Supplemental Table S1. Description of the 8 genes

	Total number of SNPs	Number of Rare variants	Median MAF	Mean MAF	Coding length
PLCH2	116	113	2.25E-04	8.47E-03	29212
KANK4	68	57	2.25E-04	5.20E-02	83247
GALNT2	57	48	2.25E-04	2.03E-02	214921
GBP3	33	27	2.25E-04	6.01E-02	16184
IQGAP3	132	125	2.25E-04	1.50E-02	47200
ACTN2	87	81	2.25E-04	1.34E-02	77790
SCLT1	59	55	2.25E-04	8.22E-03	209614
MDN1	347	311	2.25E-04	2.22E-02	176213

Supplemental Table S2. Average type 1 error rates of the statistics for testing interaction between two genes with both common and rare variants

Model	Sample Size	0.05	0.01	0.001
Model 1	500	0.0516	0.0104	0.0013
	1000	0.0502	0.0103	0.0011
	2000	0.0475	0.0095	0.0009
	3000	0.0472	0.0097	0.0011
	4000	0.0476	0.0098	0.0012
	5000	0.0455	0.0089	0.0011
Model 2	500	0.0495	0.0103	0.0011
	1000	0.0493	0.0106	0.0010
	2000	0.0495	0.0102	0.0011
	3000	0.0470	0.0092	0.0009
	4000	0.0476	0.0096	0.0009
	5000	0.0465	0.0092	0.0008
Model 3	500	0.0518	0.0115	0.0013
	1000	0.0503	0.0102	0.0011
	2000	0.0493	0.0099	0.0009
	3000	0.0465	0.0097	0.0011
	4000	0.0471	0.0099	0.0009
	5000	0.0470	0.0094	0.0009

Supplemental Table S3. Type 1 error rates of the statistics for testing interaction between genes: *GBP3* and *KANK4* with rare variants and existence of LD between them

Model	Sample Size	0.05	0.01	0.001
Model 1	500	0.0478	0.0106	0.0012
	1000	0.0432	0.0094	0.0006
	2000	0.0494	0.0112	0.0014
	3000	0.0524	0.0098	0.0012
	4000	0.0516	0.0094	0.0010
	5000	0.0522	0.0118	0.0008
Model 2	500	0.0474	0.0096	0.0004
	1000	0.0448	0.0106	0.0012
	2000	0.0506	0.0096	0.0008
	3000	0.0496	0.0084	0.0010
	4000	0.0538	0.0120	0.0014
	5000	0.0514	0.0118	0.0012
Model 3	500	0.0430	0.0084	0.0010
	1000	0.0466	0.0068	0.0008
	2000	0.0490	0.0100	0.0008
	3000	0.0526	0.0082	0.0014
	4000	0.0492	0.0100	0.0012
	5000	0.0530	0.0080	0.0012

Supplemental Table S4. The interaction models: 0 and r stand for a quantitative trait mean given the genotypes.

Models	First locus	Second locus		
		$Q_{h_2}Q_{h_2}$	$Q_{h_2}q_{h_2}$	$q_{h_2}q_{h_2}$
Dominant OR Dominant	$Q_{h_1}Q_{h_1}$	r	r	r
	$Q_{h_1}q_{h_1}$	r	r	r
	$q_{h_1}q_{h_1}$	r	r	0
Dominant AND Dominant	$Q_{h_1}Q_{h_1}$	r	r	0
	$Q_{h_1}q_{h_1}$	r	r	0
	$q_{h_1}q_{h_1}$	0	0	0
Recessive OR Recessive	$Q_{h_1}Q_{h_1}$	r	r	r
	$Q_{h_1}q_{h_1}$	r	0	0
	$q_{h_1}q_{h_1}$	r	0	0
Threshold	$Q_{h_1}Q_{h_1}$	r	r	0
	$Q_{h_1}q_{h_1}$	r	0	0
	$q_{h_1}q_{h_1}$	0	0	0

Supplemental Table 5. P-values of 130 pairs of mildly interacted genes (Exile file: Supplemental Table S5)

Supplemental Table S6. MAF of SNPs from genes *BMF* and *BHMT2* and P-value of their interactions

BMF	MAF	*BHMT2*	MAF	P-value
rs16970349	0.053483	rs61058144	0.010787	5.52E-08
rs16970349	0.053483	rs60166823	0.010562	6.83E-08
rs16970349	0.053483	rs143035984	0.000449	6.29E-04
rs16970349	0.053483	rs59804781	0.004944	4.37E-02
rs148420422	0.000674	rs60158007	0.000225	3.91E-02
rs148420422	0.000674	rs59804781	0.004944	2.55E-02
rs148420422	0.000674	rs145099848	0.000899	3.19E-02
rs143072589	0.000225	rs60158007	0.000225	4.20E-02

Supplemental Figure 1

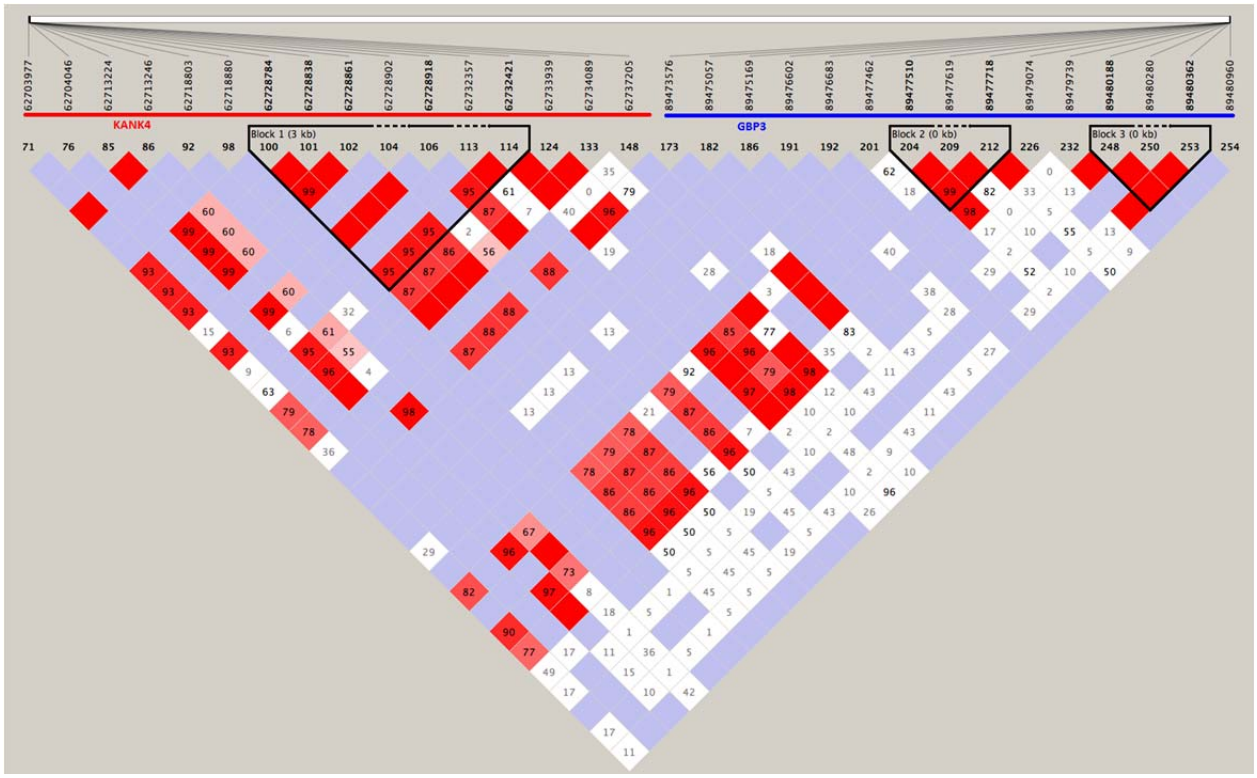

Figure S1: The LD map of genes: *GBP3* and *KANK4*.

Supplemental Figure 2

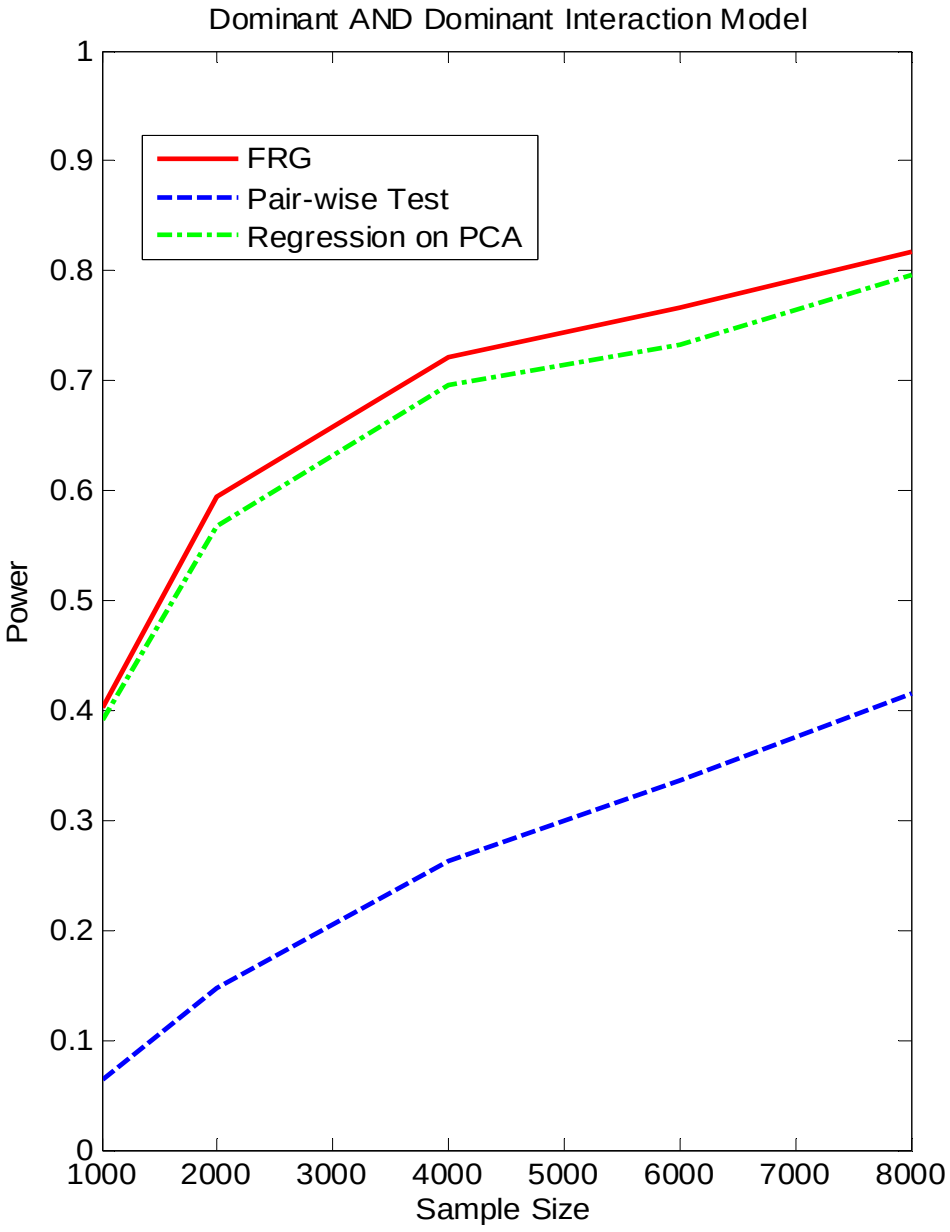

Figure S2: Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests where permutations were used to adjust for multiple testing for testing interaction between two genomic regions that consist of rare variants for a quantitative trait as a function of the sample size at the significance level $\alpha = 0.05$ under the Dominant AND Dominant model, assuming the relative risk parameter $r = 1$.

Supplemental Figure 3

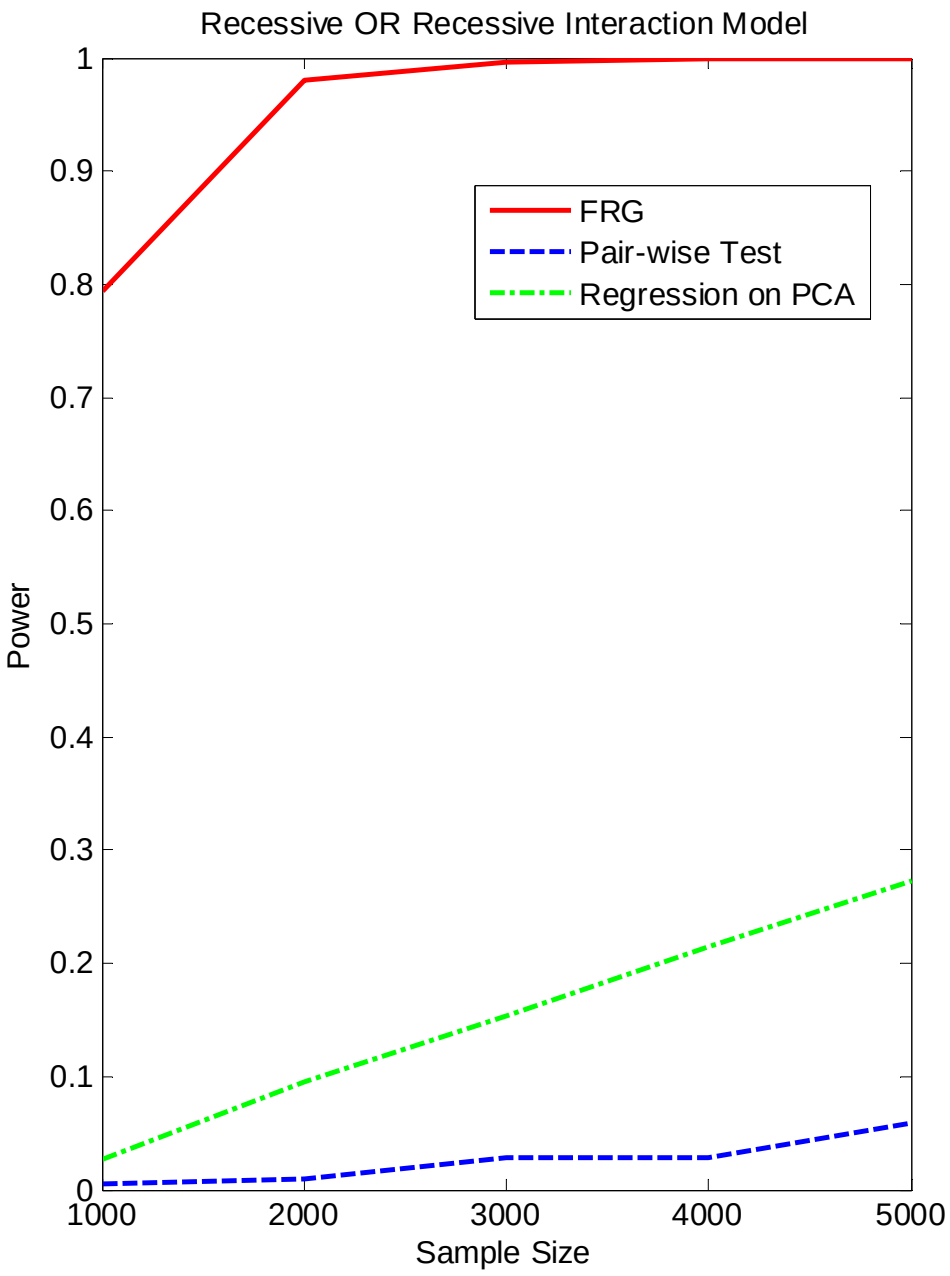

Figure S3. Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests where permutations were used to adjust for multiple testing for testing interaction between two genomic regions that consist of rare variants for a quantitative trait as a function of the sample size at the significance level $\alpha = 0.05$ under the Recessive OR Recessive model, assuming the relative risk parameter $r = 0.1$.

Supplemental Figure 4

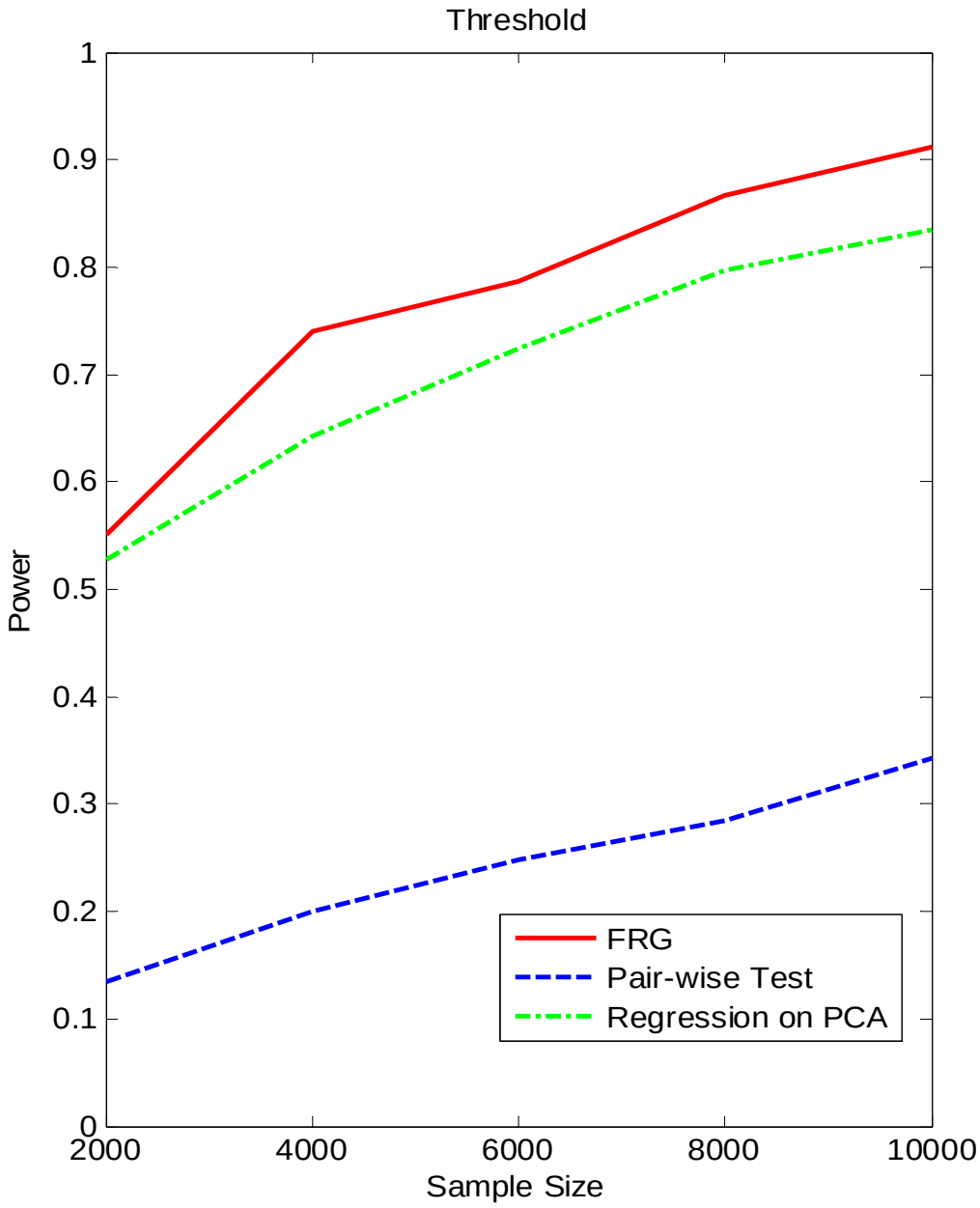

Figure S4. Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests where permutations were used to adjust for multiple testing for testing interaction between two genomic regions that consist of rare variants for a quantitative trait as a function of the sample size at the significance level $\alpha = 0.05$ under the Threshold model, assuming the relative risk parameter $r = 1$.

Supplemental Note 1

Similar to the Kempthorne model (Mao et al. 2006), we assume that the SNPs between two genomic regions are in linkage equilibrium. To find genetic effect and interaction effect functions, we minimize the following objective function using variation of theory (Struwe 1990):

$$F[\alpha(t),\beta(s),\gamma(t,s)] = E\{[Y-\alpha_0-\int_T \alpha(t)(X(t)-E(X(t))dt-\int_S \beta(s)(X(s)-E(X(s))ds- \int_T\int_S \gamma(t,s)(X(t)-E(X(t))(X(s)-E(X(s))dtds]^2\} \quad \text{(S1)}$$

The first variation of $F(\alpha(t),\beta(s),\gamma(t,s))$ at $\alpha(t)$ is given by

$$\begin{aligned}\delta F[h] = \frac{\partial F[\alpha(t)+\varepsilon h(t),\beta(s),\gamma(t,s)]}{\partial \varepsilon} &= E\{[Y-\alpha_0-\int_T \alpha(t)(X(t)-E(X(t))dt-\int_S \beta(s)(X(s)-E(X(s))ds- \\ &\int_T\int_S \gamma(t,s)(X(t)-E(X(t))(X(s)-E(X(s))dtds]\int_U h(u)X(u)du\} \\ &= -\int_U h(u)\mathrm{cov}(Y,X(u))du+\int_U\int_T h(u)R(t,u)\alpha(t)dtdu.\end{aligned}$$

By the first necessary condition for a relative minimum of a functional, we have

$$\delta F[h] = \int_U \{\int_T R(t,u)\alpha(t)dt-\mathrm{cov}(Y,X(u))\}h(u)du = 0. \quad \text{(S2)}$$

Substituting $h(u) = \int_T R(t,u)\alpha(t)dt-\mathrm{cov}(Y,X(u))$ into equation (S2) yields the following integral equation:

$$\int_T R(t,u)\alpha(t)dt = \mathrm{cov}(Y,X(u)). \quad \text{(S3)}$$

Similarly, we can obtain the integral equations:

$$\begin{aligned}&\int_S R(s,v)\beta(s)dv = \mathrm{cov}(Y,X(v)) \\ &\int_T\int_S R(u,t)\gamma(t,s)R(s,v)dtds = \mathrm{cov}(Y,X(u)X(v)).\end{aligned} \quad \text{(S4)}$$

When we consider only one SNP in the genomic region, equation (S3) is reduced to

$$Var(X)\alpha = \mathrm{cov}(Y,X).$$

Let M_1 and m_1 be two alleles in the first locus with allele frequencies p_1 and q_1, respectively. Similarly, we define two alleles M_2 and m_2 with frequencies p_2 and q_2 for the second locus. Nine genotypic values are denoted in the following Table 1.

Table 1**.** Notations for nine genotypic values at two loci.

	M_2M_2	M_2m_2	m_2m_2
M_1M_1	G_{00}	G_{01}	G_{02}
M_1m_1	G_{10}	G_{11}	G_{12}
m_1m_1	G_{20}	G_{21}	G_{22}

Let $G_{0,.}, G_{1,.}, G_{2,.}$ be the genotypic values of the genotypes M_1M_1, M_1m_1 and m_1m_1, $G_{.,0}, G_{.,1}, G_{.,2}$ be the genotypic values of the genotypes M_2M_2, M_2m_2 and m_2m_2, respectively.

Consider a standard quantitative genetic model:

$$\begin{aligned} G_{0,.} &= \mu + 2q_1\alpha + e_1 \\ G_{1,.} &= \mu + (q_1 - p_1)\alpha + e_2 \\ G_{2,.} &= \mu - 2p_1\alpha + e_3. \end{aligned} \qquad \text{(S5)}$$

Clearly, from model equation (S5) we have

$$X_1 = \begin{cases} 2q_1 & M_1M_1 \\ (q_1 - p_1) & M_1m_1 \\ -2p_1 & m_1m_1 \end{cases}$$

It follows from the above equations that

$\text{var}(X_1) = 2p_1q_1$ and

$\text{cov}(Y, X_1) = 2p_1q_1[p_1G_{0,.} + (q_1 - p_1)G_{1,.} - q_1G_{2,.}]$, which implies that

$$\hat{\alpha} = p_1G_{0,.} + (q_1 - p_1)G_{1,.} - q_1G_{2,.}. \qquad \text{(S6)}$$

Equation (S6) gives the standard estimation of genetic additive effect or substitution effect.

Now we consider simplification of interaction effect when each genomic region has only one SNP. In such case, the model (1) and equation (S4) are reduced to

$Y = \alpha_0 + X_1\alpha + X_2\beta + X_1X_2\gamma + \varepsilon$ and

$\mathrm{var}(X_1X_2)\gamma = \mathrm{cov}(Y, X_1X_2)$.

After some algebra, we obtain

$\mathrm{var}(X_1, X_2) = 4p_1q_1p_2q_2$ and

$$\mathrm{cov}(Y, X_1X_2) = 4p_1q_1p_2q_2\{p_1[p_2G_{00} + (q_2 - p_2)G_{01} - q_2G_{02}] + (q_1 - p_1)[p_2G_{10} + (q_2 - p_2)G_{11} - q_2G_{12}] - q_1[p_2G_{20} + (q_2 - p_2)G_{21} - q_2G_{22}]\}.$$

Thus, we obtain

$$\gamma = p_1[p_2G_{00} + (q_2 - p_2)G_{01} - q_2G_{02}] + (q_1 - p_1)[p_2G_{10} + (q_2 - p_2)G_{11} - q_2G_{12}] - q_1[p_2G_{20} + (q_2 - p_2)G_{21} - q_2G_{22}],$$

which coincides with the interaction results in the traditional quantitative genetics.

Supplemental Note 2

The classical concept of genetic additive variance and interaction variance can be extended to functional model. When sequence data are considered in the genomic regions the covariance functions will take linkage disequilibrium between SNPs into account. We first briefly review some concepts of classical quantitative genetics. For simplicity, we assume that $G_0 = a, G_1 = d$ and $G_2 = -a$ (Falconer 1989). Then, the average effect of the gene substitution is

$$\hat{\alpha} = p_1 G_{0,.} + (q_1 - p_1) G_{1,.} - q_1 G_{2,.} = p_1 a + (q_1 - p_1) d + q_1 a = a + (q_1 - p_1) d .$$

which coincides with the standard results in the quantitative genetics.

Let μ be overall population mean, α_1 and α_2 be the respective genic effects, the statistical model for the three genotypic values can be expressed as

$$\begin{aligned} a &= \mu + 2\alpha_1 + e_1 \\ d &= \mu + \alpha_1 + \alpha_2 + e_2 \\ -a &= \mu + 2\alpha_2 + e_3 \end{aligned} \qquad \text{(L1)}$$

where e_1, e_2 and e_3 are the respective deviations of the genotypic values from their expectations on the basis of a perfect fit of the model. From classical quantitative genetics theory we have that

$$\begin{aligned} \hat{\alpha}_1 &= q\alpha \\ \hat{\alpha}_2 &= -p\alpha. \end{aligned}$$

Therefore, the model (L1) can be written as

$$\begin{aligned} a &= \mu + 2q\alpha + e_1 \\ d &= \mu + (q - p)\alpha + e_2 \\ -a &= \mu - 2p\alpha + e_3 \end{aligned} \qquad \text{(L2)}$$

Three terms in equation (L1) make contribution to the genetic additive variance:

$$\begin{aligned}\sigma_A^2 &= 4p^2\alpha_1^2 + 2pq(\alpha_1+\alpha_2)^2 + 4q^2\alpha_2^2 \\ &= 4p^2(q\alpha)^2 + 2pq(q-p)^2\alpha^2 + 4q^2(-p\alpha)^2 \\ &= 2pq\alpha^2(2pq + (q-p)^2 + 2pq) \\ &= 2pq\alpha^2.\end{aligned} \tag{L3}$$

In the text we define the indicator variable for the genotype as

$$x = \begin{cases} 0, & mm \\ 1, & Mm \\ 2, & MM. \end{cases} \tag{L4}$$

We add $-2p$ in the equation (L4) we obtain

$$x = \begin{cases} -2p, & mm \\ q-p, & Mm \\ 2q, & MM. \end{cases} \tag{L5}$$

The model (L2) can be rewritten as

$$y = \mu + x\alpha + e. \tag{L6}$$

From model (L6), the additive genetic variance is given by

$$\sigma_A^2 = \text{var}(x\alpha) = \text{var}(x)\alpha^2. \tag{L7}$$

But,

$$E[x] = (-2p)q^2 + (q-p)2pq + 2qp^2 = 2pq(-q+q-p+p) = 0$$

and

$$\begin{aligned}\text{var}(x) = E[x^2] &= (-2p)^2q^2 + (q-p)^2 2pq + (2q)^2p^2 \\ &= 2pq[2pq + (q-p)^2 + 2pq] = 2pq.\end{aligned} \tag{L8}$$

Substituting equation (L8) into equation (L7) yields

$$\sigma_A^2 = 2pq\alpha^2. \tag{L9}$$

Extension of equation (L7) to sequence data (multiple densely distributed SNPs) in a genomic region and using stochastic calculus yields:

$$\mathrm{var}(\int_T x(t)\alpha(t)dt) = \int\int_{T\,T} \alpha(s)R_1(s,t)\alpha(t)dsdt\,. \qquad \text{(L10)}$$

When only one SNP is located in the region, the covariance function $R(s,t)$ is reduced to $\mathrm{var}(x) = 2pq$ and $\alpha(s) = \alpha(t) = \alpha$. Then, from the model (L10) we obtain

$$\mathrm{var}(\int_T x(t)\alpha(t)dt) = \int\int_{T\,T} \alpha(s)R_1(s,t)\alpha(t)dsdt = \mathrm{var}(x)\alpha^2 = 2pq\alpha^2\,,$$

which exactly corresponds to the formula for additive genetic variance in the classical quantitative genetics (L9) (Falconer 1989). When sequence data are considered in the genomic regions the covariance functions will take linkage disequilibrium between SNPs into account. Similarly, we have

$$\mathrm{var}(\int_S x(s)\beta(s)ds = \int\int_{S\,S} \beta(u)R(u,v)\beta(v)dudv \text{ and}$$

$$\mathrm{var}(\int\int_{T\,S} \gamma(t,s)x(t)x(s)dsdt) = \int\int\int\int_{T\,S\,T\,S} \gamma(t,s)R_1(t,u)R_2(s,v)\gamma(u,v)dsdtdudv.$$

In summary, the variance of the overall genetic and interaction effects in two genomic regions can be defined as

$$\begin{aligned}
&\mathrm{var}(\int_T x(t)\alpha(t)dt) = \int\int_{T\,T} \alpha(s)R(s,t)\alpha(t)dsdt \\
&\mathrm{var}(\int_S x(s)\beta(s)ds = \int\int_{S\,S} \beta(u)R(u,v)\beta(v)dudv \qquad \text{(L11)} \\
&\mathrm{var}(\int\int_{T\,S} \gamma(s,t)x(s)x(t)dsdt = \int\int\int\int_{T\,S\,T\,S} \gamma(s,t)R(s,u)R(t,v)\gamma(u,v)dsdtdudv.
\end{aligned}$$

By the similar arguments, if we assume that each genomic region has only one SNP, then equation (L11) is reduced to

$$\sigma^2_{A_1} = 2p_1q_1\alpha^2$$
$$\sigma^2_{A_2} = 2p_2q_2\beta^2$$
$$\sigma^2_I = 4p_1q_1p_2q_2\gamma^2,$$

which are the standard results in quantitative genetics. This demonstrates that the formulas for genetic effect at single SNP are special cases of the proposed genetic effect models for genomic regions.

Supplemental Note 3

Genetic additive and additive interaction variances

Let $R(t,s)$ be the covariance function of the genotype profile $X(t)$. By Karhunen-Loeve expansion (Ash and Gardner 1975), the covariance function $R(t,s)$ can be expanded in terms of orthonormal eigenfunctions $\phi_j(t)$ (functional principal components) and non-increasing eigenvalues λ_j:

$$R(t,s) = \sum_{j=1}^{\infty} \lambda_j \phi_j(t) \phi_j(s) \tag{A1}$$

and the i th centered random genotype profile can be expanded as

$$x_i(t) = \sum_{j=1}^{\infty} \xi_{ij} \phi_j(t), \tag{A2}$$

where ξ_{ij} are uncorrelated random variables with zero mean and variances $E[\xi_{ij}^2] = \lambda_j$.

It follows from equation (A1) that

$$\begin{aligned} \int_T R(t,s)\phi_j(t)dt &= \int_T \sum_{k=1}^{\infty} \lambda_k \phi_k(t)\phi_k(s)\phi_j(t)dt \\ &= \sum_{k=1}^{\infty} \lambda_k \int_T \phi_k(t)\phi_j(t)dt \phi_k(s) \\ &= \lambda_j \phi_j(s). \end{aligned} \tag{A3}$$

Recall that the genetic effect functions can also be expanded in terms of eigenfunctions:

$$\alpha(t) = \sum_j \alpha_j \phi_j(t), \beta(s) = \sum_k \beta_k \psi_k(s) \text{ and } \gamma(t,s) = \sum_j \sum_k \gamma_{jk} \phi_j(t) \psi_k(s). \tag{A4}$$

From equations (2) and (A4) we know that the genetic additive variance $\sigma^2_{A_1}$ can be expressed as

$$\begin{aligned}\sigma^2_{A_1} &= \int_T \int_T \alpha(s) R(s,t) \alpha(t) ds dt \\ &= \int_T \int_S \sum_{k=1}^{\infty} \alpha_k \phi_k(s) R(t,s) \sum_{j=1}^{\infty} \alpha_j \phi_j(t).\end{aligned} \tag{A5}$$

Substituting equation (A3) into equation (A5) yields

$$\begin{aligned}\sigma^2_{A_1} &= \int_S \sum_{k=1}^{\infty} \alpha_k \phi_k(s) ds \sum_{j=1}^{\infty} \alpha_j \int_T R(t,s) \phi_j(t) dt \\ &= \int_S \sum_{k=1}^{\infty} \alpha_k \phi_k(s) \sum_{j=1}^{\infty} \alpha_j \lambda_j \phi_j(s) ds \\ &= \sum_{k=1}^{\infty} \sum_{j=1}^{\infty} \alpha_k \alpha_j \lambda_j \int_S \phi_k(s) \phi_j(s) ds \\ &= \sum_{j=1}^{\infty} \lambda_j \alpha_j^2 .\end{aligned} \tag{A6}$$

Similarly, we have

$$\sigma^2_{A_2} = \sum_{k=1}^{\infty} \theta_k \beta_k^2 . \tag{A7}$$

Next we consider estimation of the variance of genetic interaction effect $\sigma^2_{A\times A}$. It follows from equations (2) and (A4) that

$$\begin{aligned}\sigma^2_{A\times A} &= \int_T \int_S \int_T \int_S \gamma(t,s) R_1(t,u) R_2(s,v) \gamma(u,v) ds dt du dv. \\ &= \int_T \int_S \int_T \int_S [\sum_{j=1}^{\infty} \sum_{k=1}^{\infty} \gamma_{jk} \phi_j(t) \psi_k(s) R_1(t,u) \sum_{i=1}^{\infty} \sum_{l=1}^{\infty} \gamma_{il} \phi_i(u) \psi_l(v) R_2(s,v)] dt ds du dv \\ &= \sum_{j=1}^{\infty} \sum_{k=1}^{\infty} \sum_{i=1}^{\infty} \sum_{l=1}^{\infty} \gamma_{jk} \gamma_{il} \lambda_j \theta_l \int_T \phi_i(u) \phi_j(u) du \int_S \psi_k(s) \psi_l(s) ds \\ &= \sum_{j=1}^{\infty} \sum_{k=1}^{\infty} \lambda_j \theta_k \gamma_{jk}^2 .\end{aligned} \tag{A8}$$

Supplemental Note 4

The genetic models for type 1 error calculations

We assumed the three models: model 1(without marginal effects), model 2 (with marginal effect of one gene) and model 3 with marginal effects of two genes to generate a phenotype

Model 1 (without marginal effects):

$$y_i = \mu + \varepsilon_i, i = 1,2,\cdots,n,$$

where μ is an overall mean, ε_i are independent and identically distributed normal variables with mean zero and variance $\sigma^2 = 1$.

Model 2 (with marginal effect of one gene):

$$y_i = \mu + \sum_{j=1}^{k_1} x_{ij}\alpha_j + \varepsilon_i,$$

where x_{ij} is an indicator variable for the genotype at the j th SNP in the first gene, $\alpha_j = (1-p_j)(r-1)$, p_j is the frequency of the minor allele, and r is a risk parameter and is equal to 1.2. We assume that 20% of the variants to be risk variants.

Model 3 (with marginal effects of two genes):

$$y_i = \mu + \sum_{j=1}^{k_1} x_{ij}\alpha_j + \sum_{l=1}^{k_2} z_{il}\beta_l + \varepsilon_i,$$

where x_{ij} and z_{il} are indicator variables for the genotype at the j th SNP in the first gene and at the l of the SNP in the second gene, respectively, $\alpha_j = (1-p_j)(r_1-1)$, $\beta_l = (1-p_l)(r_2-1)$, p_j and p_l are the frequencies of minor alleles at the j th SNP in the first gene and at the l th SNP in the second gene, respectively, and r_1 and r_2 are risk parameters and are equal to 1.2 and 1.4, respectively. We assume that 20% of the variants to be risk variants.

Impact of the lengths of the genes and sequencing errors on the type 1 error rates of the test.

In this section, we evaluate the lengths of the genes and sequencing errors on the type 1 error rates of the test.

To examine whether the lengths of two genes will influence the type 1 error rates, we selected genes *DST* and *SYNE1* in chromosome 6 with the length of 497 k and 515 k and total of 430 and 645 SNPs, respectively, for simulations. The type 1 error rates were summarized in Supplemental Table S7. We still observed that type 1 error rates for testing interaction between two genes with large size were not appreciably different from the nominal levels. The impact of the length of the genes on the type 1 error rates was limited.

Supplemental Table S7. Type 1 error rates of the statistics for testing interaction between two large genes: *DST* and *SYNE1* with rare variants

Model	Sample Size	0.05	0.01	0.001
Model 1	500	0.0440	0.0102	0.0014
	1000	0.0450	0.0096	0.0012
	2000	0.0482	0.0098	0.0014
	3000	0.0486	0.0102	0.0016
	4000	0.0506	0.0106	0.0010
	5000	0.0494	0.0098	0.0012
Model 2	500	0.0474	0.0096	0.0012
	1000	0.0454	0.0102	0.0010
	2000	0.0466	0.0076	0.0006
	3000	0.0478	0.0086	0.0008
	4000	0.0538	0.0106	0.0006
	5000	0.0490	0.0106	0.0006
Model 3	500	0.0504	0.0078	0.0008
	1000	0.0478	0.0108	0.0016
	2000	0.0530	0.0104	0.0004
	3000	0.0482	0.0090	0.0006
	4000	0.0510	0.0092	0.0010

	5000	0.0468	0.0100	0.0010

The error rates for the new generation of sequencing technologies are higher than traditional Sanger sequencing (Harismendy et al. 2009). Variants caused by sequencing errors may bias available genotype-phenotype association tests. Investigating the impact of sequencing errors on association analyses will provide guidance for developing robust statistics for interaction tests. For simplicity, we assumed that the genotyping error rate for rare variants (frequencies < 0.05) ranges from 10^{-5} to 0.01, respectively. Supplemental Table S8 provides the type I error rates for FRG model in the presence of the variant genotype error rate.

Supplemental Table S8. Average type 1 error rates of the statistics for testing interaction between two genes with rare variants and genotyping errors

Model	Sequencing error rates	0.05	0.01	0.001
Model 1	0.0%	0.04744	0.00958	0.00098
	0.1%	0.05004	0.00964	0.00084
	1.0%	0.04620	0.00980	0.00120
Model 2	0.0%	0.04908	0.00976	0.00100
	0.1%	0.04880	0.01025	0.00090
	1.0%	0.04600	0.00920	0.00084
Model 3	0.0%	0.04728	0.00940	0.00108
	0.1%	0.04910	0.01036	0.00106
	1.0%	0.04620	0.00920	0.00080

Supplemental Note 5

In this section, we present the power pattern of the tests in several additional scenarios. In practice, the number of causal variants may be small. To study the impact of the number of causal variants on the power, we considered a simulation scenario where 10% of the rare variants were randomly chosen as causal variants. Supplemental Figure S5 plotted the power curves of

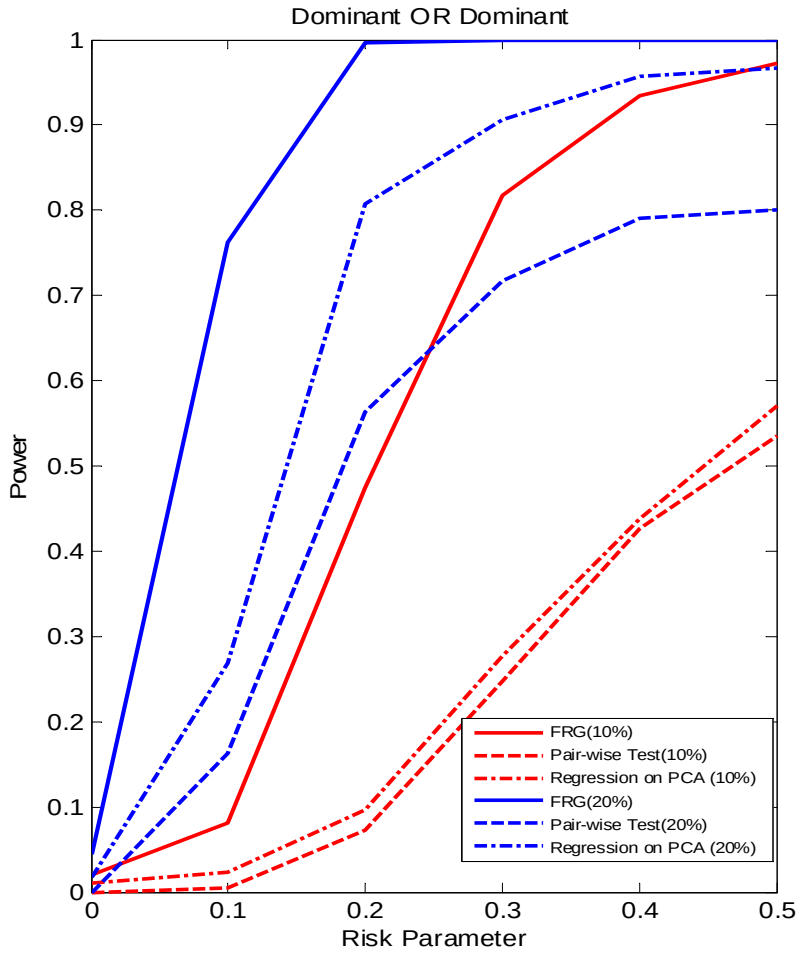

Figure S5. Power curves for three statistics: the FRG, the regression on PCA, the pair-wise interaction tests for testing interaction between two genes with 10% of the rare variants and 20% of the rare variants as causal variants under the Dominant OR Dominant model, and sample sizes of 2,000.

three statistics for testing interaction between two genes with 10% and 20% of the rare variants as causal variants under the Dominant OR Dominant interaction model . From Figure S5, we observed that low percentage of causal variants will decrease the power, but will not change the power pattern of three tests. We also observed that the FRG for risk parameters larger than 0.3 still has high power to detect interaction.

In many cases, causal variants are not randomly distributed. They may be clustered along the chromosome. To investigate the impact of the distribution of causal variants on the power we considered a scenario where the causal variants were close to each other rather than randomly distributed. We used the SNPs in genes *KANK4* with 68 SNPs (57 rare SNPs) and *GBP3* with 33 SNPs (27 rare SNPs) to simulate genotypes of individuals. The LD map and distribution of causal variants in genes *KANK4* and *GBP3* were shown in Supplemental Figure 1. The number of SNPs in this two genes were much less than the number of SNPs in the genes *IQGAP3* (132 SNPs, 125 rare SNPs) and *ACTN2* (87 SNPs, 81 rare SNPs) which were used to simulate genotypes for other power evaluation. Supplemental Figure 6 plotted the power curves of three statistics under the Dominant OR Dominant interaction model, assuming the causal variants were close to each other. Other parameters were unchanged as that in Figure 1. The results showed that the power of linked causal variants was much higher than that of randomly selected causal variants because linked causal variants had much stronger LD than unlinked causal variants. We need to point out that the power of the test using genes *KANK4* and *GBP3* was lower than using genes *IQGAP3* and *ACTN2* because the number of SNPs in genes *IQGAP3* and *ACTN2* was much larger than the number of SNPs in genes *KANK4* and *GBP3* .

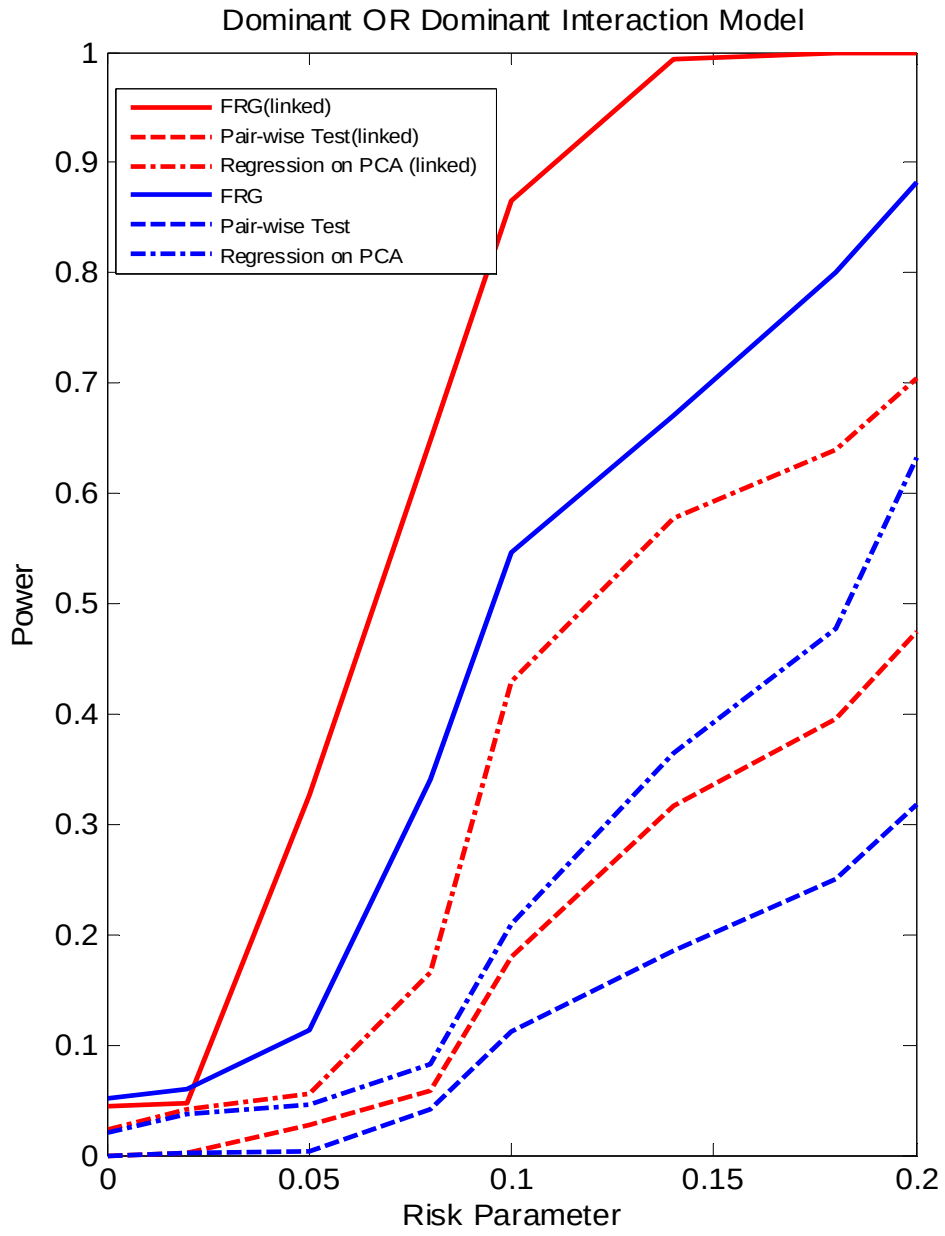

Figure S6. Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests for testing interaction between genes *KANK4* and *GBP3* with 20% of the linked rare variants and 20% of the randomly distributed rare variants as causal variants under the Dominant OR Dominant model, and sample sizes of 2,000.

Next we exam the power of tests for common variants where 20% of the common variants were chosen as causal variants. Supplemental Figures 7-10 plotted the power curves of three statistics for testing interaction between two genomic regions (or genes) which consisted of only common variants under Dominant OR Dominant, Dominant AND Dominant, Recessive OR Recessive and Threshold models, respectively. Power of all three statistics for common variants was higher than that for rare variants, but their power pattern for both common and rare variants was the similar.

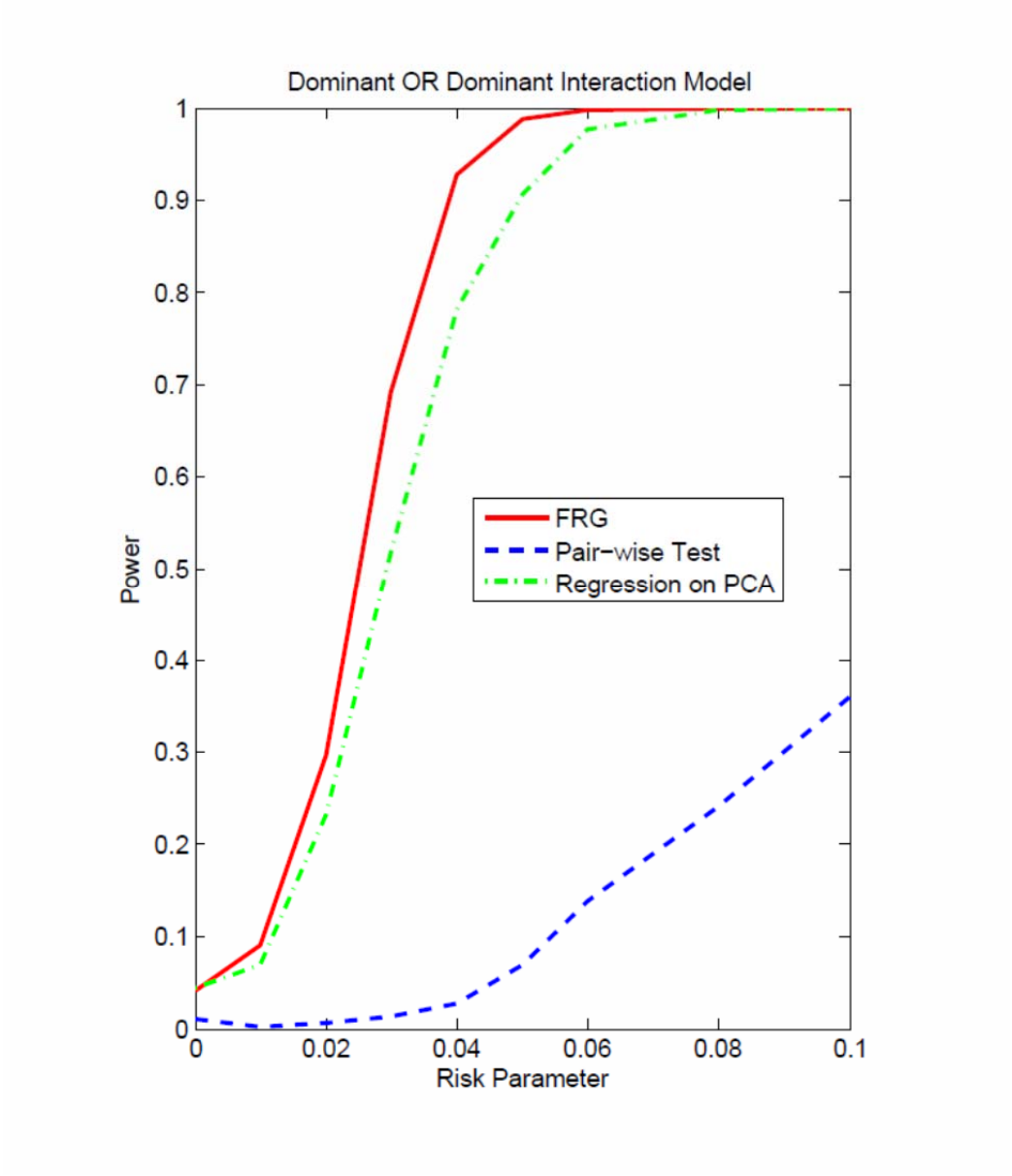

Figure S7. Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests where permutations were used to adjust for multiple testing for testing interaction between two genomic regions that consist of common variants for a quantitative trait as a function of the relative risk parameter r at the significance level $\alpha = 0.05$ under the Dominant OR Dominant interaction model, assuming sample sizes of 2,000.

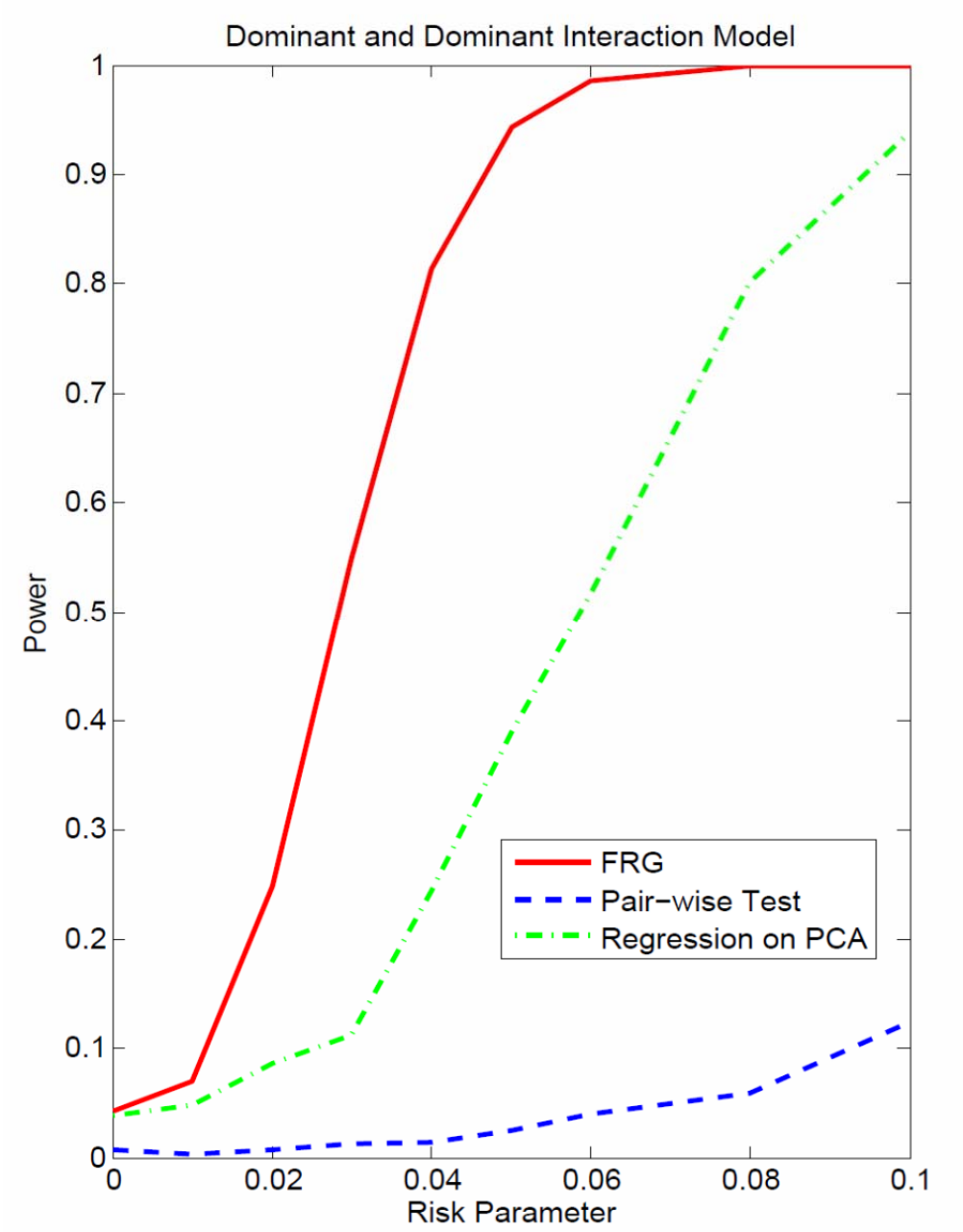

Figure S8. Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests where permutations were used to adjust for multiple testing for testing interaction between two genomic regions that consist of common variants for a quantitative trait as a function of the relative risk parameter r at the significance level $\alpha = 0.05$ under the Dominant AND Dominant interaction model, assuming sample sizes of 2,000.

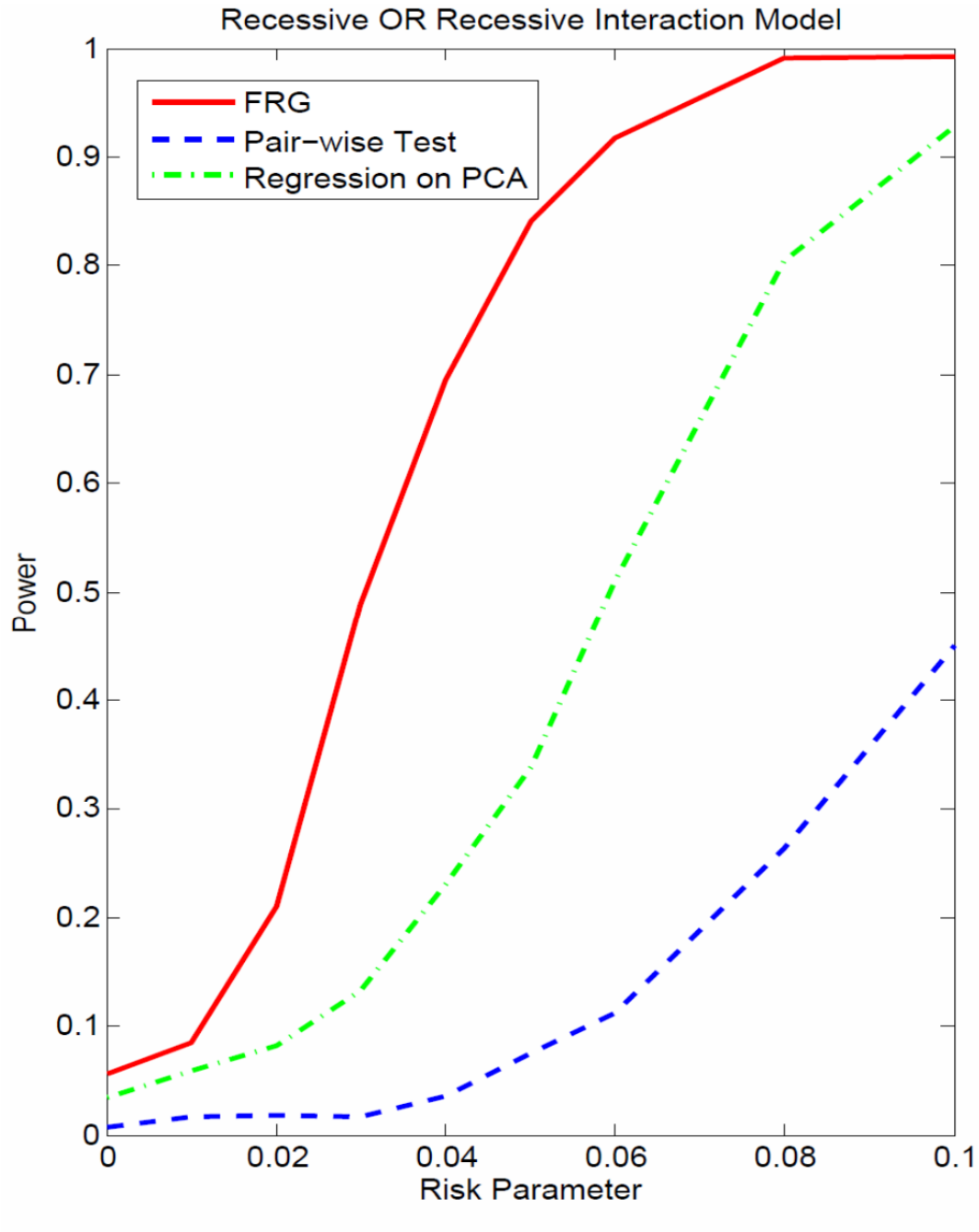

Figure S9. Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests where permutations were used to adjust for multiple testing for testing interaction between two genomic regions that consist of common variants for a quantitative trait as a function of the relative risk parameter r at the significance level $\alpha = 0.05$ under the Recessive OR Recessive interaction model, assuming sample sizes of 2,000.

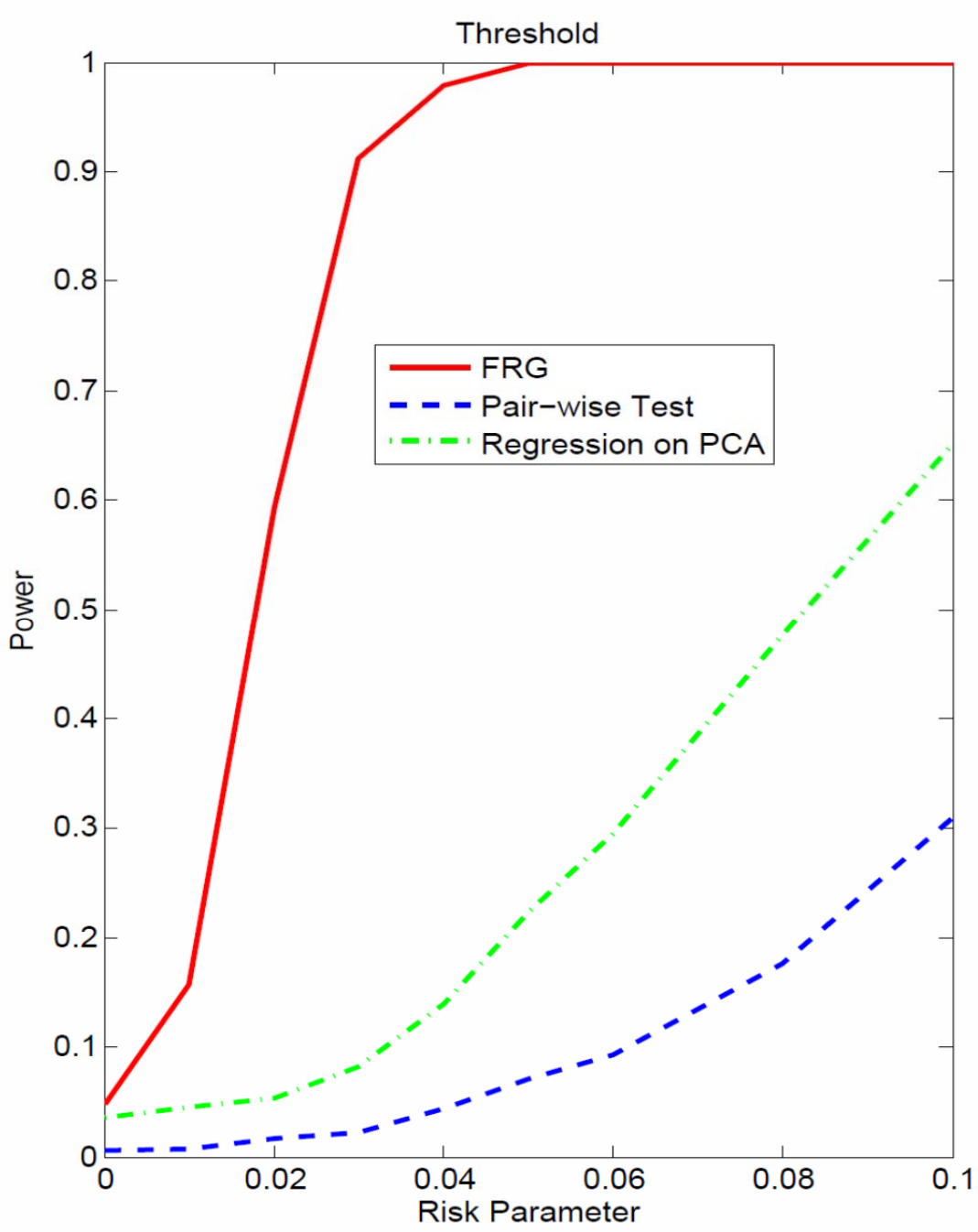

Figure S10. Power curves of three statistics: the FRG, the regression on PCA, the pair-wise interaction tests where permutations were used to adjust for multiple testing for testing interaction between two genomic regions that consist of common variants for a quantitative trait as a function of the relative risk parameter r at the significance level $\alpha = 0.05$ under the Threshold interaction model, assuming sample sizes of 2,000.

Supplemental Note 6

Interaction between loci which were identified to be associated with serum lipid levels in recent GWAS

Next we examined whether loci which were identified to be associated with serum lipid levels in recent GWAS (Aulchenko et al. 2009) were interacted with other genes to influence HDL. Specifically, we investigated 31 genes: *GALNT2, RPA2, GALNT2, PCSK9, GALNT3, APOB, GCKR, HMGCR, MLXIPL, BAZ1B, TBL2, LPL, ABCA1, APOA5, APOA4, APOA1, FADS2, FADS3, MADD, FOLH1, MVK, MMAB, LIPC, CETP, CTCF, PRMT7, GALNT1, LIPG, LDLR, DNAH11,* and *APOE*. We observed that all 31genes have trending interaction with more than one gene (P-value range from 7.81×10^{-8} to 9.97×10^{-5}) (Supplemental Table S9 and Supplemental Figure S11).

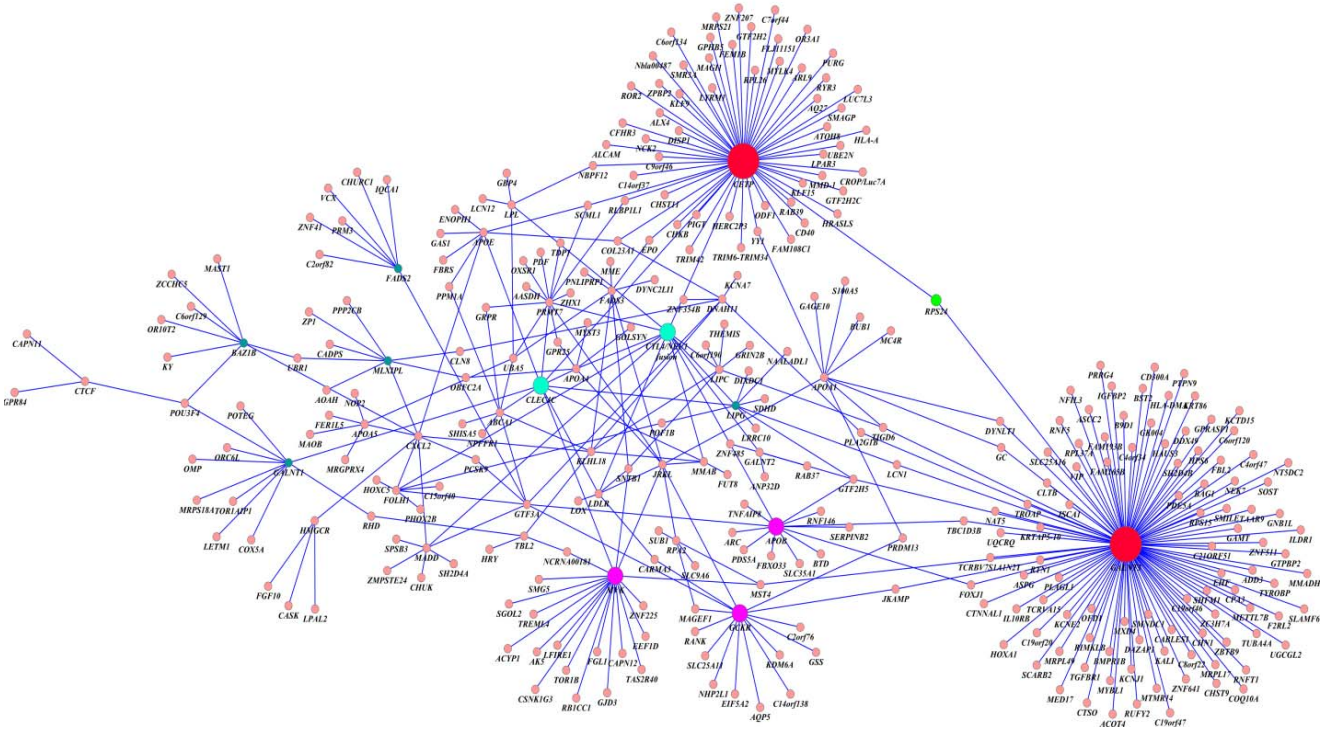

Figure S11. Networks of 384 pairs of modest interactions between 31 genes that influenced lipid levels and discovered in previous GWAS and other genes in our analysis.

For example, *GALNT3* has modest interactions with 104 genes (P-value range from 2.96×10^{-7} to 9.87×10^{-5}). Among them, KCNJ1 was reported to be associated with fasting glucose (Karnes et al. 2012), *NFATC2* was associated with type 2 diabetes (Bailey et al. 2010), *KCTD15* was associated with obesity and related vascular diseases (Winter et al. 2013), *ADD3* was associated with hypertension (Manunta et al. 2007).

To examine the patterns of interaction between genes, we plotted Supplemental Figure S12 which showed 4 pair-wise interaction between *BMF* (SNP: rs16970349) and *BHMT2* (two SNPs: rs60166823 and rs59804781) where Q represents a major allele and q represents a minor allele at a SNP. The P-value for testing interaction between *BMF* and *BHMT2* was 2.27×10^{-10}. The number of SNPs in *BMF* and *BHMT2* were 7 and 26, respectively. Out of a 182 possible pairs of SNPs, the number of pairs of SNPs with P-values less than 0.05 was 8 (Supplemental Table S6). In Supplemental Table 6 we also listed the MAF of these SNPs. Three genotypes of rs16970349 in *BMF* were represented on the *x* axis, three genotypes of two SNPs in *BHMT2* were represented in the legend, and HDL was represented on *y* axis. When we could not observe a homozygous genotype (qq) of minor allele at the SNPs in *BMF* and hence we only showed two mean HDL values for individuals with genotype QQ and Qq at SNPs in *BMF*. If only homozygous genotype QQ was observed, we used one point to represent interaction. We observed from the interaction plot Supplemental Figure 12 that these HDL lines were not parallel and that the effect of changing genotypes of SNPs in *BMF* on HDL highly depends on the genotypes of one of two SNPs in *BHMT2*.

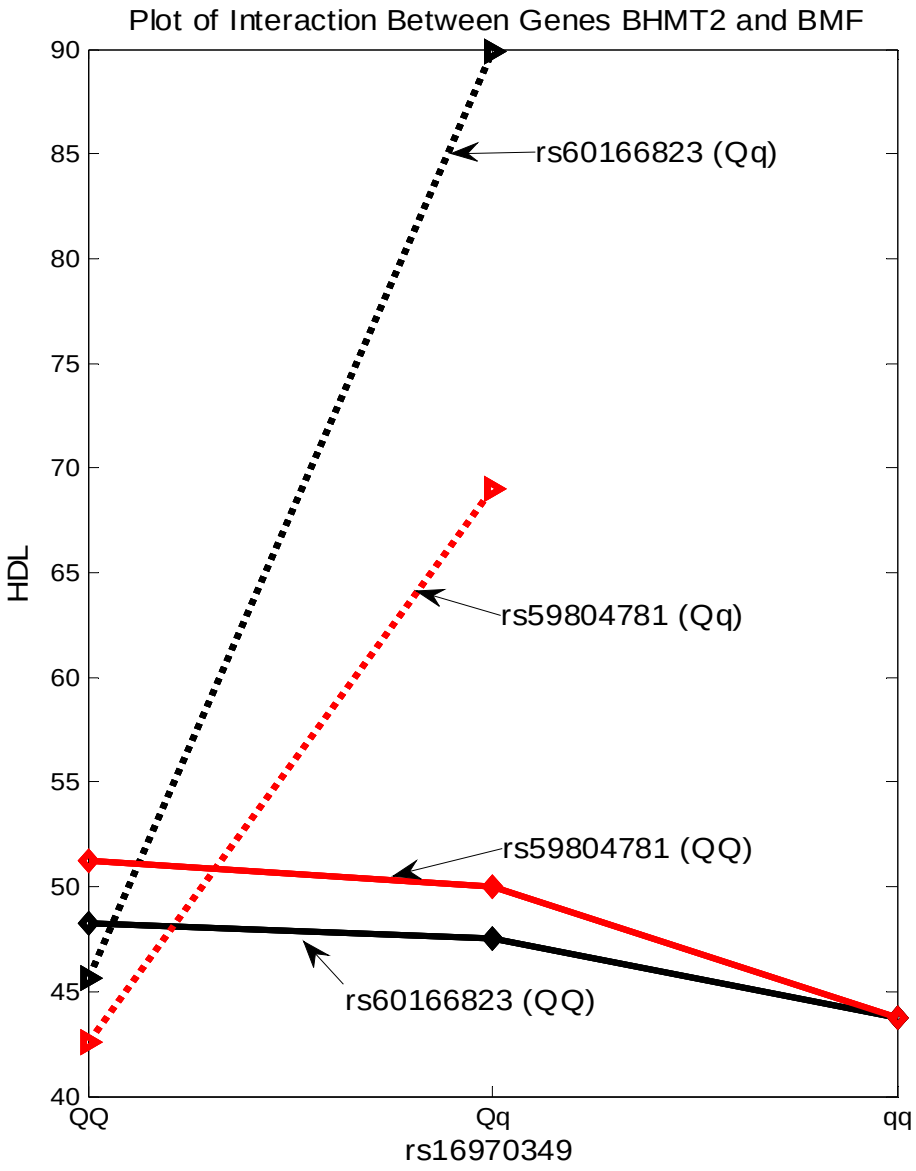

Figure S12. Plot of the interactions between *BMF* (rs16970349) and *BHMT2* (two SNPs: rs60166823, and rs59804781) where three genotypes of rs16970349 in *BMF* were represented on the *x* axis, three genotypes of two SNPs in *BHMT2* were represented in the legend, and HDL was represented on *y* axis. The effect of changing genotypes of SNPs in *BMF* on HDL highly depends on the genotypes of one of two SNPs in *BHMT2*.

Supplemental Table S9. P-values of interaction between Loci influencing lipid levels discovered in previous GWAS and other genes in our analysis (Exile file: Supplemental Table S9).

Interaction analysis when log rank transformation of the HDL was taken as a quantitative trait.

We performed the rank-based inverse normal transformation (INT)of the phenotype HDL before we test interactions using three test statistics. The P-values of testing interactions between 10 pairs of genes that were selected form Table 2 using INT were listed in Supplemental Table S10. Supplemental Table S8 showed that the patterns of P-values by two transformations look similar, the P-values calculated by FRG, in general, is much smaller than that calculated by regression on PCA and the pair-wise test.

Supplemental Table S10. P-values of 10 pairs of genes selected from Table 2 using INT transformation

Gene 1	Chr	No of SNPs	Gene 2	Chr	No of SNPs	P-value		
						FRG	PCA	Pair-wise (mim)
VSIG8	1	29	SLC35A1	6	17	1.19E-09	1.05E-01	4.42E-06
CCDC115	2	17	CALM1	14	11	2.76E-06	5.51E-05	7.47E-05
C1orf92	1	29	ITPKA	15	15	1.09E-09	1.64E-02	4.33E-09
BHMT2	5	26	BMF	15	7	1.96E-05	1.77E-01	4.21E-05
HSPA2	14	23	C18orf56	18	5	6.73E-08	4.92E-01	3.58E-06
PRDM13	6	18	TBC1D3B	17	2	1.42E-09	3.46E-09	5.07E-09
SNTB1	8	36	FLJ35776	18	6	3.53E-05	2.71E-02	1.32E-03
SNTB1	8	36	DBNDD2	20	46	1.87E-05	1.60E-03	1.39E-03
DIRC2	3	20	INSM2	14	16	4.30E-09	4.28E-02	3.84E-10
PRDM13	6	18	ATP6V1D	14	16	3.09E-08	1.03E-07	5.85E-10

References

Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM. 2009. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nat Genet* **41:** 47–55.

Bailey SD, Xie C, Do R, Montpetit A, Diaz R, Mohan V, Keavney B, Yusuf S, Gerstein HC, Engert JC, Anand S. 2010. Variation at the NFATC2 locus increases the risk of thiazolidinedione-induced edema in the Diabetes REduction Assessment with ramipril and rosiglitazone medication (DREAM) study. *Diabetes Care* **33:** 2250-2253.

Falconer DS. 1989. Introduction to Quantitative Genetics. Third Edition. Longman Scientific & Technical. New York.

Harismendy O, Ng PC, Strausberg RL, Wang X, Stockwell TB, Beeson KY, Schork NJ, Murray SS, Topol EJ, Levy S, et al. 2009. Evaluation of next generation sequencing platforms for population targeted sequencing studies. Genome Biol **10:** R32.

Karnes JH, McDonough CW, Gong Y, Vo TT, Langaee TY, Chapman AB, Gums JG, Beitelshees AL, Bailey KR, Del-Aguila JL, Boerwinkle EA, Pepine CJ, Turner ST, Johnson JA, Cooper-Dehoff RM. 2012. Association of KCNJ1 variation with change in fasting glucose and new onset diabetes during HCTZ treatment. *Pharmacogenomics J,* Aug 21. doi: 10.1038/tpj.2012.34.

Manunta P, Citterio L, Lanzani C, Ferrandi M. 2007. Adducin polymorphisms and the treatment of hypertension. *Pharmacogenomics* **8:** 465-72.

Mao Y, London NR, Ma L, Dvorkin D, Da Y. 2006. Detection of SNP epistasis effects of quantitative traits using an extended Kempthorne model. *Physiol Genomics* **28:** 46-52.

Struwe M. 1990. Variational Methods. Springer-Verlag, Berlin.

Winter Y, Sankowski R, Back T. 2013. Genetic determinants of obesity and related vascular diseases. *Vitam Horm* **91:** 29-48.