The American Journal of Human Genetics

Supplemental Data

A Joint Location-Scale Test Improves Power to Detect Associated SNPs, Gene-Sets, and Pathways

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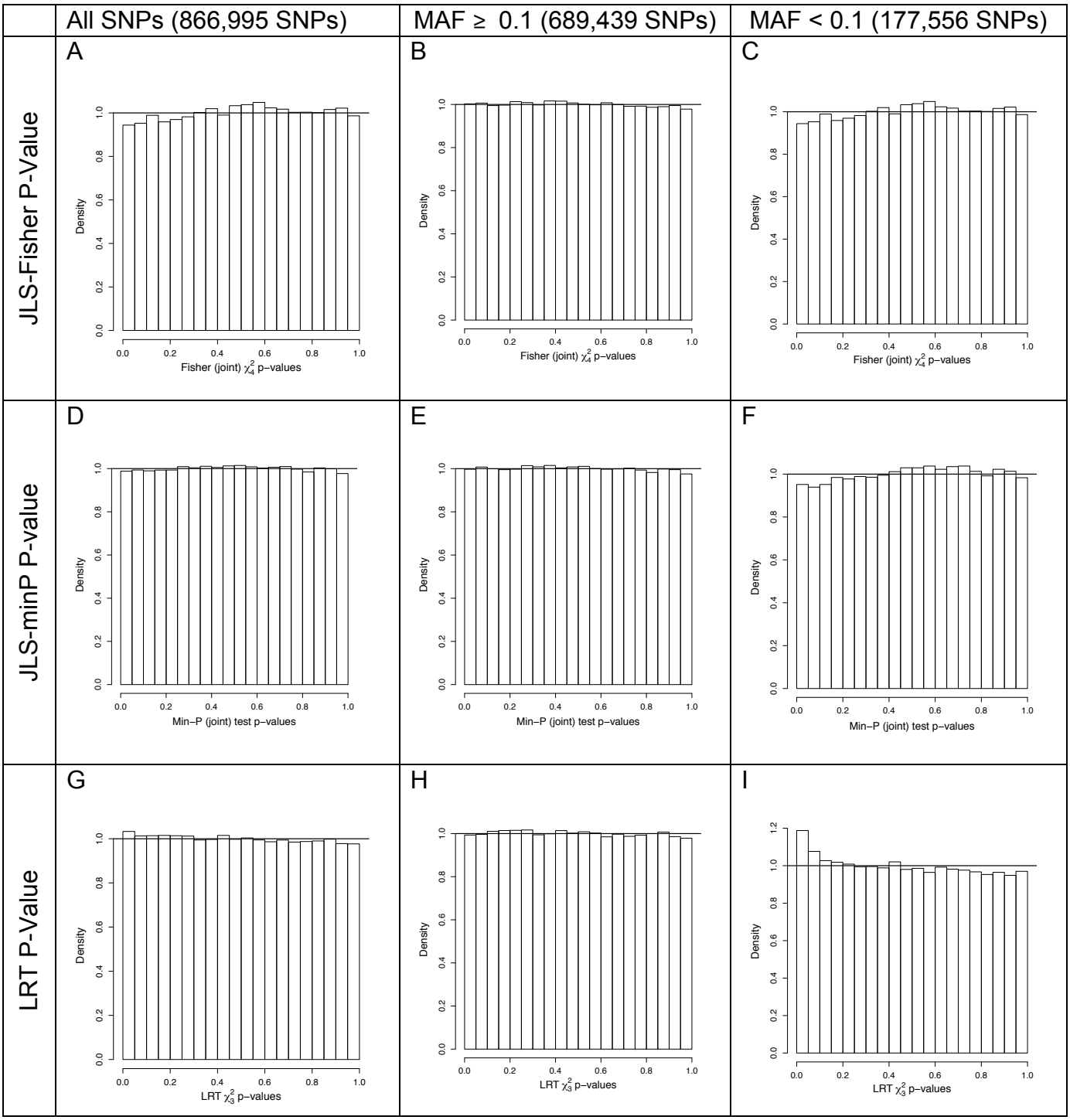

Figure S1. Type 1 error comparison of the joint testing methods – GWAS of HbA1c in type 1 diabetes (T1D). Following a permutation of the T1D HbA1c phenotype (Inverse Normal Transform on average of 38 quarterly measured values), GWAS was conducted on the DCCT/EDIC sample (n=1304 subjects) using (A-C) the JLS-Fisher test, (D-F) the JLS-minP test and (G-I) the LRT methods, across all SNPs (A,D,G), as well as stratifying by MAF ≥ 0.1 (B,E,H) and MAF < 0.1 (C,F,I).

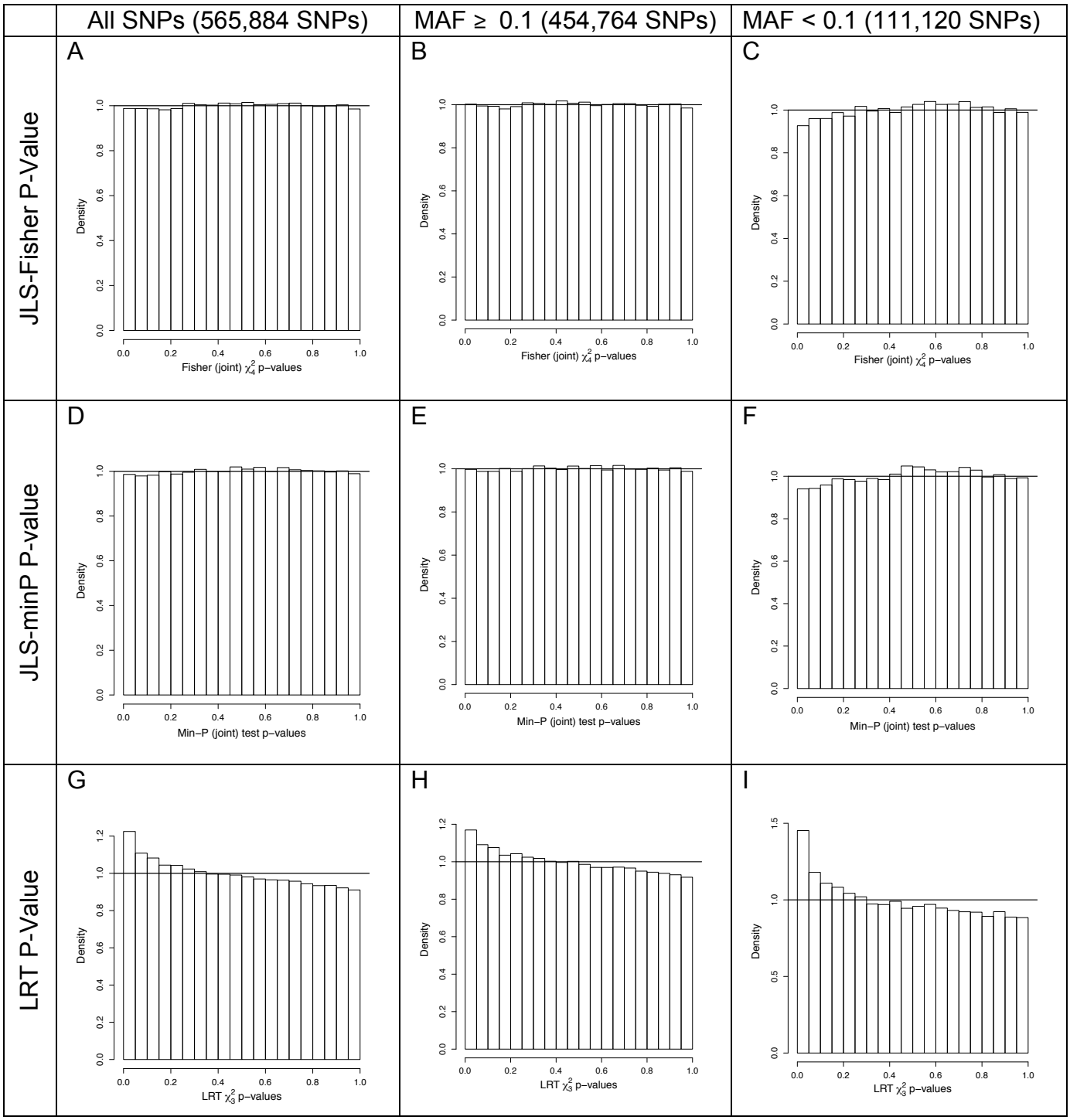

Figure S2. Type 1 error comparison of the joint testing methods – GWAS of cystic fibrosis (CF) lung disease as measured by SaKnorm. Following a permutation of SaKnorm in the CGS sample (n=1409 patients), GWAS was conducted using (A-C) the JLS-Fisher test, (D-F) the JLS-minP test and (G-I) the LRT methods across all SNPs (A,D,G), as well as stratifying by MAF ≥ 0.1 (B,E,H) and MAF<0.1 (C,F,I).

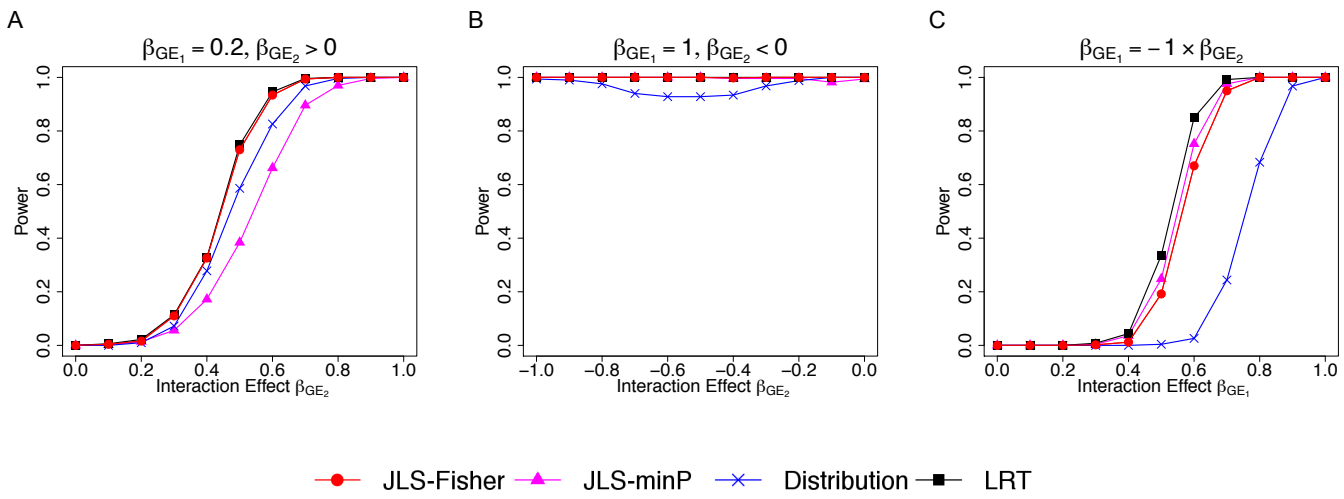

Figure S3. Power comparison under simulation Model 2 – Proposed and competing joint location-scale testing methods. Four different joint location-scale testing methods are examined: the proposed JLS-Fisher (red) and JLS-minP (purple) tests, and the distribution test (blue) of Aschard et al. [1] and the LRT (black) of Cao et al. [2]. Phenotype values for 2000 independent subjects were simulated under Model 2, $E[Y] = \beta_{E1}E_1 + \beta_{E2}E_2 + \beta_{GE1}G{\cdot}E_1 + \beta_{GE2}G{\cdot}E_2$, where the MAF of G was 0.3 and the exposure variables E_1 and E_2 were simulated as Bernoulli variables with frequency=0.3. The effect of the exposure E_1, β_{E1}, was fixed at 0.3 while the interaction effect β_{GE1} varied. The effect of exposure E_2, β_{E2}, was fixed at 0.3 when the interaction effect β_{GE2} was positive, and -0.3 when β_{GE2} was negative. Results are presented for models when effects of the two interaction effects, β_{GE1} and β_{GE2}, are in the same direction (A), and when the two interaction effects are in opposite direction having different amplitude (B) or the same amplitude (C). Power was calculated at the 5×10^{-8} level based on 500 replicates. For other details see Appendix A.

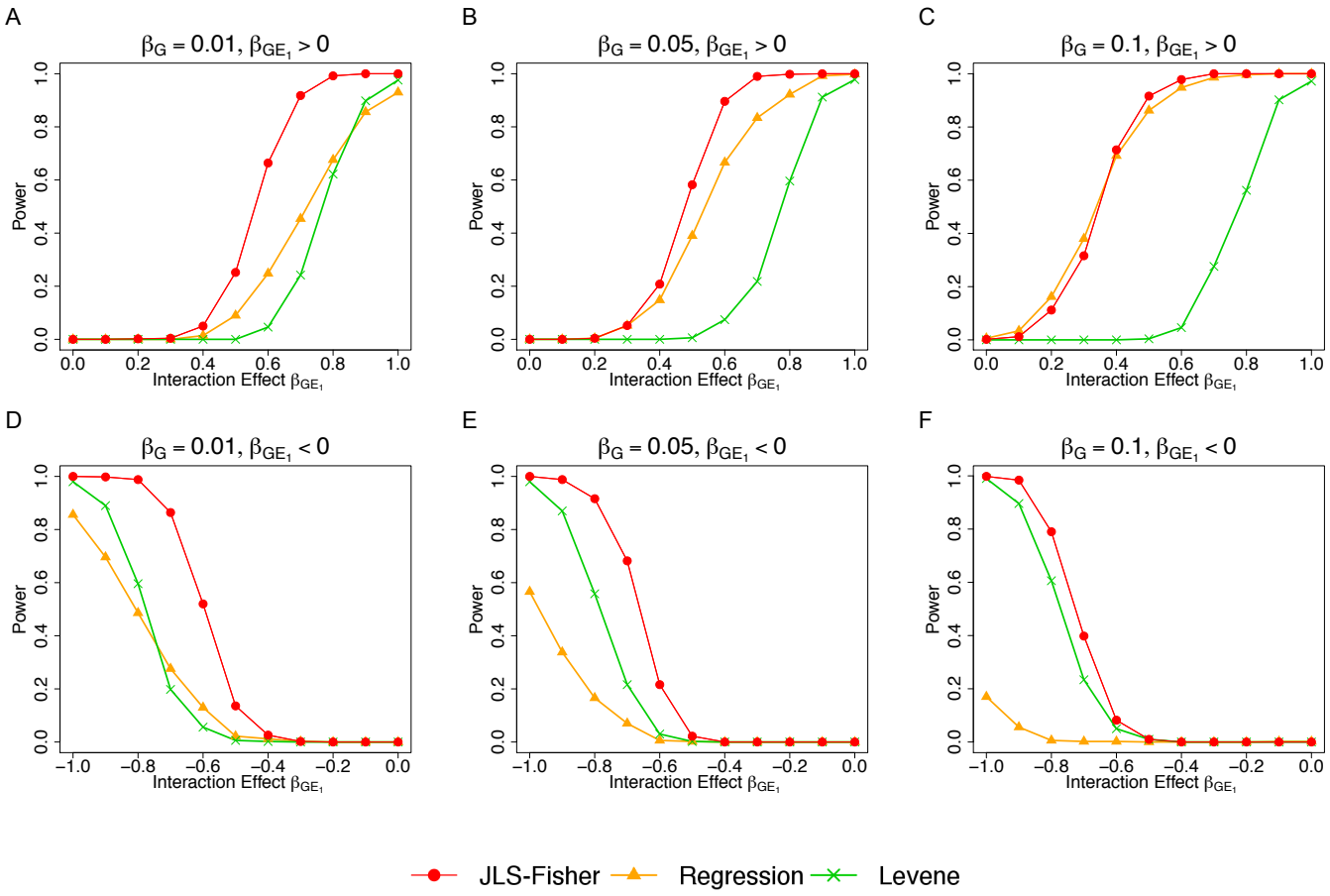

Figure S4. Power comparison under simulation Model 1 – Proposed joint location-scale testing method and individual location-only or scale-only testing methods. The proposed JLS-Fisher test (red) is compared to the individual regression location-only test (orange) and Levene's scale-only test (green). Phenotype values for 2000 independent subjects were simulated under Model 1, $E[Y] = \beta_G G + \beta_{E1} E_1 + \beta_{GE1} G \cdot E_1$, where the MAF of G was 0.3 and the exposure variable E_1 was simulated as a Bernoulli variable with frequency=0.3. The exposure effect β_{E1} was fixed at 0.3 while the other effects vary. Top panel (A)-(C) are results when the main genetic effect β_G and the interaction effect β_{GE1} are in the same direction, and the bottom panel (D)-(F) are results when β_G and β_{GE1} are in opposite direction. Power was calculated at the 5×10^{-8} level based on 500 replicates. For other details see Appendix A.

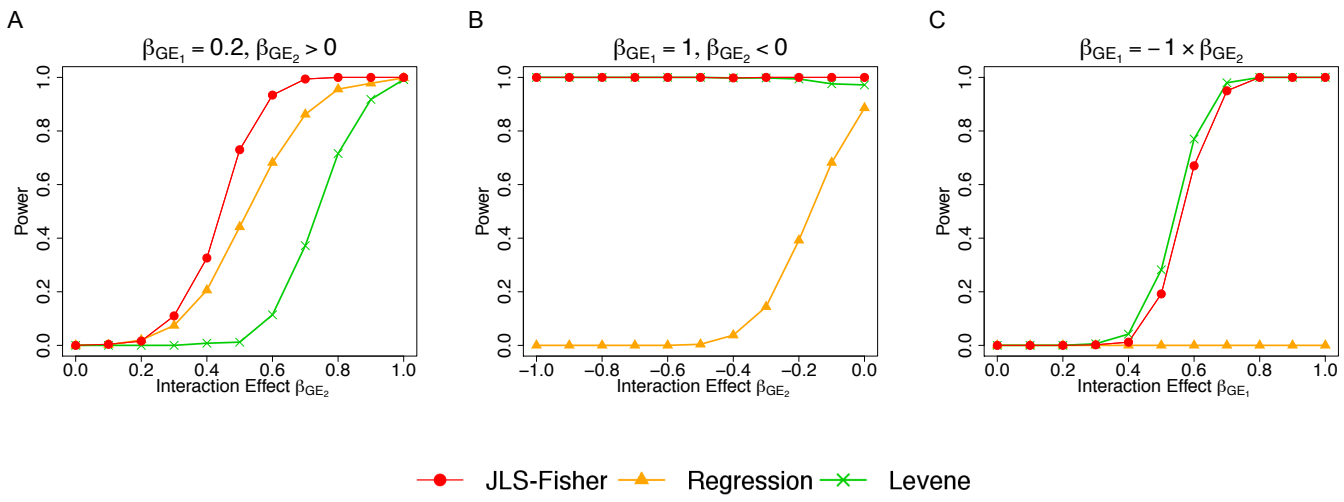

Figure S5. Power comparison under simulation Model 2 – Proposed joint location-scale testing method and individual location-only or scale-only testing methods. The proposed JLS-Fisher test (red) is compared to the individual regression location-only test (orange) and Levene's scale-only test (green). Phenotype values for 2000 independent subjects were simulated under Model 2, $E[Y] = \beta_{E1}E_1 + \beta_{E2}E_2 + \beta_{GE1}G{\cdot}E_1 + \beta_{GE2}G{\cdot}E_2$, where the MAF of G was 0.3 and the exposure variables E_1 and E_2 were simulated as Bernoulli variables with frequency=0.3. The exposure effect β_{E1} was fixed at 0.3 while the interaction effect β_{GE1} varied. The exposure effect β_{E2} was fixed at 0.3 when the interaction effect β_{GE2} was positive, and -0.3 when β_{GE2} was negative. Results are presented for models when the two interaction effects, β_{GE1} and β_{GE2}, are (A) in the same direction, and (B) when the two interaction effects are in opposite direction having different amplitude or (C) the same amplitude. Power was calculated at the $5\text{x}10^{-8}$ level based on 500 replicates. For other details see Appendix A.

Table S1. Type 1 error comparison with varied minor allele frequency (MAF). Type 1 error is presented for the regression location test (Reg), Levene's scale test (Levene), the proposed JLS-Fisher and JLS-minP joint location-scale tests, and the LRT of Cao et al. [2]. The distribution test of Aschard et al. [1] has correct type 1 error by design because it is a permutation-based testing method. Phenotype values for 2,000 independent subjects were simulated under the null model with no genetic association (Model 1 with $\beta_G = 0, \beta_E = 0, \beta_{GE} = 0$) and varied MAF (0.03-0.3) with residual variation from a normal distribution with mean 0 and standard deviation 1. Empirical type 1 error rates were calculated at the 0.05, 0.005, and 0.0005 nominal levels based on 100,000 replicates. For other details see Appendix A.

MAF=0.3					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.05005	0.04988	0.04931	0.04952	0.05144
0.005	0.00490	0.00497	0.00476	0.00488	0.00473
0.0005	0.00047	0.00056	0.00048	0.00045	0.00045
MAF=0.2					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.04983	0.04788	0.04869	0.04910	0.05060
0.005	0.00475	0.00473	0.00508	0.00481	0.00476
0.0005	0.00049	0.00054	0.00053	0.00058	0.00047
MAF=0.1					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.05101	0.04766	0.04888	0.04860	0.05254
0.005	0.00476	0.00435	0.00460	0.00452	0.00506
0.0005	0.00047	0.00058	0.00049	0.00049	0.00053
MAF=0.05					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.05026	0.04183	0.04603	0.04624	0.06130
0.005	0.00473	0.00498	0.00460	0.00524	0.00777
0.0005	0.00047	0.00064	0.00060	0.00063	0.00107
MAF=0.03					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.05026	0.04788	0.04900	0.04959	0.06722
0.005	0.00523	0.00583	0.00568	0.00592	0.01120
0.0005	0.00045	0.00110	0.00089	0.00087	0.00231

Table S2. Type 1 error comparison with varied genotypic group sizes. Type 1 error is presented for the regression location test (Reg), Levene's scale test (Levene), the proposed JLS-Fisher and JLS-minP joint location-scale tests, and the LRT of Cao et al. [2]. The distribution test of Aschard et al. [1] has correct type 1 error by design because it is a permutation-based testing method. Phenotype values for 2,000 independent subjects were simulated under the null model with no genetic association (Model 1 with $\beta_G = 0, \beta_E = 0, \beta_{GE} = 0$) with residual variation from a normal distribution with mean 0 and standard deviation 1. The genotype group sizes were fixed with respect to the smallest group size ($N_{smallest}$= N_2 = 2, 5, 7, 10, 15 or 20) for the rare homozygous group and the corresponding Hardy Weinberg equilibrium (N_0 and N_1) for the common homozygous and heterozygous groups. Empirical type 1 error rates were calculated at the 0.05, 0.005, and 0.0005 nominal levels based on 100,000 simulation replicates. For other details see Appendix A.

Group Sizes (N_0, N_1, N_2)=(1882,116,2) (MAF~0.03)					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.04890	0.05369	0.05350	0.05593	0.09134
0.005	0.00485	0.01002	0.00820	0.00862	0.02108
0.0005	0.00045	0.00221	0.00157	0.00166	0.00582
Group Sizes (N_0, N_1, N_2)=(1805,190,5) (MAF~0.05)					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.05039	0.03769	0.04263	0.04262	0.05869
0.005	0.00496	0.00356	0.00399	0.00411	0.00712
0.0005	0.00043	0.00043	0.00036	0.00042	0.00079
Group Sizes (N_0, N_1, N_2)=(1767,226,7) (MAF~0.06)					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.05087	0.04103	0.04578	0.04591	0.05677
0.005	0.00505	0.00406	0.00451	0.00488	0.00617
0.0005	0.00041	0.00061	0.00047	0.00063	0.00070
Group Sizes (N_0, N_1, N_2)=(1730,320,10) (MAF~0.07)					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.04999	0.04524	0.04776	0.04709	0.0542
0.005	0.00474	0.00506	0.00487	0.00502	0.00571
0.0005	0.00037	0.00071	0.00067	0.00065	0.00071
Group Sizes (N_0, N_1, N_2)=(1674,311,15) (MAF~0.085)					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT

0.05	0.05037	0.04539	0.04808	0.04845	0.05263
0.005	0.00486	0.00509	0.00471	0.00464	0.00553
0.0005	0.00046	0.00055	0.00050	0.00051	0.00061
Group Sizes (N_0, N_1, N_2)=(1620,360,20) (MAF~0.1)					
	Individual		Joint		
level	Reg	Levene	JLS-Fisher	JLS-MinP	LRT
0.05	0.05090	0.04704	0.04953	0.04936	0.05319
0.005	0.00514	0.00414	0.00470	0.00472	0.00500
0.0005	0.00050	0.00057	0.00046	0.00060	0.00045

Table S3. SNP-by-Age interaction effect p-values for variants in Cystic Fibrosis SaKnorm susceptibility loci. Interaction effect p-values were obtained from linear regression models including the main effects of a SNP and the Age variable, and their interaction effect. Variants were previously identified as associated with meconium ileus[3]. Age (in years) was modeled as either a continuous or dichotomous variable (using different cut points of 16yrs, 18yrs and 20yrs).

					Age Dichotomous: Cut point		
Chr	**Gene**	**SNP**	BP[a]	**Age Continuous**	16yrs	18yrs	20yrs
1	*SLC26A9*	rs7512462	204,166,218	0.23	0.23	0.29	0.52
1	*SLC26A9*	rs4077468	204,181,380	0.04	0.08	0.10	0.17
1	*SLC26A9*	rs12047830	204,183,322	0.02	0.01	0.01	0.02
1	*SLC26A9*	rs7419153	204,183,932	0.03	0.09	0.07	0.11
5	*SLC9A3*	rs17563161	550,624	0.003	0.03	0.02	0.02
X	*SLC6A14*	rs12839137	115,479,578	0.26	0.23	0.34	0.26
X	*SLC6A14*	rs5905283	115,479,909	0.21	0.31	0.26	0.36
X	*SLC6A14*	rs3788766	115,480,867	0.009	0.03	0.03	0.02

[a] hg18 assembly (March 2006; NCBI36).

Table S4. Apical gene-specific association results in the Canadian Gene Modifier Study (CGS) sample (n=1409). A list of 155 genes was annotated as described in Sun et al. [3]. Genes with gene-based JLS-Fisher p-value < 0.1 are listed in rank order.

			Gene p-value[b]		
Gene	**Chr**	**#SNPs[a]**	**JLS-Fisher**	**Location/ Regression**	**Scale/ Levene**
SLC9A3	5	10	0.0001	<0.0001	0.0238
ACY3	11	7	0.0006	0.0003	0.3233
MREG	2	37	0.0076	0.0901	0.013
SLC9A3R2	16	10	0.012	0.0696	0.0263
EZR	6	10	0.0128	0.032	0.0635
SLC46A1	17	3	0.0136	0.0118	0.2588
CRB1	1	27	0.017	0.0671	0.0398
LMO7	13	56	0.0287	0.0462	0.1386
KCNMA1	10	225	0.033	0.293	0.0152
SI	3	10	0.0461	0.336	0.0207
PTK2	8	38	0.0506	0.1196	0.0668
LCT	2	11	0.0565	0.0119	0.9008
SLC34A2	4	12	0.0617	0.036	0.4264
STXBP3	1	12	0.0635	0.3105	0.0533
AJAP1	1	50	0.0703	0.022	0.6287
ATP6V0A4	7	39	0.0734	0.7735	0.0071
DPEP1	16	2	0.0738	0.0483	0.347
MUC1	1	3	0.0776	0.0656	0.2099
SLC9A4	2	55	0.0945	0.042	0.4244
SLC22A12	11	4	0.095	0.145	0.1178
SLC9A3R1	17	8	0.0956	0.0807	0.313

[a] The number of GWAS SNPs (MAF>0.02) within ±10 kb of the boundaries of indicated gene.

[b] Permutation-based p-values for the gene. See Material and Methods for details.

Table S5. Type 1 error control with genotype uncertainty. Type 1 error is presented for the regression location test (Reg), Levene's scale test (Levene), and the proposed JLS-Fisher and JLS-minP joint location-scale tests. Simulated true genotypes were converted to probabilistic genotype data using a Dirichlet distribution with scale parameters a for the correct genotype category and $(1-a)/2$ for the other two; a was fixed at values of 1, 0.9, 0.8, 0.7, 0.6 and 0.5, corresponding to group uncertainty ranging from 0% to 50%. Based on the simulated posterior probabilities, the most-likely genotype for each subject was the genotype with the highest posterior probability (i.e. the 'hard call'). The most-likely genotypes were then used to assess type 1 error control at the 0.05, 0.005 and 0.0005 levels, using 100,000 simulated replicate samples of n = 2000, under the null model of no genetic association (i.e. $\beta_G = 0$ and $\beta_{GE} = 0$), and MAF = 0.3 for each level of genotype imputation uncertainty (a). Average concordance is the average percent of agreement between true and most-likely genotypes across the simulation replicates. For other details see Appendix A.

Uncertainty=0% (a=1); Average Concordance=1				
	Individual		Joint	
level	Reg	Levene	JLS-Fisher	JLS-MinP
0.05	0.05039	0.05032	0.05005	0.05010
0.005	0.00489	0.00478	0.00498	0.00523
0.0005	0.00054	0.00052	0.00057	0.00057
Uncertainty=10% (a=0.9); Average Concordance=0.907				
	Individual		Joint	
level	Reg	Levene	JLS-Fisher	JLS-MinP
0.05	0.05012	0.05032	0.04964	0.04996
0.005	0.00528	0.00499	0.00535	0.00530
0.0005	0.00043	0.00053	0.00066	0.00050
Uncertainty=20% (a=0.8); Average Concordance=0.808				
	Individual		Joint	
level	Reg	Levene	JLS-Fisher	JLS-MinP
0.05	0.04862	0.04865	0.04780	0.04795
0.005	0.00459	0.00475	0.00424	0.00462
0.0005	0.00039	0.00049	0.00043	0.00047
Uncertainty=30% (a=0.7); Average Concordance=0.706				
	Individual		Joint	
level	Reg	Levene	JLS-Fisher	JLS-MinP
0.05	0.05082	0.05160	0.05081	0.05187
0.005	0.00540	0.00533	0.00534	0.00523
0.0005	0.00050	0.00051	0.00053	0.00043
Uncertainty=40% (a=0.6); Average Concordance=0.605				
	Individual		Joint	

0.05	0.04942	0.04943	0.04895	0.04987
0.005	0.00502	0.00489	0.00520	0.00505
0.0005	0.00054	0.00053	0.00050	0.00048
Uncertainty=50% (a=0.5); Average Concordance=0.508				
	Individual		Joint	
level	Reg	Levene	JLS-Fisher	JLS-MinP
0.05	0.05025	0.04920	0.04967	0.04922
0.005	0.00495	0.00450	0.00500	0.00481
0.0005	0.00047	0.00040	0.00040	0.00047

Table S6. Power comparison under simulation Model 3. Power is presented for the regression location test (Reg), Levene's scale test (Levene), the proposed JLS-Fisher and JLS-minP joint location-scalue tests, the distribution (Dist.) test of Aschard et al. [1],and the LRT of Cao et al. [2]. Phenotype values for 4000 independent subjects were simulated under Model 3, E[Y] = $\beta_{GE1}G{\cdot}E_1$, where the MAF of G was 0.3. Results are presented for scenarios when the interaction effect, β_{GE1}, and exposure (E_1) frequency were chosen such that the observed marginal effect of G was fixed at 10% of the trait standard deviation. Power was calculated at the $5x10^{-8}$ level based on 500 replicates. For other details see Appendix A.

		Individual		Joint			
Freq-E_1	Int. Effect (β_{GE1})	Reg.	Levene	JLS-Fisher	JSL-minP	LRT	Dist.
0.05	2	0.010	0.110	0.406	0.090	0.996	0.916
0.1	1	0.040	0.040	0.378	0.054	0.740	0.178
0.2	0.5	0.038	0.000	0.098	0.026	0.110	0.064
0.3	0.33	0.110	0.000	0.086	0.084	0.084	0.028
0.5	0.2	0.080	0.000	0.046	0.064	0.036	0.054
1	0.1	0.076	0.000	0.042	0.060	0.036	0.040

Table S7. Power comparison under simulation Model 3 using p-values estimated based on the approximate asymptotic distribution of the test statistics vs. permutation-based method. Power is presented for the regression location-only test (Reg), Levene's scale-only test (Levene), the proposed JLS-Fisher and JLS-minP joint location-scale tests, the distribution (Dist.) test of Aschard et al. [1], and the LRT of Cao et al. [2]. Phenotype values for 1000 independent subjects were simulated under Model 3, E[Y] = $\beta_{GE1}G{\cdot}E_1$, where the MAF of G was 0.3 and the exposure variable E_1 was simulated as a Bernoulli variable with frequency=0.05, and the interaction effect β_{GE1} was fixed at 2. Power was calculated at the 0.01 significance level based on 500 replicates. For each replicate, permutation p-values were estimated from 10,000 iterations.

	Individual		Joint			
P-value estimation method	Reg.	Levene	JLS-Fisher	JLS-minP	LRT	Dist.
Asymptotic	0.190	0.266	0.390	0.288	0.932	NA
Permutation	0.192	0.268	0.364	0.288	0.698	0.812

Table S8. Minor allele frequency (MAF) of variants in Cystic Fibrosis SaKnorm susceptibility loci. Variants were previously identified as associated with meconium ileus[3]. Pediatric subsets (age cutoffs of 16, 18 and 20 years) included for comparison.

				Full Sample MAF	**Pediatric Subsets MAF**		
Chr	**Gene**	**SNP**	BP[a]	All ages (n=1409)	<16yrs (n=653)	<18yrs[b] (n=753)	<20yrs (n=830)
1	*SLC26A9*	rs7512462	204,166,218	0.41	0.38	0.39	0.39
1	*SLC26A9*	rs4077468	204,181,380	0.42	0.38	0.39	0.40
1	*SLC26A9*	rs12047830	204,183,322	0.49	0.48	0.48	0.48
1	*SLC26A9*	rs7419153	204,183,932	0.37	0.39	0.39	0.38
5	*SLC9A3*	rs17563161	550,624	0.26	0.26	0.26	0.26
X	*SLC6A14*	rs12839137	115,479,578	0.24	0.23	0.23	0.24
X	*SLC6A14*	rs5905283	115,479,909	0.49	0.51	0.50	0.49
X	*SLC6A14*	rs3788766	115,480,867	0.40	0.39	0.39	0.39

[a] hg18 assembly (March 2006; NCBI36).

[b] Pediatric subset cutoff (<18yrs) used in Li et al. [4]; subset here only includes unrelated subjects (n=753).

References

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