Supplementary Online Material

Fast set-based association analysis using summary data from GWAS identifies novel gene loci for human complex traits

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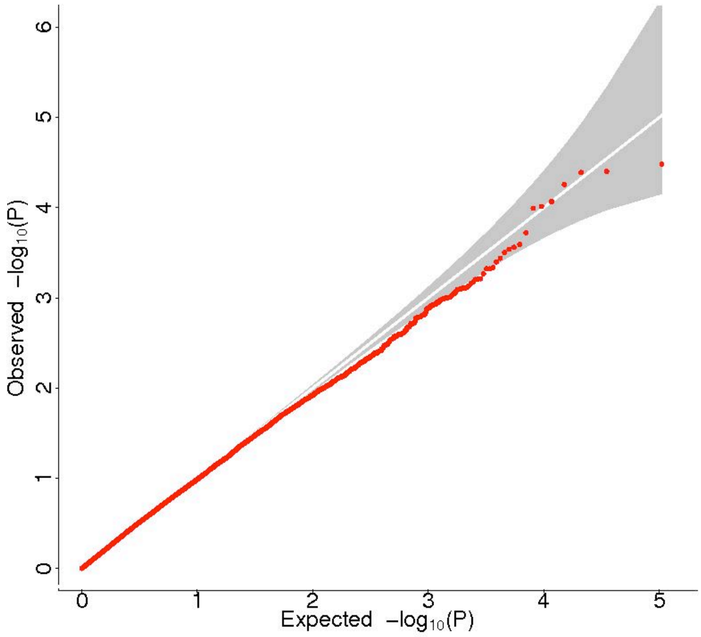
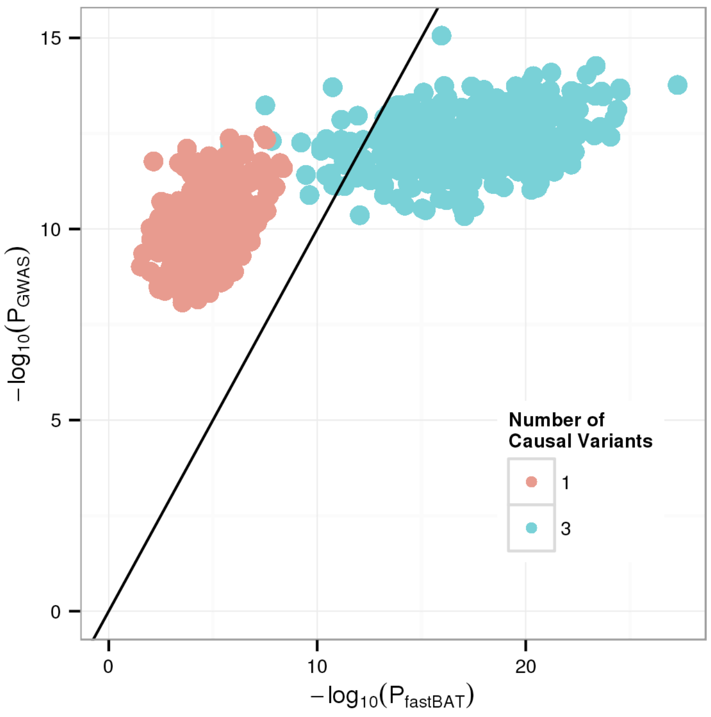
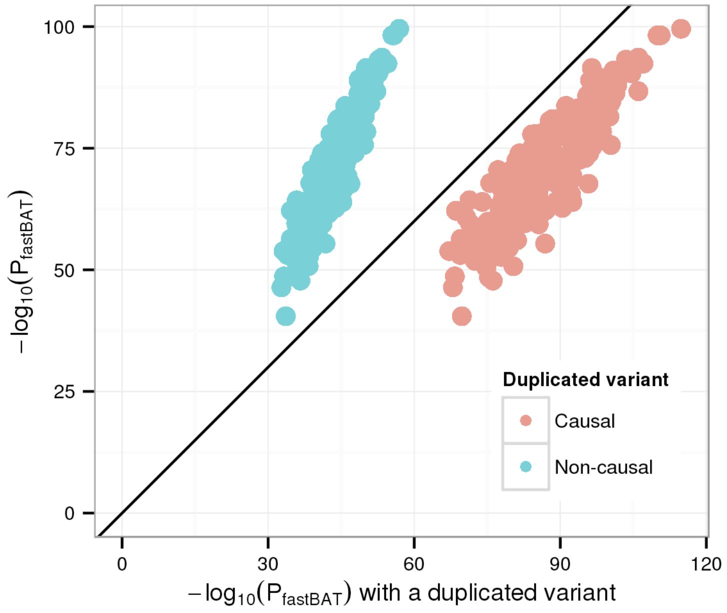


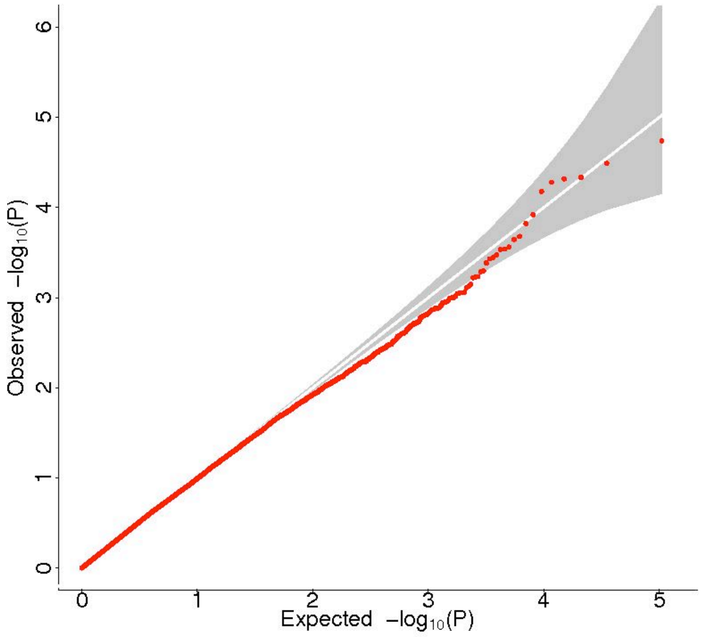
Supplementary Figure 1 QQ plot of P_{fastBAT} from simulations under the null. The simulation is based observed SNP genotypes from the ARIC cohort (Online Methods). Shown is the QQ plot of p-values from 100 simulations (526 gene sets × 100 simulation replicates) using fastBAT. Mean χ^2_1 value across 100 simulations = 0.9993 (standard error of the mean, s.e.m = 0.015), where χ^2_1 is calculated from $P_{\mathrm{fastBAT.}}$



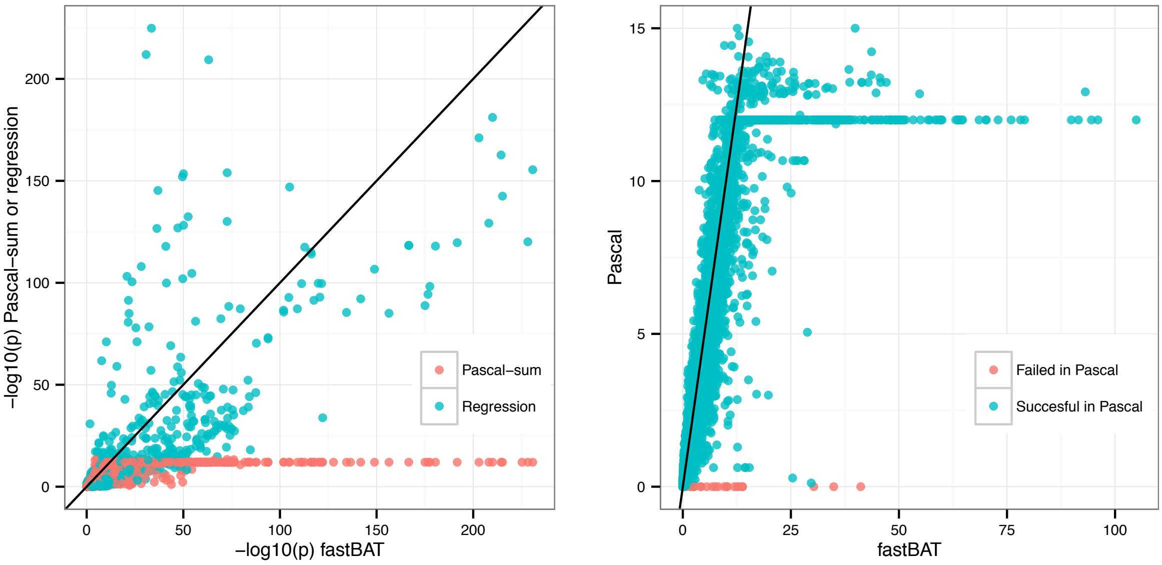
Supplementary Figure 2 Comparison between single-SNP and set-based tests**.** Results are from simulations of unlinked SNPs in 450 sets (Online Methods), each causal variant explaining 0.4% of phenotypic variance. Shown on the y-axis is the –log10(p-value) of the top associated SNP in a set and shown on the x-axis is the –log10(p-value) from the fastBAT test using all SNPs of the set. Each dot represents the average from 10 simulation replicates.



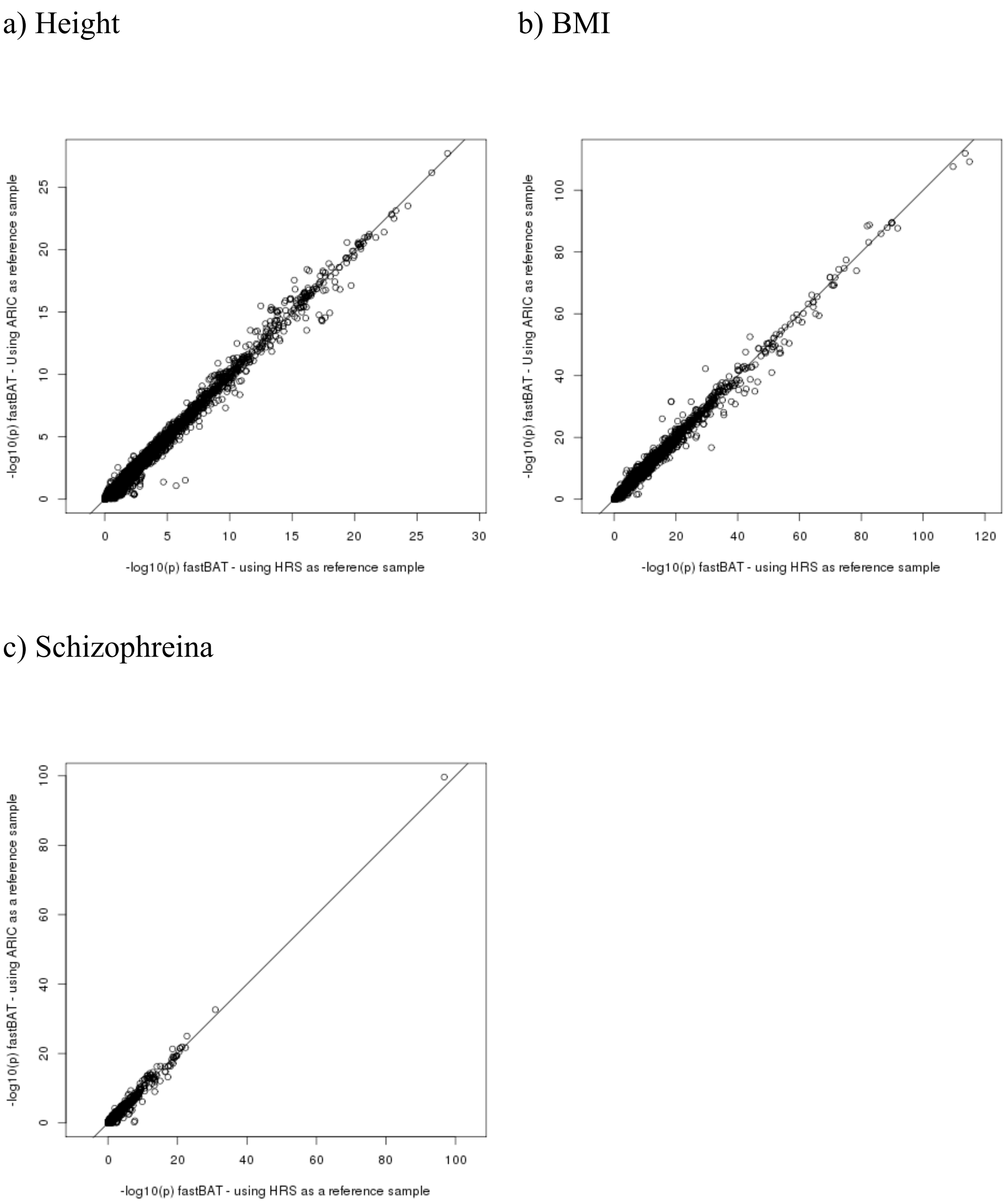
Supplementary Figure 3 The change of power by duplicating a variant in a set. Results are from simulations of unlinked SNPs in 450 sets (Online Methods). There is only one causal variant (explaining 0.4% of phenotypic variant) in each set. Shown is the –log10(p-value) from the fastBAT analysis with the original set plotted against that with the an additional variant in perfect LD with the causal (in red) or non-causal (in blue) in the original set. Each dot represents the average from 10 simulation replicates.



Supplementary Figure 4 QQ plot of P_{fastBAT} from simulations under the null. Shown is the QQ plot of p-values from LD-pruned fastBAT (r^2 threshold of 0.9) analyses of data simulated in **Supplementary Figure 1**. Mean $\chi^2{}_1$ value across 100 simulations = 0.9996 (s.e.m. = 0.015), where $\chi^2{}_1$ is calculated from $P_{\text{fastBAT.}}$



Supplementary Figure 5 Comparison between fastBAT and Pascal using simulations and analysis of real data. Shown are the results from gene-based analyses using the latest versions of fastBAT (GCTA version 1.25.2) and Pascal (downloaded on 27 Jan 2016) with default options. A gene region is defined as ±50Kb of UTRs. We used the 1KGP-EUR data as the reference set for LD estimation. For Pascal analysis, we used the 1KGP-EUR data in Pascal format, which were downloaded as part of the Pascal package. For fastBAT analysis, we used the 1KGP-EUR data (Phase 3; SNP inclusion criteria: missingness rate < 0.05, HWE p > 1×10^{-6} and MAF > 0.0025) downloaded from 1KGP website (**URLs**). In panel (a), the GWAS data were generated from 50 simulations based on real genotypes. In each simulation replicate, we randomly sampled 10 common SNPs (MAF ≥ 0.01) on chromosome 22 as causal variants from the 1KGP-imputed GERA study[1] (n = 53,991 unrelated individuals of European ancestry), and generated a phenotype based on the simulated causal variants, where each causal variant explains 0.4% of phenotypic variation. Regression: linear regression analysis of phenotype on causal variant(s) in each gene region, which is is used as the gold standard for the comparison between fastBAT and Pascal. In panel (b), we used the GWAS summary data from the GIANT meta-analysis for height[2]. There were 30 genes (highlighted in red) for which Pascal could not determine a p-value. It shows in both panels (a) and (b) that p-values from Pascal are bounded at about 1×10^{-15}. This might not be an issue for gene discovery but could potentially be a problem to prioritise the top associated genes for follow-up functional studies. fastBAT also outperforms Pascal in computational efficiency, e.g. for the analysis of height data, fastBAT took 40 sec using 302MB memory, and Pascal took 502 sec using 5,152MB memory (mean values quantified from 50 repeats on identical hardware).

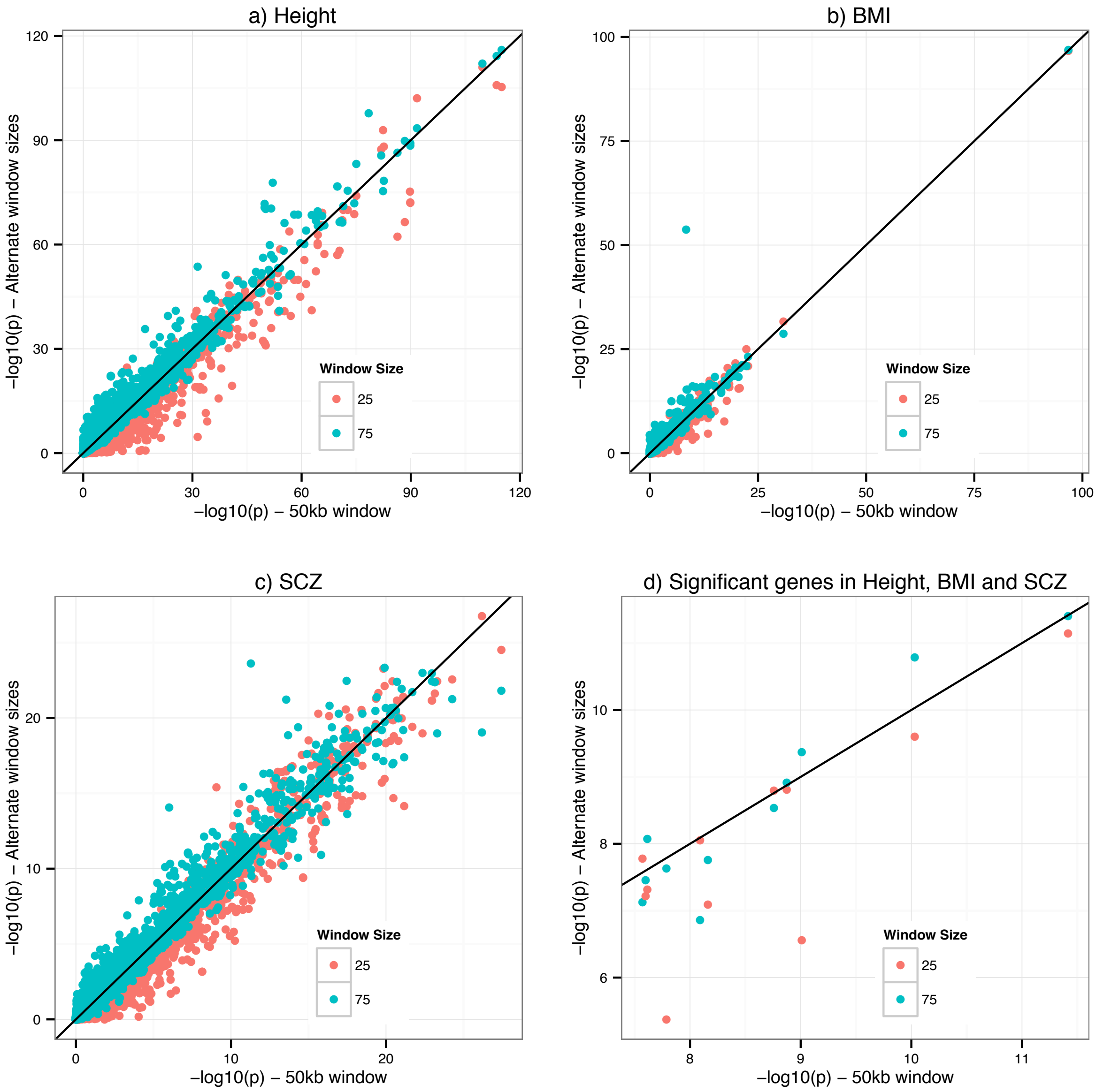


a) Height

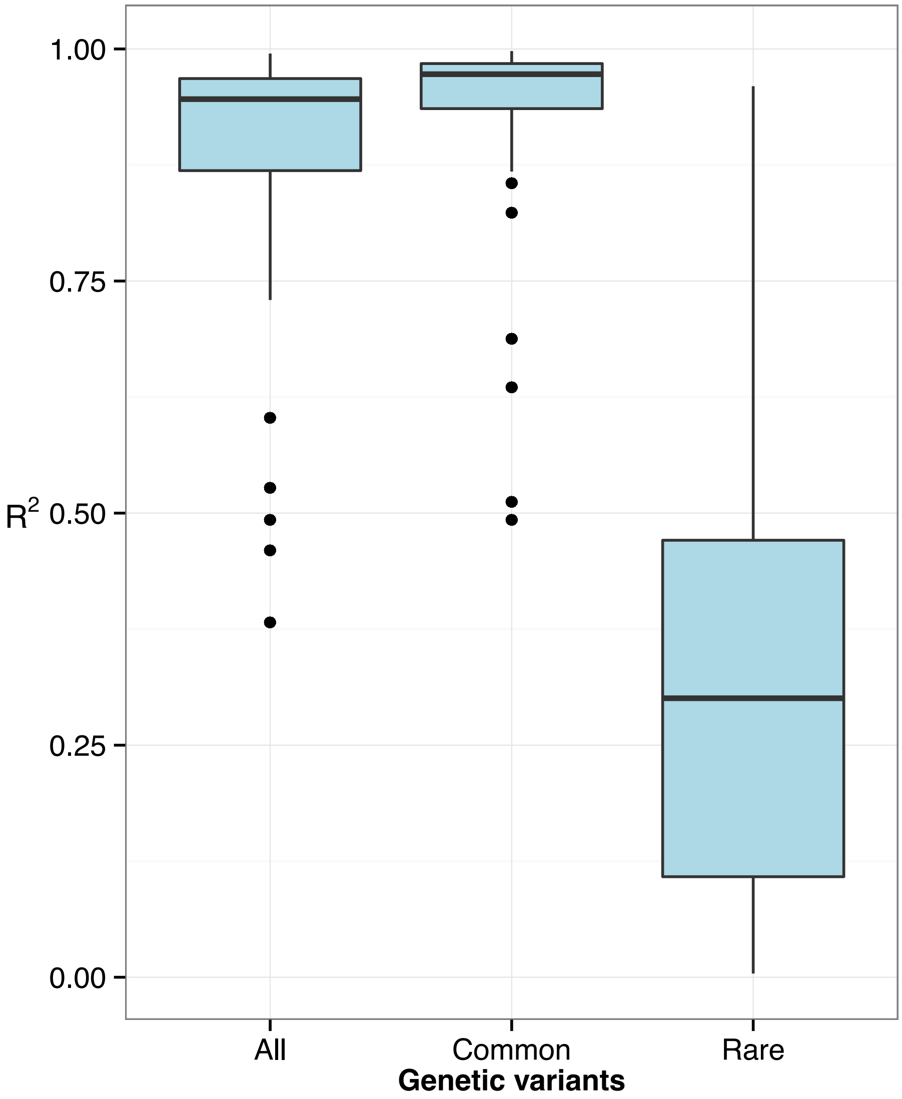
b) BMI

c) Schizophreina

Supplementary Figure 6 Robustness of fastBAT to variation in LD. Shown are the results from the fastBAT analysis of the latest GWAS data using the 1KGP-imputed ARIC data as a reference for LD estimation vs. that using the 1KGP-imputed HRS data for (a) height, (b) BMI, and (c) schizophrenia.



Supplementary Figure 7 Analyses of height, BMI and schizophrenia data with different gene window sizes. In all panels the x-axis shows the -log10(p-value) calculated by fastBAT for a gene using the default window size, i.e. ±50kb of the UTRs. On the y-axis, shown are the –log10(p-value) calculated by fastBAT for the alternate window sizes of 25kb and 75kb. The first three panels show the results of all genes for (a) height, (b) schizophrenia and (c) BMI, respectively. Panel (d) is a combined set of the novel genes for three traits that passed the commonly used GWAS threshold p-value ($P_{\text{fastBAT}} < 5\times10^{-8}$) with a 50kb window (**Table 1**), and their respective p-values with alternate windows. All but one of the novel genes were still significant to a genome-wide significance level ($P_{\text{fastBAT}} < 2\times10^{-6}$) with the alternate window sizes.



Supplementary Figure 8 Correlation between –log10(P_{fastBAT}) using WGS data and that using 1KGP-imputed data. Shown are the results from simulations based on UK10K-WGS data (**Supplemental Note**). In each simulation replicate, a quantitative trait is simulated using WGS data, and the analysis was performed using both WGS and 1KGP-imputed data (see **Supplemental Note** for details). Common: variants with MAF ≥ 0.01. Rare: variants with MAF < 0.01.

Supplementary Table 1 Mean χ^2_1 values calculated from fastBAT analysis with and without LD pruning using simulations. The simulations are based on UK10K-WGS data (Online Methods). The χ^2_1 value is calculated from P_{fastBAT}. The mean χ^2_1 value is calculated from 500 simulation replicates.

Distribution of causal variants	**No LD pruning**		**LD pruning with r^2 threshold = 0.99**		**LD pruning with r^2 threshold = 0.9**	
	All	**Causal**	**All**	**Causal**	**All**	**Causal**
Random	1.216	4.27	1.229	4.34	1.235	4.13
Clustered	1.229	12.72	1.247	13.34	1.261	13.41

Supplementary Table 2 Descriptive summary of the GWAS summary data

Version	Trait	Sample size	Number of SNPs	Reference
Latest	Height	253,288	2.6M	Wood et al. 2014 Nature Genetics[2]
	BMI	339,224	2.6M	Locke et al. 2015 Nature[3]
	SCZ	36,989 vs. 113,075	9.4M	PGC 2014 Nature[4]
Earlier	Height	133,653	2.7M	Lango Allen et al. 2010 Nature[5]
	SCZ	9,394 vs. 12,462	1.3M	PGC 2011 Nature Genetics [6]

Supplementary Table 3 "Novel" loci identified by fastBAT analyses using the earlier version of GWAS data for height and schizophrenia. "earlier": the earlier version of GWAS data (**Supplementary Table 2**). "latest": the latest version of GWAS data (**Supplementary Table 2**). "Top P_{GWAS}": p-value of the top associated SNP in GWAS.

Trait	**Chr**	**Gene**	**Top P_{GWAS} (earlier)**	**P_{fastBAT} (earlier)**	**Top P_{GWAS} (latest)**	**P_{fastBAT} (latest)**
SCZ	1	SDCCAG8	3.26E-06	1.45E-06	3.73E-09	1.46E-10
SCZ	3	MUSTN1	2.25E-06	1.10E-06	4.26E-11	2.71E-11
SCZ	7	MAD1L1	5.06E-08	5.41E-08	6.43E-14	5.27E-11
SCZ	12	CACNA1C	5.06E-08	1.88E-07	3.217E-18	2.06E-18
Height	1	SKI	3.07E-06	1.51E-06	3.30E-17	2.16E-19
Height	2	BOK-AS1	7.05E-06	2.49E-07	1.40E-12	1.02E-16
Height	3	HDAC11	1.15E-07	1.04E-06	3.10E-16	5.41E-15
Height	3	SHOX2	4.87E-07	4.72E-07	9.70E-13	3.03E-11
Height	3	ZBTB20	4.49E-06	4.19E-07	6.60E-11	2.20E-16
Height	4	TACC3	6.02E-08	1.91E-07	1.80E-18	6.06E-20
Height	7	RPS2P32	5.56E-08	4.90E-08	3.50E-26	1.96E-28
Height	8	ENPP2	1.29E-06	8.33E-07	3.50E-13	1.05E-16
Height	11	SENCR	6.75E-07	4.89E-07	2.70E-14	1.35E-14
Height	11	TEAD1	6.07E-08	1.46E-07	1.10E-15	1.28E-16
Height	12	CCDC53	1.09E-07	4.18E-08	1.50E-19	1.42E-20
Height	14	RAD51B	3.99E-06	2.20E-07	3.80E-14	2.81E-17
Height	16	HAGHL	2.40E-07	5.87E-08	1.40E-18	4.56E-20
Height	17	GIT1	1.58E-06	3.97E-07	1.10E-12	3.50E-13
Height	17	KCNJ12	2.00E-07	3.20E-08	8.40E-14	9.43E-14
Height	17	UBE2Z	1.86E-07	1.93E-06	1.50E-16	7.10E-15
Height	19	ADAMTS10	2.52E-07	6.80E-07	1.40E-18	1.17E-18
Height	19	INSR	1.72E-06	7.76E-08	7.20E-18	7.65E-22
Height	19	NFIC	2.25E-07	6.53E-08	1.30E-20	1.83E-25

Supplementary Table 4 Novel gene loci identified by fastBAT for height, BMI and schizophrenia at a genome-wide significance level (P < 2e-6). "Top P_{GWAS}": p-value of the top associated SNP in GWAS.

Trait	Chr	Gene	Top associated SNP	Top P_{GWAS}	P_{fastBAT}
Height	3	THRB	rs2360960	1.20E-07	1.33E-09
Height	3	FOXP1	rs7617596	2.10E-07	1.74E-09
Height	22	UBE2L3	rs5754217	8.50E-08	6.89E-09
Height	8	RBPMS	rs2979510	5.80E-08	8.11E-09
Height	6	MIR6780B	rs2487663	1.70E-07	1.63E-08
Height	7	CALU	rs1043595	1.20E-07	2.68E-08
Height	2	PCBP1	rs6546568	1.20E-07	6.23E-08
Height	2	COL6A3	rs6719451	3.30E-07	6.36E-08
Height	19	APOC4	rs2288911	2.80E-07	7.40E-08
Height	7	AUTS2	rs10262697	2.70E-07	7.93E-08
Height	1	TIPRL	rs10737541	5.50E-07	9.86E-08
Height	20	TOMM34	rs2180292	9.30E-07	1.67E-07
Height	1	CNIH4	rs12754832	5.50E-08	1.79E-07
Height	8	TOX	rs3780001	1.50E-06	1.95E-07
Height	16	WWOX	rs4444350	1.40E-07	2.04E-07
Height	11	SYTL2	rs290195	8.30E-08	2.46E-07
Height	3	MECOM	rs2014590	6.40E-07	3.02E-07
Height	1	RBM34	rs4551650	3.90E-07	3.02E-07
Height	7	KDM7A	rs7797205	8.00E-08	3.13E-07
Height	12	C12orf10	rs7398691	1.30E-07	3.31E-07
Height	6	KIAA1586	rs720884	6.00E-07	3.59E-07
Height	17	ZNF18	rs7216812	1.40E-06	3.80E-07
Height	18	PSMA8	rs4800724	7.60E-07	3.83E-07
Height	12	E2F7	rs310796	2.80E-07	4.13E-07
Height	22	SCUBE1	rs998409	1.50E-06	5.16E-07
Height	6	STX7	rs7743622	7.10E-08	5.48E-07
Height	2	ATOH8	rs1465821	7.80E-07	5.82E-07
Height	5	SRFBP1	rs12153375	2.20E-07	6.02E-07
Height	16	TEKT5	rs8057807	8.50E-08	6.03E-07
Height	20	MYBL2	rs387769	8.00E-06	6.18E-07
Height	19	ARHGAP33	rs2280743	1.50E-06	6.23E-07
Height	4	SLC7A11	rs4863767	1.30E-06	6.86E-07
Height	22	TUG1	rs5749202	5.20E-08	7.31E-07
Height	13	LINC00462	rs12871822	5.70E-08	7.43E-07
Height	4	LIMCH1	rs11726922	1.20E-07	7.71E-07
Height	8	ZHX2	rs4128589	6.30E-07	7.80E-07
Height	10	DLG5	rs1248690	2.20E-07	8.65E-07
Height	8	TMEM74	rs7007200	8.80E-08	9.12E-07
Height	6	TRAM2	rs614570	1.90E-06	9.19E-07
Height	17	MED9	rs7946	8.90E-07	9.73E-07
Height	2	LOC101060091	rs6542180	1.20E-07	1.16E-06

Height	2	LOC101927619	rs2058051	1.30E-07	1.21E-06
Height	12	WBP11	rs16910259	1.10E-06	1.21E-06
Height	9	FBXW2	rs4836831	3.20E-07	1.36E-06
Height	3	ALCAM	rs9288807	1.10E-07	1.47E-06
Height	8	SLC45A4	rs11167042	1.10E-07	1.51E-06
Height	4	LOC101927007	rs4447843	9.90E-07	1.55E-06
Height	3	FAM198A	rs865842	1.90E-07	1.55E-06
Height	7	CHCHD3	rs12537090	1.10E-06	1.65E-06
Height	2	LY75	rs1344632	2.30E-07	1.75E-06
SCZ	3	FOXP1	rs7372960	1.25E-07	3.83E-12
SCZ	10	ZNF365	rs72829007	1.61E-07	9.32E-11
SCZ	15	AP3B2	rs113272695	1.70E-07	2.51E-08
SCZ	3	RBMS3	rs1506297	2.66E-07	5.86E-08
SCZ	18	KCNG2	rs56775891	2.17E-07	9.66E-08
SCZ	16	CPNE7	rs34753377	1.06E-07	9.75E-08
SCZ	17	RPTOR	rs8066384	1.12E-06	1.13E-07
SCZ	8	WHSC1L1	rs112537273	3.70E-07	1.13E-07
SCZ	21	DOPEY2	rs2284641	1.13E-05	1.73E-07
SCZ	8	ZDHHC2	rs17687067	1.14E-07	2.36E-07
SCZ	2	DLX1	rs1001780	8.07E-06	3.40E-07
SCZ	17	LOC388436	rs4273100	7.77e-07	3.53e-07
SCZ	2	MRPL33	rs12474906	1.01E-07	3.56E-07
SCZ	5	ADAMTS2	rs1826864	8.44E-05	3.76E-07
SCZ	8	PSD3	rs6984438	2.66E-07	4.81E-07
SCZ	1	CNTN2	rs16937	8.69E-07	6.42E-07
SCZ	19	SLC39A3	rs57549656	1.22E-06	6.75E-07
SCZ	10	SORCS3	rs11192193	2.54E-06	1.03E-06
SCZ	9	FAM120A	rs12554020	3.96E-06	1.04E-06
SCZ	11	OTUB1	rs571171	1.93E-06	1.18E-06
SCZ	9	KDM4C	rs2026714	1.88E-07	1.37E-06
SCZ	8	MSRA	rs73191547	9.05E-08	1.38E-06
SCZ	4	CXXC4	rs2905627	5.17E-07	1.42E-06
SCZ	2	BCL11A	rs7599488	3.11E-07	1.44E-06
SCZ	16	NMRAL1	rs6500602	2.79E-07	1.57E-06
SCZ	7	CALN1	rs1914395	5.20E-07	1.60E-06
SCZ	15	FAM214A	rs4776059	1.06E-05	1.89E-06
SCZ	14	RTN1	rs12431410	4.22E-07	1.91E-06
SCZ	2	ERBB4	rs16846200	1.62E-05	1.92E-06
BMI	19	SCAMP4	rs11672550	1.33E-07	9.77E-10
BMI	7	DTX2P1-UPK3BP1-PMS2P11	rs6955651	6.19E-08	2.43E-08
BMI	17	TACO1	rs8075273	1.55E-07	2.04E-07
BMI	9	TRUB2	rs2270204	3.22E-07	5.30E-07
BMI	7	CALCR	rs9641123	1.83E-07	7.57E-07
BMI	17	CBX1	rs3764400	5.54E-07	8.86E-07
BMI	2	USP37	rs7607369	1.10E-07	1.08E-06
BMI	19	ZNF536	rs33439	7.58E-07	1.27E-06

Supplementary Table 5 Numbers of "novel" gene loci discovered by fastBAT – with and without LD-pruning – as well as Pascal-Sum and Pascal-Max using the earlier version of GWAS data for height and schizophrenia. A "novel" gene is "replicated" if p-value of the top associated SNP in the gene region is < 5e-8 in the latest GWAS data. fastBAT analyses were performed using the 1KGP-imputed HRS as the reference sample for LD, and Pascal analyses were performed with 1KG-EUR data set provided in the Pascal software package.

	Height		**SCZ**	
	Discovered	Replicated	Discovered	Replicated
fastBAT with LD pruning (default)	19	19	4	4
fastBAT without LD pruning	16	16	4	4
Pascal-Sum (default)	15	15	4	3
Pascal-Max	10	9	3	1

Supplemental Note

1. Comparison between fastBAT with sequence and imputed variants

We performed simulations based on WGS data from the UK10K project[7], of which there were 17.6M genetic variants across 3,781 unrelated individuals after quality control[8]. Following the strategy proposed in Yang et al.[8], we extracted the set of SNPs that can be found on an Illumina CoreExome array from the UK10K data, and used IMPUTE2 (ref[9]) to impute the subset of SNPs to 1000 Genome Project (1KGP) reference panels[10]. The imputed SNPs with Hardy-Weinberg Equilibrium (HWE) test p-value < 1e-6 or minor allele counts < 3 were removed from the analysis.

Following Yang et al.[8], to quantify the variation at sequence variants that can be captured by 1KGP-imputed variants, we simulated traits using WGS data and performed the analysis using 1KGP-imputed data. For the ease of computation, we only used data on chromosome 1. We simulated a quantitative trait using the GCTA simulation function (50 causal variants with a total heritability of 10%) under two scenarios, I) causal variants were sampled at random from all the sequence variants; II) causal variants are clustered in a few randomly sampled genomic regions (see Online Method for the method to simulated clustered causal variants). We then performed the fastBAT analysis for the simulated trait using the 1KGP-imputed genotypes and compared the result with that using WGS data.

2. Benchmarking Performance

We compared the computational performance of the three implementations, PLINK-set, VEGAS (offline version) and GCTA-fastBAT by re-running the analysis presented in **Fig. 1a** on identical hardware, recording the execution time and maximum memory usage, and reported the mean results of 10 executions. We ran a gene-based PLINK-set test (10^6 permutations) with the individual-level genotype and phenotype data in the ARIC cohort (chromosome 22). The GCTA-fastBAT and VEGAS (command-line version) analyses were performed using the summary statistics. On average, PLINK-set used ~38 hours to complete the analysis (note that the set-based test implemented in PLINK2 is much faster than PLINK-set but still much slower than fastBAT), VEGAS (default parameters) took 36 minutes, and GCTA-fastBAT (using only a single thread) completed in 8 seconds (see the table below). The LD-pruned fastBAT analysis has slightly higher memory requirements than that without LD-pruning but it is still orders magnitude faster than PLINK-set and VEGAS (see the table below).

	Time	**RAM**
PLINK-set	38 hours	10GB
VEGAS	36 min	1.2GB
fastBAT	8.6 sec	48MB
fastBAT with LD pruning	7.9 sec	424MB

3. Running fastBAT

A complete manual is available at the GCTA website (**URLs**). The implementation of fastBAT in GCTA uses a PLINK binary file as the reference set for LD estimation. If no reference for LD is available it is possible to use the HapMap3 or 1KGP data (**URLs**). A list of gene coordinates is available from the PLINK website (**URLs**) and mirrored on the GCTA website (**URLs**).

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HRS: HRS is supported by the National Institute on Aging (NIA U01AG009740). The genotyping was funded separately by the National Institute on Aging (RC2 AG036495, RC4 AG039029). Genotyping was conducted by the NIH Center for Inherited Disease Research (CIDR) at Johns Hopkins University. Genotyping quality control and final preparation of the data were performed by the Genetics Coordinating Center at the University of Washington.

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