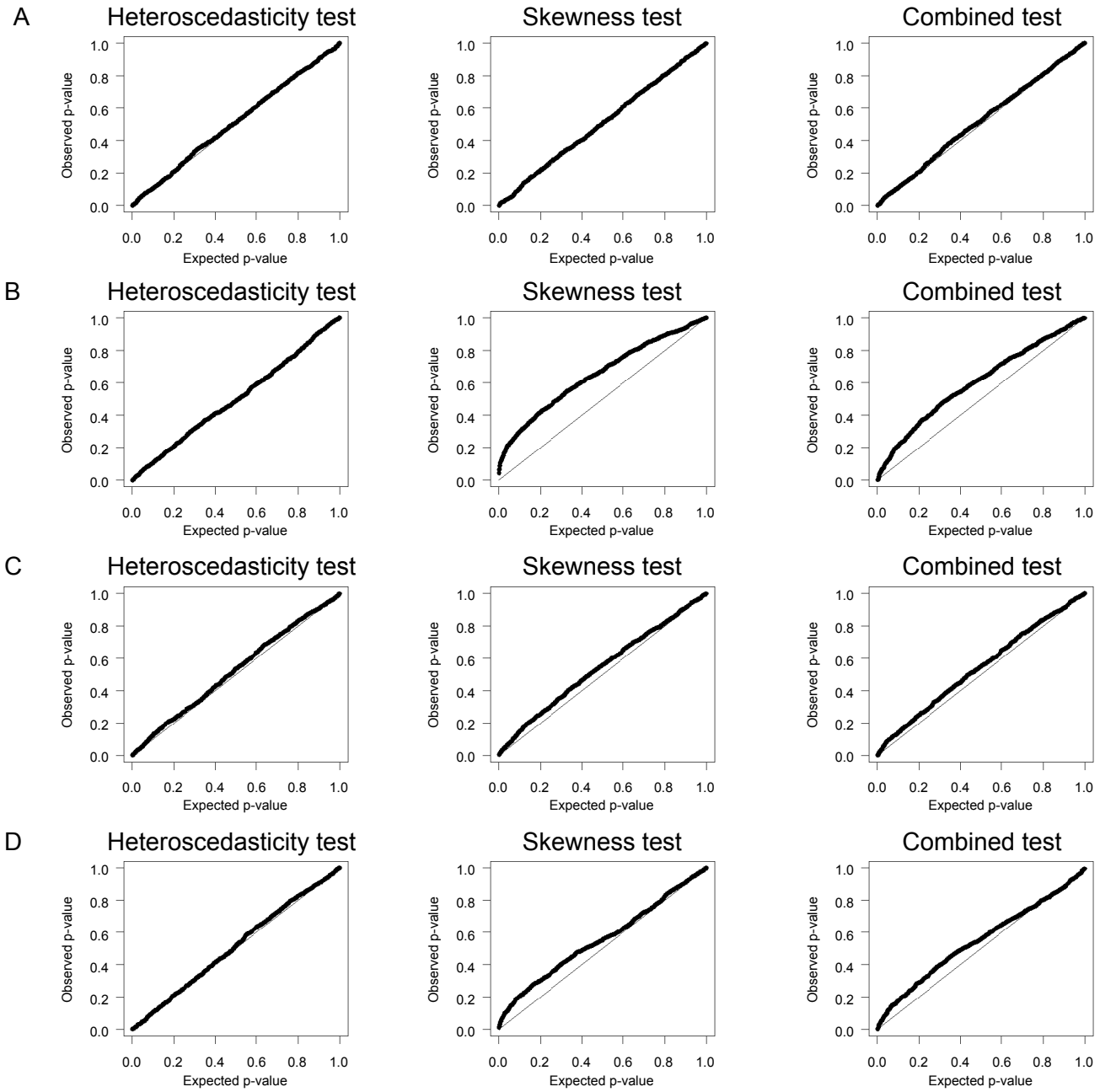
Supplemental Figure 4

Type I error rate of the tests for synthetic association is estimated using simulation. Quantile-quantile plots of the nominal p-value were computed under the "null hypothesis" for synthetic association, by assuming indirect association of a marker completely correlated with a causal variant. Under each model, we performed 1000 simulation trials of generating 5000 individuals.

When the strength of association R^2_{mrk} = 0.00592, and the marker allele frequency p_A = 0.5 (**A**) or 0.05 (**B**), or when R^2_{mrk} = 0.05 under the respective frequencies (**C**, **D**), the nominal p-value of the heteroscedasticity test was distributed as expected, but the skewness test and the combined test tended to be conservative (i.e., the actual type I error rate was smaller than the nominal p-value). We did not calibrate the bias. The bias was due to the application of inverse normal transformation to the whole QT distribution.

In the last two rows of figures, the strength of association was as large as R^2_{mrk} = 0.1, which is exceptional for GWA signals, and the marker allele frequency p_A = 0.5 (**E**) or 0.05 (**F**). In the case with lower allele frequency (**F**), the tests were anticonservative (i.e., the actual type I error rate was larger than the nominal p-value), thus invalid. Spurious heteroscedasiticy or skewness could be caused by inverse normal transformation when the effect-size was large.

There was no correlation (p > 0.05) between the heteroscedasticity and the skewness test p-values, or their log-transforms, when R^2_{mrk} = 0.00592 or when p_A = 0.5. When p_A = 0.05, there was weak correlations of coefficient –0.06 or –0.20 under R^2_{mrk} = 0.05 or 0.1 respectively.



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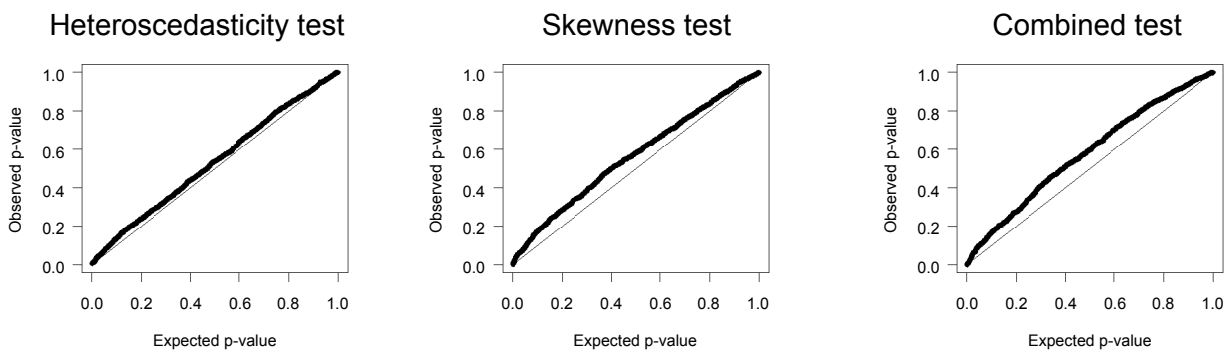
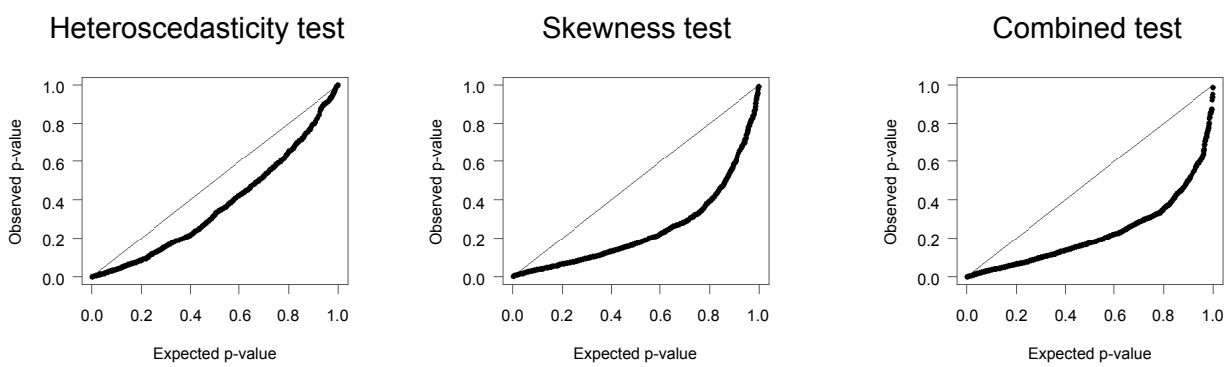
Supplemental Figure 4 (continued)

E

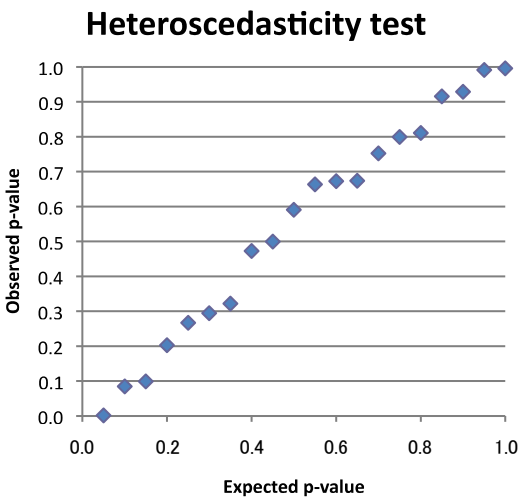


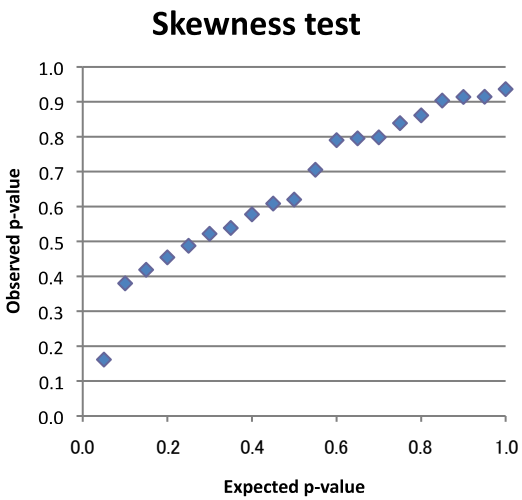
F

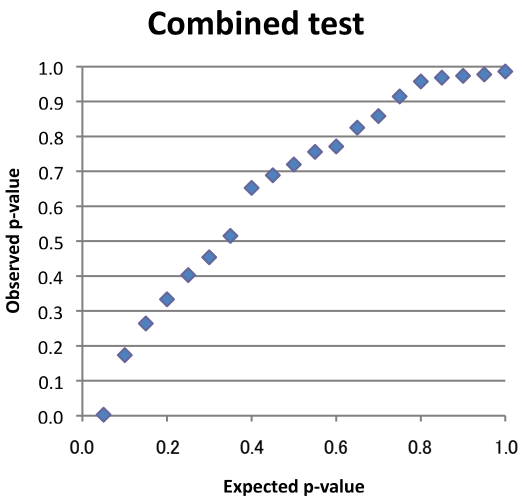


Supplemental Figure 5

Empirical distribution of the test statistics for synthetic association in gene expression data. Quantile-quantile plots are shown for the tests applied to the data of SNP genotype and gene expression in lymphoblastoid cell lines of the HapMap CEU individuals (*N*=60). From the reported significant associations (Stranger et al. 2007, *Science* 315:848, Table S2), those with R^2 ≤0.35 and data available from a public database (http://www.ncbi.nlm.nih.gov/gtex/test/GTEX2/gtex.cgi) were extracted. Inverse normal transformation was applied to the expression level before testing synthetic association (see Methods). Since inverse normal transformation can cause spurious signal when the association is very strong (for example, if the range of QT is disjoint among different genotypes), such cases were excluded by requiring R^2 ≤0.35. The test statistic for heteroscedasticity was distributed as expected. The test for skewness tended to be conservative possibly due to the inverse normal transformation. See Supplemental Table 2 for details.







Supplemental Figure 6

Plots for d_i, the effect size of B_i vs b_i alleles, under the four genetic models used for simulation. Horizontal and vertical axes represent frequency of the marker allele *A*, and the total frequency of causal alleles B_i linked to allele *A*, respectively. The effect-size of a causal variant with total frequency in the analyzed range (>0.5%) is unlikely to exceed three-sigma, as long as the QT distribution is normal. Thus, we neglect the black region of the parameter space, where d_i >3. The strength of the marker association (R^2_{mrk}) is 0.00592.