



Association between genetic risk scores and risk of narcolepsy: a case-control study

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Background: Genome-wide association studies (GWASs) have identified a large number of single-nucleotide polymorphisms (SNPs) associated with narcolepsy. However, the sum impact of these SNPs on defining the genomic risk of narcolepsy remains unknown. In the present study, we investigated the associations between genetic risk scores (GRSs) and narcolepsy along with their predictive power

Methods: A case-control study consisting of 903 narcolepsy patients and 1,981 healthy control subjects was performed. Thirty-two SNPs previously reported to confer susceptibility to narcolepsy were assessed for their association with narcolepsy risk. Subsequently, we constructed four GRS groups comprising reported narcolepsy susceptibility SNPs located in different genomic regions, and tested their association with narcolepsy risk using a regression model. Receiver operating characteristic (ROC) curves were used to examine the discriminatory power of the GRSs for predicting narcolepsy.

Results: Nine individual SNPs were significantly associated with narcolepsy after Bonferroni correction. All four GRSs were strongly associated with narcolepsy risk even when GRSs were constructed using SNPs located outside the previously implicated human leukocyte antigen (HLA) region on chromosome 6. The odds ratio (OR) for narcolepsy risk increased with the number of genetic loci implicated, ranging from an OR of 2.016 (95% CI, 1.657–2.456) to an OR of 4.298 (95% CI, 3.378–5.481). GRS4, constructed using the narcolepsy-associated SNPs identified in the Chinese population, was most closely associated with narcolepsy risk.

Conclusions: The results suggest that the GRS method for combining common genetic variations can significantly associate GRS scores with narcolepsy risk and may facilitate narcolepsy risk stratification for prevention trials, both for HLA-DQB1*06:02-positive and -negative individuals.

Keywords: Case-control study; genetic risk score (GRS); single-nucleotide polymorphisms (SNPs)

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Introduction

Narcolepsy is a life-long neurological disorder characterized by excessive daytime sleepiness, cataplexy, sleep paralysis, hallucinations, and disrupted nocturnal sleep (1). While narcolepsy can manifest at any age, symptoms typically

start between 15 and 30 years of age, and the prevalence of narcolepsy ranges from 0.002% to 0.167% worldwide. In order to identify patients at risk for narcolepsy and to facilitate the development of enhanced screening or preventive measures, research has focused on genetic risk loci marked by individual single-nucleotide polymorphisms

(SNPs) (2-9). Recently, the genetic risk of narcolepsy has been evaluated based on the *HLA-DQB1*06:02* marker, because 10–40% of healthy individuals are also carriers, it represents an important but seemingly imperfect predictor of narcolepsy (10-12). In addition to *HLA-DQB1*06:02*, many other genes have been implicated in narcolepsy risk in international SNP-based genome-wide association studies (GWASs), including carnitine palmitoyltransferase 1B (*CPT1B*), choline kinase- β (*CHKB*), T cell receptor alpha (*TCRA*), purinergic receptor P2Y, G-protein coupled 11 (*P2RY11*), tumor necrosis factor superfamily member 4 (*TNSF4*), cathepsin H (*CTSH*), T cell receptor beta (*TCRB*), zinc finger protein 365 (*ZNF365*), chemokine receptor1 (*CCR1*), chemokine receptor3 (*CCR3*), eukaryotic initiation factors 3G (*EIF3G*), and interleukin 10 receptor, beta (*IL10RB*) (2-9,13). SNPs conferring narcolepsy susceptibility in the human leukocyte antigen (HLA) and other regions have also been identified (4,14,15).

The studies referenced above have provided a rich compendium of genetic variations potentially underlying narcolepsy and prepared the grounds for building new risk prediction tools. However, the effects of common SNPs identified by GWASs are usually minor and of unclear clinical significance. In order to complement GWASs and uncover potential instances of “missing heritability”, several studies investigated the benefits of conflating genomic risk estimates obtained from SNP genotyping into a genetic risk score (GRS) to predict the risk of diseases such as cancer (16), diabetes (17), schizophrenia (18), coronary artery disease (19), Alzheimer’s disease (20), and leukemia (21). These studies have repeatedly demonstrated significant associations between GRSs and disease risk. As for narcolepsy, although GWASs have been performed, few studies have investigated the aggregate effects of genetic variants on narcolepsy risk in large study samples. We therefore selected SNPs discovered in recently reported narcolepsy susceptibility loci (including variants in European and Asian ancestry reported in previous case-control studies) and investigated the association of these SNPs, along with their related GRSs, with narcolepsy risk in a Chinese population.

Methods

Study site and population

We performed a case-control study comprising 1,062 narcolepsy patients and 1,997 healthy control subjects. The patients were recruited from the sleep laboratory of Peking

University People’s Hospital, a unit that receives referrals from all over China. The narcolepsy patients had either hypocretin deficiency (CSF hypocretin-1 ≤ 110 pg/mL) or clear-cut cataplexy and *HLA-DQB1*06:02* (3). Subjects were all Chinese, mostly of Han descent (95%). The majority of study patients were male (68%). The controls were Chinese university employees and students (41% male), and were free of narcolepsy as determined by clinical examination and medical history. Trained interviewers used structured questionnaires to collect information on demographic variables, medical history, and medication. Informed consent (in accordance with governing institutions) was obtained from all subjects. The research protocols were approved by the Institutional Review Board on Medical Human Subjects Research at the Peking University People’s Hospital.

Genotyping and quality control

SNP genotyping and quality control have been described in detail elsewhere (4). In brief, samples were genotyped and analyzed using the Affymetrix Axiom CHB array and the Affymetrix Genotyping Console. All samples used in the current study achieved a call rate of >99%. In total, 903 narcolepsy cases and 1,981 healthy controls were included for further analysis.

Genetic loci selection

We selected 40 previously discovered narcolepsy risk loci from GWAS studies published before September, 2018 (2,4-6,8,9,14,15,22,23). The narcolepsy susceptibility loci identified in both the Chinese population and European population were selected. The final candidate SNPs were in approximate linkage equilibrium with all pairwise $r^2 < 0.25$ based on linkage disequilibrium (LD) data from the HapMap Chinese/Japanese combined reference set (CHB/JPT) (24). When a selected variant was not on the Affymetrix Axiom CHB array, an analysis of LD in a 10–20 kb window was performed using Ensembl 1,000 Genomes LD data to identify suitable proxies on the array with an $r^2 \geq 0.8$. SNPs whose call rate was lower than 99% or that violated the Hardy-Weinberg equilibrium (HWE) test were excluded. The final list of candidate SNPs is shown in *Table 1*. Among the candidate SNPs, 17 included in the GRS were directly genotyped while the remaining SNPs were assessed based on LD with the reported index SNPs with $r^2 \geq 0.8$.

Table 1 SNPs selected for the study

Chromosome	Gene	SNP	Tag SNP	r ²	LD [†]	Allele	Risk allele	Missing (%)	MAF [‡]	P for HWE [§]
1	<i>TNFSF4</i>	rs7553711	rs4090391	0.999	1	C/T	T	0.5	0.08	0.057
1	<i>MIR-552</i>	rs10915020	rs12026171	0.928	1	A/G	G	0.2	0.06	0.681
3	<i>CCR1/CCR3</i>	rs3181077	–	–	–	C/T	C	0.1	0.11	0.246
4	<i>BTBD9</i>	rs3923809	–	–	–	A/G	G	0.5	0.40	0.999
7	<i>TRB</i>	rs2854536	–	–	–	T/C	T	0.3	0.24	0.48
8	<i>UBXN2B</i>	rs2859998	rs6993992	0.999	1	T/C	T	0.3	0.47	0.999
10	<i>ZNF365</i>	rs10995245	–	–	–	G/A	A	0.2	0.31	0.507
10	<i>A1CF</i>	rs4290173	rs16911668	0.956	1	A/G	A	0.1	0.12	0.172
12	<i>TEAD4</i>	rs12425451	rs12322530	0.947	1	G/A	G	0.3	0.09	0.283
14	<i>TRA</i>	rs1154155	–	–	–	T/G	T	0.8	0.45	0.682
14		rs12587781	rs1154153	0.999	1	T/C	T	0.2	0.36	0.264
14		rs1263646	rs1263647	0.981	1	A/G	A	0.2	0.46	0.470
15	<i>CTSH</i>	rs12148472	–	–	–	T/C	C	0.1	0.05	1.000
15		rs3825932	–	–	–	T/C	T	0.1	0.11	0.745
19	<i>DNMT1</i>	rs2290684	rs2114724	0.999	1	C/T	T	0.0	0.29	0.406
19		rs6511570	rs7253062	0.999	1	G/A	G	0.2	0.25	0.999
20	<i>NFATC2</i>	rs8119787	–	–	–	A/G	G	0.1	0.37	0.841
21	<i>IL10RB- IFNAR1</i>	rs2834118	rs2834113	0.934	0.966	T/G	G	0.6	0.16	0.176
21		rs2252931	rs2834188	0.999	1	A/G	A	0.3	0.28	0.625
22	<i>CPT1B-CHKB</i>	rs5770917	rs5770911	0.999	1	C/T	C	0.2	0.23	0.237
1	<i>DHS</i>	rs213038	rs213026	0.961	1	C/T	T	0.0	0.39	0.847
6	<i>HLA</i>	rs1338829	rs9444828	0.869	0.979	C/T	T	0.2	0.48	0.927
6		rs473267	–	–	–	C/T	T	0.2	0.34	0.919
11		rs290183	rs7122887	0.959	1	C/T	C	0.1	0.36	0.924
18		rs1786783	rs524513	0.821	0.935	C/T	T	0.1	0.08	0.644
20		rs4810966	rs169160	0.896	1	T/C	T	0.5	0.36	0.698
6		rs9274477	–	–	–	A/G	A	0.14	0.30	0.243
6		rs9271117	–	–	–	T/C	C	0.42	0.34	0.899
6		rs2858884	rs2859090	0.999	1	G/A	G	0.10	0.06	0.209
6		rs3117242	rs3117221	0.999	1	C/T	T	0.24	0.46	0.237
6		rs2523882	rs2517455	0.962	1	C/T	C	0.21	0.44	0.164
6		rs3129907	rs3129932	0.999	1	C/G	G	0.76	0.14	0.093

[†]LD, linkage disequilibrium; [‡]MAF, minor allele frequency; [§]HWE, Hardy-Weinberg equilibrium. SNPs, single-nucleotide polymorphisms.

Table 2 SNPs included in the GRSs

GRS category	SNPs involved in the GRS
GRS1	rs4090391, rs12026171, rs3181077, rs3923809, rs2854536, rs6993992, rs10995245, rs16911668, rs12322530, rs1154155, rs12148472, rs7253062, rs8119787, rs2834113, rs2834188, rs5770911
GRS2	rs9274477, rs3117221, rs2517455, rs3129932
GRS3	rs4090391, rs12026171, rs3181077, rs3923809, rs2854536, rs6993992, rs10995245, rs16911668, rs12322530, rs1154155, rs12148472, rs7253062, rs8119787, rs2834113, rs2834188, rs5770911, rs213026, rs9444828, rs473267, rs2859090, rs7122887, rs524513, rs169160, rs9274477, rs3117221, rs2517455, rs3129932
GRS4	rs1154155, rs2854536, rs2834188, rs10995245, rs9274477

SNPs, single-nucleotide polymorphisms; GRSs, genetic risk scores.

Statistical analyses

We used the χ^2 test to assess whether the SNPs were in HWE and to determine the differences in genotype frequencies between narcolepsy patients and controls. Statistical significance was set to a two-sided P value of less than 0.05. The experiment-wide significance level was derived via Bonferroni correction for the number of lead SNPs.

For the construction of individual GRSs, a genetic score was calculated based on a personal genotype of the SNPs that are associated with narcolepsy, and unweighted GRSs were calculated by adding the number of risk alleles across each SNP. We constructed four different GRSs (the SNPs included in GRSs were shown in *Table 2*):

(I) GRS1 excluded the SNPs in the HLA region; (II) GRS2 was limited to the SNPs from the HLA region; (III) GRS3 included all selected SNPs; and (IV) GRS4 only included the SNPs reported in the Chinese population. We considered the GRSs as continuous variables and as tertiles defining low risk, intermediate risk, and high risk classes. Categorical variables were shown in proportions. The associations of the GRSs with narcolepsy risk were evaluated using the logistic regression model adjusted by gender. Odds ratios, 95% CI, and P values were calculated.

We further examined the ability of the four GRSs to discriminate between patients and controls by using receiver-operating characteristics (ROC) curves adjusted for gender. We used Haploview (version 4.2) to calculate the linkage disequilibrium of selected SNPs. The subsequent statistical analyses were performed by using R (version 3.5.0).

Results

The demographic and clinical data of the narcolepsy patients are presented in *Table 3*. Among these patients, 860

(95.2%) were of Chinese Han ethnicity, 285 (31.6%) were women, 618 (68.4%) were men, and 18 (2.0%) had a family history of narcolepsy. Among the healthy controls, 678 (34.2%) were men, and 1,303 (65.8%) were women. The flow chart of the inclusion process is shown in *Figure 1*.

Association between individual SNP and narcolepsy risk

We examined the association between the individual genetic variant and narcolepsy risk. Fourteen loci, including *TRB* (rs2854536), *ZNF365* (rs10995245), *DNMT1* (rs2114724, rs7253062), *IL10RB-IFNAR1* (rs2834113, rs2834188), *TRA* (rs1154155, rs1154153, rs1263647), and HLA region (rs9274477, rs9271117, rs2859090, rs3129932, rs2517455) showed significant associations with incident narcolepsy, with P values ranging from <0.001 to 0.005. After the Bonferroni correction, *TRB* (rs2854536), *ZNF365* (rs10995245), *TRA* (rs1154155, rs1154153, rs1263647), and HLA region (rs9274477, rs9271117, rs2859090, rs3129932) remained statistically significant. The association of each individual SNP with narcolepsy risk is summarized in *Tables 4* and *S1*.

Combined genetic effects on narcolepsy risk

We examined the association between the GRSs and narcolepsy risk and observed that the GRSs were significantly associated with narcolepsy risk. *Table 5* shows the odds ratios (ORs) for the association between incident narcolepsy and GRSs, comparing the narcolepsy risk of individuals in the low polygenic load categories with the narcolepsy risk of individuals in the intermediate and high polygenic load category. Individuals who carry a higher polygenic load have higher odds of narcolepsy compared with patients carrying a low polygenic load. The OR of

Table 3 Demographic and clinical variables of the narcolepsy patients and controls

Variables	Cases	Controls
Male, n (%)	618 (68.4)	678 (34.2)
Chinese, n (%)	903 (100.0)	1,981 (100.0)
Diagnostic delay (years), median [Q1, Q3]	2 [0–8]	NA
Age of onset (years), median [Q1, Q3]	9 [7–13]	NA
Inflammation before onset, n (%)	39 (4.3)	NA
Cataplexy, n (%)	903 (100.0)	0 (0.0)
Sleep paralysis, n (%)	331 (36.7)	0 (0.0)
Hypnagogic hallucinations, n (%)	525 (58.1)	0 (0.0)
Number of symptoms, n (%)		
1	284 (31.5)	0 (0.0)
2	356 (39.4)	0 (0.0)
3	242 (26.8)	0 (0.0)
Familial history, n (%)	18 (2.0)	0 (0.0)
The self-report impairment of life quality, n (%)		
No impairment	99 (11.0)	1,981 (100.0)
Small impairment	244 (27.0)	0 (0.0)
Great impairment	254 (28.1)	0 (0.0)
CSF hypocretin (pg/mL), median (Q1, Q3)	17.8 (12.7–30.5)	NA

REM, rapid eye movement; CSF, cerebral spinal fluid.

narcolepsy risk increased as the genetic load for narcolepsy increased, ranging from an OR of 2.016 (95% CI, 1.657–2.456) to an OR of 4.298 (95% CI, 3.378–5.481). Among the four GRSs, GRS4 (constructed from the narcolepsy susceptibility SNPs identified in the Chinese population) was most closely associated with narcolepsy risk with an OR of 4.298 (95% CI, 3.378–5.481) followed by GRS3 constructed using all narcolepsy-associated SNPs.

The discriminatory power of GRS

To further refine these results, we examined the discriminatory power of the GRSs for individuals at high risk of narcolepsy by using the ROC curve. The discriminatory ability of the model using GRS1, measured as area under the curve (AUC), was 0.709. The other models using GRS2, GRS3, and GRS4 showed modestly improved discrimination with AUCs of 0.715, 0.723, and 0.736, respectively (Figure 2). These results showed that the GRS constructed from the narcolepsy susceptibility SNPs reported in the Chinese population provided greater discrimination than other GRSs. At a cutoff of 0.335, which was determined according to the maximum of the Youden Index, GRS4 had a sensitivity of 70.9% and a specificity of 65.1% in discriminating narcolepsy patients from healthy controls.

Discussion

The present study reports two main findings: first, we

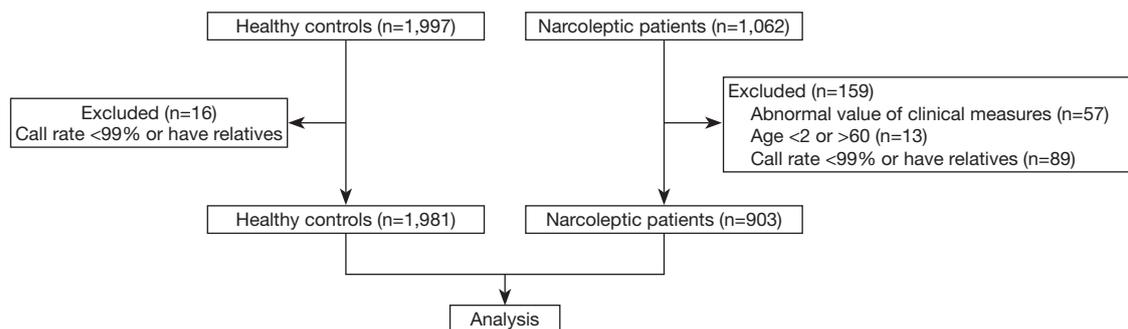
**Figure 1** The flow chart of the inclusion process.

Table 4 Association of individual SNPs with narcolepsy risk

Gene	SNP	Allele	Control, N (%)	Case, N (%)	P	P [†]		
<i>TRB</i>	rs2854536	TT	1,087 (55.1)	554 (61.6)	<0.001	0.005		
		TC	764 (38.7)	317 (35.2)				
		CC	123 (6.2)	29 (3.2)				
<i>ZNF365</i>	rs10995245	T	2,938 (74.4)	1,425 (79.2)	<0.001	0.003		
		C	1,010 (25.6)	375 (20.8)				
		GG	998 (50.5)	375 (41.5)			<0.001	<0.001
		GA	821 (41.5)	427 (47.3)				
		AA	157 (7.9)	101 (11.2)				
<i>TRA</i>	rs1154155	G	2,817 (71.3)	1,177 (65.2)	<0.001	<0.001		
		A	1,135 (28.7)	629 (34.8)				
		TT	518 (26.3)	317 (35.5)			<0.001	<0.001
		TG	975 (49.5)	479 (53.7)				
		GG	476 (24.2)	96 (10.8)				
	rs1154153	T	2,011 (51.1)	1,113 (62.4)	<0.001	<0.001		
		G	1,927 (48.9)	671 (37.6)				
		TT	760 (38.5)	430 (47.6)			<0.001	<0.001
		TC	912 (46.2)	403 (44.6)				
		CC	304 (15.4)	70 (7.8)				
	rs1263647	T	2,432 (61.5)	1,263 (69.9)	<0.001	<0.001		
		C	1,520 (38.5)	543 (30.1)				
		AA	505 (25.6)	310 (34.3)			<0.001	<0.001
		AG	1,003 (50.8)	469 (51.9)				
		GG	468 (23.7)	124 (13.7)				
<i>DNMT1</i>	rs2114724	A	2,013 (50.9)	1,089 (60.3)	<0.001	<0.001		
		G	1,939 (49.1)	717 (39.7)				
		CC	1,003 (50.7)	426 (47.2)			0.072	1
		CT	823 (41.6)	387 (42.9)				
		TT	154 (7.8)	90 (10.0)				
	rs7253062	C	2,829 (71.4)	1,239 (68.6)	0.031	1		
		T	1,131 (28.6)	567 (31.4)				
		GG	1,085 (54.9)	539 (59.8)			0.025	0.825
		GA	759 (38.4)	317 (35.2)				
		AA	133 (6.7)	45 (5.0)				
		G	2,929 (74.1)	1,395 (77.4)			0.007	0.231
A	1,025 (25.9)	407 (22.6)						

Table 4 (Continued)

Table 4 (Continued)

Gene	SNP	Allele	Control, N (%)	Case, N (%)	P	P [†]		
<i>IL10RB-IFNAR1</i>	rs2834113	TT	1,386 (70.3)	627 (70.0)	0.021	0.693		
		TG	543 (27.5)	234 (26.1)				
		GG	42 (2.1)	35 (3.9)				
	rs2834188	T	3,315 (84.1)	1,488 (83.0)	0.333	1		
		G	627 (15.9)	304 (17.0)				
		AA	983 (49.8)	496 (54.9)			0.006	0.198
		AG	812 (41.2)	352 (39.0)				
		GG	177 (9.0)	55 (6.1)				
		A	2,778 (70.4)	1,344 (74.4)			0.002	0.066
		G	1,166 (29.6)	462 (25.6)				
<i>HLA</i>	rs9274477	AA	876 (44.3)	512 (56.7)	<0.001	<0.001		
		AG	862 (43.6)	391 (43.3)				
		GG	239 (12.1)	0 (0)				
		A	2,614 (66.1)	1,415 (78.3)			<0.001	<0.001
		G	1,340 (33.9)	391 (21.7)				
	rs9271117	TT	1,190 (60.3)	37 (24.5)	<0.001	<0.001		
		TC	683 (34.6)	642 (71.4)				
		CC	100 (5.1)	220 (4.1)				
		T	3,063 (77.6)	716 (39.8)			<0.001	<0.001
		C	883 (22.4)	1,082 (60.2)				
	rs2859090	GG	1,689 (85.3)	835 (92.6)	<0.001	<0.001		
		GA	274 (13.8)	67 (7.4)				
		AA	16 (0.8)	0 (0)				
		G	3,652 (92.3)	1,767 (96.3)			<0.001	<0.001
		A	306 (7.7)	67 (3.7)				
	rs3117221	CC	581 (29.4)	254 (28.2)	0.367	1		
		CT	1,006 (50.9)	448 (49.8)				
		TT	390 (19.7)	198 (22.0)				
		C	2,168 (54.8)	956 (53.1)			0.236	1
		T	1,786 (45.2)	844 (46.9)				
	rs2517455	CC	607 (30.7)	300 (33.2)	0.037	1		
		CT	947 (47.9)	447 (49.5)				
		TT	421 (21.3)	156 (17.3)				
		C	2,161 (54.7)	1,047 (58.0)			0.022	0.726
		T	1,789 (45.3)	759 (42.0)				
	rs3129932	CC	1,547 (78.7)	595 (66.4)	<0.001	<0.001		
		CG	385 (19.6)	270 (30.1)				
		GG	34 (1.7)	31 (3.5)				
C		3,479 (88.5)	1,460 (81.5)	<0.001			<0.001	
G		453 (11.5)	332 (18.5)					

†, after Bonferroni correction. SNPs, single-nucleotide polymorphisms.

Table 5 Association of GRSs with the risk of narcolepsy

Risk group	Control, N=1,981 (%)	Case, N=903 (%)	Crude model		Adjusted model ¹	
			Odds ratio (95% CI)	P	Odds ratio (95% CI)	P
GRS1 [†]			1.151 (1.111–1.193)	<0.001	1.150 (1.108–1.194)	<0.001
Low risk (GRS1 <16)	774 (39.1)	249 (27.6)	1		1	
Intermediate risk (16 ≤ GRS1 <18)	672 (33.9)	307 (34.0)	1.420 (1.167–1.729)	0.002	1.431 (1.164–1.760)	<0.001
High risk (GRS1 ≥18)	535 (27.0)	347 (38.4)	2.016 (1.657–2.456)	<0.001	2.027 (1.648–2.495)	<0.001
GRS2 [‡]			1.357 (1.274–1.447)	<0.001	1.343 (1.256–1.437)	<0.001
Low risk (GRS2 <4)	998 (50.4)	307 (34.0)	1		1	
Intermediate risk (4 ≤ GRS2 <5)	544 (27.5)	285 (31.6)	1.703 (1.405–2.064)	<0.001	1.717 (1.403–2.102)	<0.001
High risk (GRS2 ≥5)	439 (22.2)	311 (34.4)	2.303 (1.898–2.796)	<0.001	2.222 (1.813–2.727)	<0.001
GRS3 [§]			1.149 (1.119–1.181)	<0.001	1.152 (1.120–1.185)	<0.001
Low risk (GRS3 <25)	925 (46.7)	279 (30.9)	1		1	
Intermediate risk (25 ≤ GRS3 <28)	688 (34.7)	337 (37.3)	1.623 (1.348–1.958)	<0.001	1.620 (1.332–1.973)	<0.001
High risk (GRS3 ≥28)	368 (18.6)	287 (31.8)	2.586 (2.109–3.173)	<0.001	2.602 (2.097–3.232)	<0.001
GRS4			1.449 (1.367–1.536)	<0.001	1.442 (1.357–1.534)	<0.001
Low risk (GRS4 <6)	818 (41.3)	192 (21.3)	1		1	
Intermediate risk (6 ≤ GRS4 <8)	937 (47.3)	483 (53.5)	2.196 (1.816–2.664)	<0.001	2.174 (1.782–2.661)	<0.001
High risk (GRS4 ≥8)	226 (11.4)	228 (25.2)	4.298 (3.378–5.481)	<0.001	4.157 (3.224–5.371)	<0.001

[†], SNPs outside the HLA region; [‡], SNPs within the HLA region; [§], all 32 SNPs selected; ^{||}, SNPs reported in the Chinese population; ¹, adjusted for gender. GRSs, genetic risk scores; HLA, human leukocyte antigen.

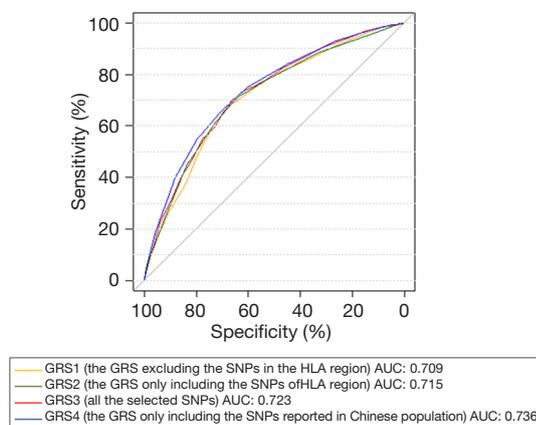


Figure 2 ROC curve of the GRS models for discriminating narcoleptic patients. ROC, receiver operating characteristic; GRS, genetic risk score.

confirmed the significant associations between reported individual SNPs and narcolepsy risk. Second, we found that the GRSs constructed from SNPs that were located

in different regions showed significant associations with narcolepsy. Together, these findings buttress the feasibility of discovering genetic biomarkers for disease risk prediction or prognosis.

To the best of our knowledge, the present study is the first detailed and large-scale genetic study of narcolepsy in the Chinese population and the largest study applying the GRS approach to evaluate the polygenic risk of narcolepsy. Major strengths of the present study include the following: (I) we constructed several GRSs from SNPs from different regions and compared their discriminate power; (II) we created GRSs using common narcolepsy susceptibility variants from both Asian and European populations. It is thus likely that our findings apply to multiple ethnicities.

We assessed the association of the reported narcolepsy-related variants at 32 loci in the Chinese ethnic group. The trends observed with 14 narcolepsy-associated loci were consistent with previous studies (2-4,25-27). In particular, nine loci, including *TRB*, *ZNF365*, *TRA*, and SNPs within the HLA region, showed significant

association with narcolepsy risk after Bonferroni correction. While these variants were mostly identified in European populations, they each showed association with broader global populations, including groups of Asian ancestry (4,9). On the other hand, we were unable to confirm, in our Chinese sample, the individual associations for several genetic variants previously reported to be associated with narcolepsy risk. This could be due to insufficient statistical power to identify variants with small individual effects or to the diverse genetic backgrounds of a variety of ethnicities.

GRSs have been previously employed to increase statistical power when investigating the association with disease-related phenotypes. Our results confirmed the slight increase in narcolepsy risk carried by genetic variants identified by GWAS and justify the use of GRSs to increase the statistical power to identify these associations. The results were consistent with previous studies indicating that the combined effects of these genetic loci had a more sizable influence on narcolepsy risk (28,29). The significant association between GRSs with narcolepsy indicated that disease risk is not only associated with the variants in the HLA region but that SNPs outside the HLA region also play an important role. Our results did not entirely replicate previous studies that emphasized the importance of HLA genes in narcolepsy (10,12,30). This indicates contribution of genetic factors besides those implicated in the dysfunction of the immune system (9,31-33). GRSs constructed without HLA-DQB1*06:02 could be a promising tool for the risk stratification of HLA-DQB1*06:02-negative populations. Among the four GRSs assembled in our study, GRS4 had the best predictive power for narcolepsy risk, followed by GRS3. Since all SNPs included in GRS4 were identified in the Chinese population, the genetic variation underlying narcolepsy may differ across different ethnicities.

The high predictive power of GRS3 constructed from all narcolepsy-associated SNPs indicates that narcolepsy is a polygenic disease involving many common genetic variants of small effect rather than being driven by rare monogenic mutations (34-36). This finding adds further credence to the concept that GRSs are superior to individual variants in identifying those subgroups at a higher risk of narcolepsy that might require earlier intervention and monitoring.

Our study encountered several limitations. First, larger sample sizes will be needed to identify additional narcoleptic genetic factors that remain unknown. As a second limitation, GRSs were validated only in the Chinese population, although narcolepsy-associated loci are deemed similar across ethnicities, informative SNPs for these loci

could vary across ethnicities. In future studies, we will focus on increasing sample sizes to identify additional genetic factors of narcolepsy and on validating GRSs across populations. Moreover, non-genetic factors may contribute to the onset of narcolepsy, and we will thus also explore gene-environment interactions in future studies.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Informed consent (in accordance with governing institutions) was obtained from all subjects. The research protocols were approved by the Institutional Review Board on Medical Human Subjects Research at the Peking University People's Hospital.

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Supplementary

Table S1 The association of an individual SNP with the risk of narcolepsy

Gene	SNP	Allele	Control, N (%)	Case, N (%)	P	P*
<i>TNFSF4</i>	rs4090391	CC	1,672 (84.7)	749 (83.5)	0.303	0.999
		CT	295 (15.0)	142 (15.8)		
		TT	6 (0.3)	6 (0.7)		
		C	3,639 (92.2)	1,640 (91.4)		
		T	307 (7.8)	154 (8.6)		
<i>MIR-552</i>	rs12026171	AA	1,748 (88.4)	797 (88.4)	0.991	0.999
		AG	223 (11.3)	102 (11.4)		
		GG	6 (0.3)	3 (0.3)		
		A	3,719 (94.1)	1,696 (94.0)		
		G	235 (5.9)	108 (6.0)		
<i>CCR1/CCR3</i>	rs3181077	CC	1,572 (79.5)	741 (82.1)	0.249	0.999
		CT	377 (19.1)	152 (16.8)		
		TT	29 (1.5)	10 (1.1)		
		C	3,521 (89.0)	1,634 (90.5)		
		T	435 (11.0)	172 (9.5)		
<i>BTBD9</i>	rs3923809	AA	708 (36.0)	324 (35.9)	0.384	0.999
		AG	946 (48.0)	416 (46.1)		
		GG	315 (16.0)	162 (18.0)		
		A	2,362 (60.0)	1,064 (59.0)		
		G	1,576 (40.0)	740 (41.0)		
<i>UBXN2B</i>	rs6993992	TT	559 (28.3)	253 (28.1)	0.996	0.999
		TC	984 (49.8)	448 (49.8)		
		CC	433 (21.9)	198 (22.0)		
		T	2,102 (53.2)	954 (53.1)		
		C	1,850 (46.8)	844 (46.9)		
<i>A1CF</i>	rs16911668	AA	1,532 (77.4)	721 (79.8)	0.096	0.999
		AG	411 (20.8)	174 (19.3)		
		GG	36 (1.8)	8 (0.9)		
		A	3,475 (87.8)	1,616 (89.5)		
		G	483 (12.2)	190 (10.5)		
<i>TEAD4</i>	rs12322530	GG	1,665 (84.3)	736 (81.9)	0.247	0.999
		GA	302 (15.3)	157 (17.5)		
		AA	9 (0.5)	6 (0.7)		
		G	3,632 (91.9)	1,629 (90.6)		
		A	320 (8.1)	169 (9.4)		
<i>CTSH</i>	rs12148472	TT	1,804 (91.1)	805 (89.3)	0.342	0.999
		TC	173 (8.7)	94 (10.4)		
		CC	4 (0.2)	2 (0.2)		
		T	3,781 (95.4)	1,704 (94.6)		
		C	181 (4.6)	98 (5.4)		
	rs3825932	TT	1,562 (79.0)	727 (80.6)	0.343	0.999
		TC	393 (19.9)	169 (18.7)		
		CC	23 (1.2)	6 (0.7)		
		T	3,517 (88.9)	1,623 (90.0)		
		C	439 (11.1)	181 (10.0)		
<i>NFATC2</i>	rs8119787	AA	789 (39.9)	343 (38.0)	0.487	0.999
		AG	916 (46.3)	440 (48.7)		
		GG	272 (13.8)	120 (13.3)		
		A	2,494 (63.1)	1,126 (62.3)		
		G	1,460 (36.9)	680 (37.7)		
<i>CPT1B-CHKB</i>	rs5770911	CC	1,165 (59.0)	535 (59.2)	0.989	0.999
		CT	692 (35.0)	314 (34.8)		
		TT	119 (6.0)	54 (6.0)		
		C	3,022 (76.5)	1,384 (76.6)		
		T	930 (23.5)	422 (23.4)		
<i>DHS</i>	rs213026	CC	727 (36.7)	343 (38.0)	0.469	0.999
		CT	942 (47.6)	408 (45.2)		
		TT	311 (15.7)	152 (16.8)		
		C	2,396 (60.5)	1,094 (60.6)		
		T	1,564 (39.5)	712 (39.4)		
	rs9444828	CC	540 (27.3)	244 (27.1)	0.932	0.999
		CT	988 (50.0)	447 (49.6)		
		TT	448 (22.7)	210 (23.3)		
		C	2,068 (52.3)	935 (51.9)		
		T	1,884 (47.7)	867 (48.1)		
	rs473267	CC	870 (44.0)	401 (44.5)	0.482	0.999
		CT	882 (44.6)	385 (42.7)		
		TT	226 (11.4)	115 (12.8)		
		C	2,622 (66.3)	1,187 (65.9)		
		T	1,334 (33.7)	615 (34.1)		
	rs7122887	CC	811 (41.0)	378 (41.9)	0.878	0.999
		CT	909 (46.0)	412 (45.6)		
		TT	258 (13.0)	113 (12.5)		
		C	2,531 (64.0)	1,168 (64.7)		
		T	1,425 (36.0)	638 (35.3)		
rs524513	CC	1,679 (84.9)	750 (83.1)	0.123	0.999	
	CT	285 (14.4)	139 (15.4)			
	TT	14 (0.7)	13 (1.4)			
	C	3,643 (92.1)	1,639 (90.9)			
	T	313 (7.9)	165 (9.1)			
rs169160	TT	795 (40.3)	372 (41.4)	0.723	0.999	
	TC	908 (46.0)	412 (45.9)			
	CC	270 (13.7)	114 (12.7)			
	T	2,498 (63.3)	1,156 (64.4)			
	C	1,448 (36.7)	640 (35.6)			

*, after Bonferroni correction. SNP, single-nucleotide polymorphism.