RESEARCH ARTICLE

Differences in clinical and genetic characteristics between early- and late-onset narcolepsy in a Han Chinese cohort

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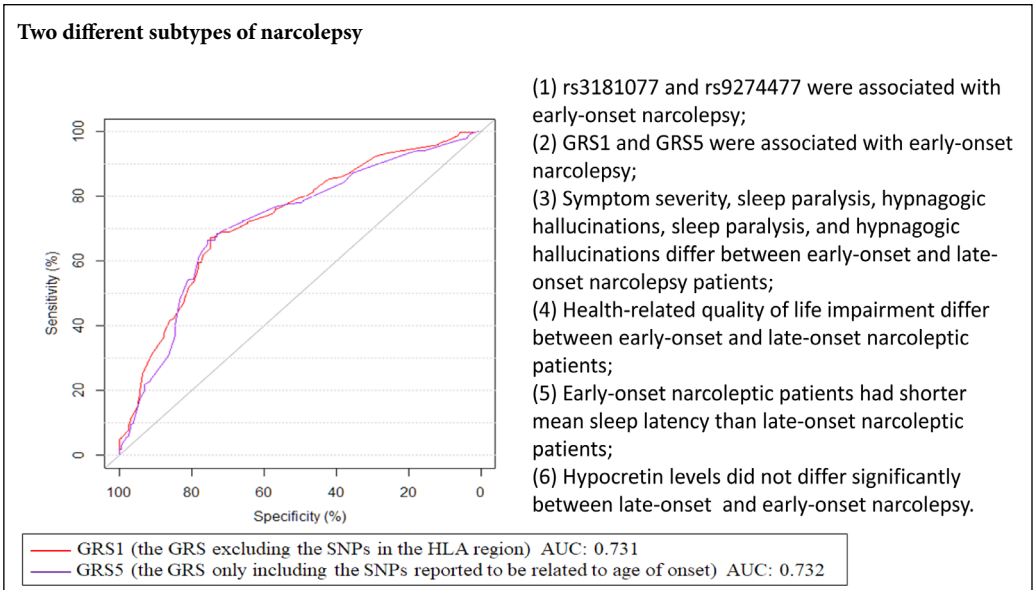
Graphical Abstract



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Abstract

Early- and late-onset narcolepsy constitutes two distinct diagnostic subgroups. However, it is not clear whether symptomology and genetic risk factors differ between early- and late-onset narcoleptics. This study compared clinical data and single-nucleotide polymorphisms (SNPs) between early- and late-onset patients in a large cohort of 899 Han Chinese narcolepsy patients. Blood, cerebrospinal fluid, and clinical data were prospectively collected from patients, and patients were genotyped for 40 previously reported narcolepsy risk-conferring SNPs. Genetic risk scores (GRSs), associations of five different sets of SNPs (GRS1–GRS5) with early- and late-onset narcolepsy, were evaluated using logistic regression and receiver operating characteristic curves. Mean sleep latency was significantly shorter in early-onset cases than in late-onset cases. Symptom severity was greater among late-onset patients, with higher rates of sleep paralysis, hypnagogic hallucinations, health-related quality of life impairment, and concurrent presentation with four or more symptoms. Hypocretin levels did not differ significantly between early- and late-onset cases. Only rs3181077 (*CCR1/CCR3*) and rs9274477 (*HLA-DQB1*) were more prevalent among early-onset cases. Only GRS1 (26 SNPs; OR = 1.513, 95% CI: 0.893–2.585; $P < 0.05$) and GRS5 (6 SNPs; OR = 1.893, 95% CI: 1.204–2.993; $P < 0.05$) were associated with early-onset narcolepsy, with areas under the receiver operating characteristic curves of 0.731 and 0.732, respectively. Neither GRS1 nor GRS5 included SNPs in HLA regions. Our results indicate that symptomology and genetic risk factors differ between early- and late-onset narcolepsy. This protocol was approved by the Institutional Review Board (IRB) Panels on Medical Human Subjects at Peking University People's Hospital, China (approval No. Yuanlunshenlinyi 86) in October 2011.

Key Words: *case-control studies; clinical features; genetic association studies; genetic load; genetic loci; genetic phenomena; hypothalamic diseases; precision medicine; risk assessment; single nucleotide polymorphism*

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Introduction

Narcolepsy is a chronic neurological disorder that is caused by hypothalamic degeneration or damage. It is characterized by excessive daytime sleepiness, cataplexy, sleep paralysis, and peri-sleep hallucinations (Akintomide and Rickards, 2011, 2014). Although patients younger than 10 years of age have been diagnosed with narcolepsy, patients are most often diagnosed between the ages of 15 and 30 years (Akintomide and Rickards, 2011; Leschziner, 2014); however, epidemiological studies show that two distinct peaks of narcolepsy incidence occur at approximately 15 and 35 years of age, thus giving rise to early- and late-onset diagnostic categories (Dauvilliers et al., 2001b, 2007; Ohayon et al., 2002; Silber et al., 2002; Longstreth et al., 2009; Thorpy and Krieger, 2014; Wu et al., 2014). However, the mean ages of patients in the early- and late-onset groups exhibit substantial variation between different study populations and locations, with differences between the mean early and late ages of 10 to 27 years (Dauvilliers et al., 2001b; Han et al., 2011a; Erdem et al., 2012; Dong et al., 2013; Wu et al., 2014). This suggests that multiple factors contribute to narcolepsy age at onset.

Some investigations using the multiple sleep latency test for narcolepsy diagnosis showed that early-onset narcolepsy patients have shorter mean sleep latency (Dauvilliers et al., 2004; Nevsimalova et al., 2009) and fewer sleep-onset rapid eye movement periods, compared with late-onset cases (Nevsimalova et al., 2009), but these findings are not replicated in all polysomnography studies of narcolepsy (Challamel et al., 1994). Studies in Europe and North America have reported a family history of narcolepsy in significantly more early-onset narcolepsy patients than in late-onset patients (Dauvilliers et al., 2001b; Nevsimalova et al., 2009; Fox et al., 2016). However, studies of childhood narcolepsy in China have reported negligible rates of narcolepsy family history (Han et al., 2001, 2011b). These findings suggest that genetic contributions to age of narcolepsy onset might vary between different populations.

Genome-wide association studies are widely used for identifying genetic variants associated with disease risk. Multiple genome-wide association studies of different populations have identified a variety of single-nucleotide polymorphisms (SNPs) that are associated with narcolepsy age of onset (Dauvilliers et al., 2001a; Dong et al., 2013; Han et al., 2013a; Tafti et al., 2014; Fox et al., 2016), and various statistical methods have been used to assess the combined effect of multiple genetic variants on the probability of diagnosis, treatment response, and outcome (Spiliopoulou et al., 2015). In the analysis of genome-wide association study data for determining disease risk, the use of a genetic risk score (GRS) to represent a combined genetic contribution to disease risk (Dudbridge, 2013; Bailey and Igo, 2016) has indicated that greater genetic burden contributes to younger age at onset of diseases with well-characterized underlying genetic components (de Miguel-Yanes et al., 2011; Vecoli et al., 2014; Theriault et al., 2018).

However, previous narcolepsy studies have had the following limitations. (1) The underlying basis of differences in age at onset is unclear. (2) Whether these differences are more acute in younger patients has not been determined (Stores, 2006; Stores et al., 2006). (3) Whether the severity of clinical narcolepsy symptoms differs between early- and late-onset cases has not been thoroughly investigated. (4) No comprehensive analysis of SNPs has been performed to evaluate their combined contributions to the age of narcolepsy onset.

To overcome the shortcomings of previous studies, we performed this study to: (1) compare differences in clinical features and genetic load between early-onset and late-onset narcolepsy; (2) construct several GRSs, and to test the ability of these GRSs to discriminate early- *versus* late-onset narcolepsy patients.

We screened 40 SNPs previously shown to be associated with narcolepsy risk in a large cohort of Han Chinese narcolepsy patients, and used a GRS-based analysis to determine the differences between their combined genetic contributions to the risk of early-onset and late-onset narcolepsy. We believe our results will help identify early-onset narcolepsy more effectively, so that measures to prevent the progression of hypothalamic degeneration and damage can be taken as soon as possible.

Materials and Methods

Study population

We recruited 1062 unrelated narcolepsy patients treated at the Sleep Laboratory of the Peking University People's Hospital, China and a case-only study was performed. The Sleep Laboratory received patients from all regions of China. All participants completed a structured questionnaire to record demographic variables and medical history. Participants were then interviewed to ensure that the questionnaires were completed according to the given instructions.

All participants (n = 1062) underwent physical examination, venous blood collection and polysomnography, which consisted of the standard multiple sleep latency test (Carskadon et al., 1986). Demographic variables included sex, age, and ethnicity, and the clinical variables included body-mass index (BMI), diagnosis delay, age at onset, pre-onset inflammation, cataplexy, sleep paralysis, hypnagogic hallucinations, family history of narcolepsy, health-related quality of life impairment, cerebrospinal fluid level of hypocretin, mean sleep latency, and rapid eye movement latency. Written informed consent was obtained from all patients prior to participation in the study and the study was conducted before the start of standard treatment.

The research protocols were approved by the Institutional Review Board (IRB) Panels on Medical Human Subjects at Peking University People's Hospital, China (approval No. Yuanlunshenlinyi 86) in October 2011. Our protocols were performed in accordance with the *Declaration of Helsinki* regarding ethical guidelines for medical research involving human subjects, as described in our previous studies (Han et al., 2013b; Ouyang et al., 2019).

Inclusion criteria included: (a) mean sleep latency < 8 minutes and ≥ 2 sleep-onset rapid eye movement periods, as determined by the multiple sleep latency test (Han et al., 2011a, 2013a), and (b) had either (i) cerebrospinal fluid hypocretin 1 ≤ 110 pg/mL or (ii) clear-cut cataplexy and HLA-DQB1*06:02. (c) Clinical data included (i) frequency of excessive daytime sleepiness, cataplexy, sleep paralysis,

hypnagogic hallucinations, and disturbed nocturnal sleep; (ii) age at disease onset, which was defined as the age at which narcolepsy symptoms first occurred; and (iii) the mean sleep latency interval according to the multiple sleep latency test. Exclusion criteria included (a) genetic relationships with other participants; (b) noncooperation with the study.

Genotyping and quality control

SNP genotyping and quality control were performed as described previously (Han et al., 2013b). Blood (< 10 mL) was collected from an arm vein and whole blood was collected for further analysis. Samples were genotyped and analyzed using the Affymetrix Axiom CHB array and the Affymetrix Genotyping Console (Affymetrix, Santa Clara, CA, USA). All samples achieved a call rate of > 99%. Conclusive genotyping data were obtained for all participants.

SNP screening

We genotyped 40 SNPs in our study population that were previously shown to be associated with narcolepsy in genome-wide association studies of North American, European, and Asian populations (Douglas, 1998; Miyagawa et al., 2008; Hor et al., 2010; Shimada et al., 2010; Kornum et al., 2011; Han et al., 2012, 2013b; Faraco et al., 2013; Tafti et al., 2014; Holm et al., 2015; Toyoda et al., 2015). Useful linkage disequilibrium was confirmed for each final candidate SNP by a pairwise $r^2 < 0.25$ in the HapMap Chinese/Japanese combined reference data (CHB/JPT) using Haploview (version 4.2, Broad Institute, USA) (Johnson et al., 2008). SNPs with a call rate < 99% and those that violated the Hardy-Weinberg equilibrium test were excluded from further analysis. For variants that were present in the study population, but were not included the Affymetrix Axiom CHB array, a linkage disequilibrium analysis was performed with a 10–20 kb window using the Ensembl linkage disequilibrium data for the Chinese population in the 1000 Genomes Browser to identify proxy loci ($r^2 \geq 0.8$). The selected SNPs ($n = 32$) are presented in **Additional Table 1**.

Statistical analysis

Statistical analyses were performed using R software (version 3.5.0; R Development Core Team, University of Auckland, New Zealand). The linkage disequilibrium of selected SNPs was evaluated using Haploview (version 4.2; Broad Institute, Cambridge, MA, USA). Early-onset and late-onset were defined as first experiencing narcolepsy symptoms at an age of ≤ 15 years or > 15 years, respectively, which was consistent with the bimodal distribution of narcolepsy age at onset previously reported in China (Dauvilliers et al., 2007). Categorical variables are presented as the number and percentage of patients, and continuous variables not conforming to normal distribution are presented as the mean and range. A chi-squared test was used to assess Hardy-Weinberg equilibrium and to determine differences in genotype frequencies between early- and late-onset narcolepsy patients. A chi-squared test or Wilcoxon rank-sum test was used to compare values of quantitative traits between groups for data with or without normal distributions, respectively. Statistical significance was set to a two-sided $P < 0.05$ for the clinical variables. For differences in genotype frequencies, significance was set at $P < 0.05$ after Bonferroni correction. The risk of narcolepsy associated with different combinations of risk-conferring SNPs was evaluated based on the GRS of various SNP sets. A GRS was defined as the total number of risk-conferring SNPs in a person.

To evaluate the combined genetic contribution of various SNPs to the risk of early-onset narcolepsy based on GRS, the selected SNPs were grouped into GRS sets 1 to 5. These groupings were based on the results of SNP screening. This study used unweighted GRSs, which did not consider differences in the strength of associations between different SNPs and narcolepsy. Association of GRSs with early- and late-onset narcolepsy was evaluated using logistic regression models. Treating the GRSs as continuous variables, according to the distribution of scores in the population, the risk tertiles were stratified as low, intermediate, and high-risk categories. The models were adjusted for potential confounders, which included sex, ethnicity, pre-onset inflammation, and BMI. Crude and adjusted odds ratios (ORs), 95% confidence intervals (CIs), and P-values were calculated for comparisons of risk between early- and late-onset narcolepsy patients and between the different risk categories within each GRS set. The low-risk category was utilized as the control. A value of $P < 0.05$ was considered statistically significant. A receiver operating characteristic (ROC) curve analysis was used to confirm the discriminative capability of the logistic regression models based on calculations of the area under the ROC curve, sensitivity, and specificity.

Results

Demographic and clinical characteristics

The test flow chart is presented in **Figure 1**. The demographic and clinical data are presented in **Additional Table 2**. The study population included 899 narcolepsy patients. Among these, 856 (95.2%) were of Han Chinese ethnicity, 284 (31.6%) were women, and 615 (68.4%) were men. The mean age in the early- and late-onset groups were 8 and 22 years, respectively. The rate of cataplexy sleep paralysis, cerebrospinal fluid hypocretin levels, gender, and family history of narcolepsy did not differ significantly between the early- and late-onset cases ($P > 0.05$ for all), whereas BMI ($P < 0.001$), pre-onset inflammation ($P = 0.004$), sleep paralysis ($P < 0.001$), hypnagogic hallucinations ($P < 0.001$), health-related quality of life impairment ($P = 0.011$), mean sleep latency ($P < 0.001$), and rapid eye movement latency ($P < 0.001$) were significantly different between late- and early-onset cases.

Differences in SNP frequency based on age at onset

In the initial analysis, the frequencies of nine of the selected SNPs differed significantly between early- and late-onset narcoleptic patients, with P values ranging from < 0.001 to 0.043 (**Additional Table 3**). These included rs3181077 in *CCR1/CCR3*; rs6993992 in *UBXN2B*; rs1154153 and rs1263647 in *TRA*; rs12148472 in *CTSH*; rs473267 in *PGM3*; and rs9274477, rs9271117, and rs2859090 in *HLA-DQB1*. However, after Bonferroni correction, only rs3181077 and rs9274477 remained significantly different between early- and late-onset cases.

Combined effects of multiple SNPs on the age of narcolepsy onset

The combined contribution of five different groups of SNPs to age of narcolepsy onset was evaluated using logistic regression models and ROC analysis. The GRS1 set included all of the selected SNPs, except those in the HLA regions (a total of 26 SNPs). The GRS2 set contained only the selected SNPs in the HLA regions, which included rs9274477, rs9271117, rs2859090, rs3117221, rs2517455, and rs3129932. The GRS3 set included all of the 32 selected SNPs. The GRS4 set contained only the selected SNPs that were previously reported in Chinese populations, which included rs2859998, rs12425451, rs1154155, rs5770917, rs9274477, and rs3129907 (Han et al., 2012, 2013b). The GRS5 set contained only non-HLA SNPs with frequencies that differed significantly between early- and late-onset narcoleptics according to our initial analysis (without Bonferroni correction; **Additional Table 3**), which included rs3181077, rs6993992, rs1154153, rs1263647, rs12148472, and rs473267.

The logistic regression analysis results are presented in **Additional Table 4**. Analysis of early-onset narcolepsy risk, clearly showed that crude OR progressively increased with increasing genetic load across the three early-onset risk categories for GRS1, GRS2, and GRS3, with the lowest crude ORs in the low-risk categories and the highest crude ORs in the high-risk categories of these three GRS sets. However, while the overall, intermediate-risk, and high-risk crude ORs for GRS1 were significant for early-onset narcolepsy (P = 0.003, P = 0.013, and P = 0.006, respectively), only the overall crude OR for early-onset narcolepsy was significant for GRS5 (P = 0.029), and none of the crude ORs for early-onset narcolepsy were significant for GRS3 (P > 0.05 for all). After adjustment for potential confounders, the overall, intermediate-risk, and high-risk ORs for early-onset narcolepsy remained significant for GRS1 (P = 0.002, P = 0.007, and P = 0.006, respectively). Both the overall and high-risk ORs for early-onset narcolepsy were significant for GRS5 after adjustment (P = 0.010 and P = 0.038, respectively). None of the adjusted ORs for early-onset narcolepsy were significant for GRS2, GRS3, or GRS4 (P > 0.05 for all).

Discriminative capability of GRS for determining risk of early-onset narcolepsy

A ROC analysis was performed to confirm the discriminative capability of the GRS1 and GRS5 logistic regression models. As shown in **Figure 2**, the areas under the ROC curve of GRS1 and GRS5 for predicting early-onset narcolepsy were 0.731 and 0.732, respectively. Using a cut-off value of 0.902, ROC analysis demonstrated a sensitivity of 78.4% and a specificity of 65.0% for the prediction of early-onset narcolepsy patients.

Discussion

In this study, the genotypes and clinical variables of a large sample of Chinese narcolepsy patients were examined. In addition, GRSs based on different combinations of selected risk-conferring SNPs were investigated to determine if they could be useful for predicting the risk of early-onset narcolepsy. Our results demonstrated that both the clinical features of narcolepsy and the genetic load of risk-conferring SNPs can differ between early-onset and late-onset cases, as evidenced by significant differences in certain clinical variables between early- and late-onset cases, including sleep paralysis, hypnagogic hallucinations, total number of symptoms, mean sleep latency, and rapid eye movement latency, as well as significant differences in the genotype frequencies for rs3181077 (*CCR1/CCR3*) and rs9274477 (*HLA-DQB1*). Our analysis of the clinical variables also showed that early-onset narcolepsy patients typically exhibit less severe symptoms and less health-related quality of life impairment than late-onset patients. Furthermore, our logistic regression and ROC analyses of the contribution of different groups of SNPs to narcolepsy risk based on GRS showed that GRS1 (26 SNPs) and GRS5 (6 SNPs) were significantly associated with the risk of early-onset narcolepsy, and both demonstrated significant discriminative capacity for identifying patients at high risk of early-onset narcolepsy.

Although few comparisons have been made between early- and late-onset narcoleptics, early studies in North America and Europe suggested that clinical symptoms might be more severe in pediatric narcolepsy cases (Young et al., 1988; Challamel et al., 1994). Sleep paralysis, hypnagogic hallucinations, and presentation with four or more narcolepsy symptoms were, however, significantly more prevalent among our late-onset cases, indicating that clinical manifestations were more severe in older Chinese narcoleptics. In two European studies, Nevsimalova et al. (2009) reported no significant differences in sleep paralysis and hypnagogic hallucinations based on age at onset, while Dauvilliers et al. (2004) reported that sleep paralysis occurred significantly more often among patients aged 25 to 65 years, whereas hypnagogic hallucinations did not differ significantly based on age at onset. The differences in these findings could be due to differences in genetic factors or environmental influences between European and Chinese populations. The size of our sample (n = 899) might also have contributed to such differences, because the sample sizes of the two European studies were much smaller (n = 26 and 58, for Dauvilliers et al., 2004 and Nevsimalova et al., 2009 respectively). A difference in diagnostic delay between Europe and China could also have influenced these differences, but we know of no study that provides evidence to support this.

Our stratified analysis of the multiple sleep latency test results showed that Chinese patients with early-onset narcolepsy had significantly shorter mean sleep latency than their late-onset narcolepsy counterparts. This was consistent with the findings of European studies, which found that mean sleep latency progressively increased with increasing age (Dauvilliers et al., 2004; Nevsimalova et al., 2009). Nevsimalova et al. (2009) also reported that BMI increased with increasing age-at-onset, a trend that we also observed in our Chinese patients. A family history of narcolepsy did not differ significantly between our early-onset and late-onset cases, which is consistent with the findings of a previous study of pediatric narcolepsy in China (Han et al., 2001), despite the potential for familial patterns in narcolepsy risk-conferring genotypes (Guilleminault et al., 1989). The highest level of health-related quality of life impairment was significantly

more prevalent among our late-onset patients. Although a number of studies have investigated the impact of narcolepsy on health-related quality of life (Goswami, 1998; Daniels et al., 2001; Dodel et al., 2007; Rocca et al., 2016), we know of no other study that has compared health-related quality of life between early- and late-onset narcoleptics. However, the more severe clinical symptoms among our late-onset patients possibly contributed to greater impairment of their health-related quality of life.

In this study, pre-onset inflammation was significantly more common among early-onset cases. This finding indicates a role for inflammation as a trigger event for narcolepsy in young people. Studies in both China and Europe found that the incidence of narcolepsy increased following the H1N1 influenza pandemic in 2009 (Han et al., 2011a; Dauvilliers et al., 2013; Heier et al., 2013; Szakács et al., 2013). In Europe, an adverse effect of the H1N1 vaccine, Pandemrix, was suggested (Szakács et al., 2013; Sturkenboom, 2015), whereas, in China, it was thought to have occurred as a result of the high density of people infected with wild-type H1N1 in Beijing during the 2009 pandemic (Ahmed et al., 2014). In a genome-wide association study that investigated the effects of the 2009 H1N1 pandemic, the HLA variant, DQB1*03:01 (rs7744020), was associated with early onset following the pandemic, but this association ceased approximately 2 years afterward (Han et al., 2013a). Given that our study was conducted more than 2 years following the 2009 pandemic, our results indicate that sources of inflammation other than H1N1 infection may also trigger the types of autoimmune events that have long been thought to contribute to narcolepsy onset (Black 3rd, 2005; Liguori et al., 2014; Partinen et al., 2014).

In our investigation of genetic risk factors for early- and late-onset narcolepsy, we genotyped 32 SNPs in our narcolepsy cohort that were previously shown to be associated with narcolepsy in North American, European, and Asian populations (Douglas, 1998; Miyagawa et al., 2008; Hor et al., 2010; Shimada et al., 2010; Kornum et al., 2011; Han et al., 2012, 2013b; Faraco et al., 2013; Tafti et al., 2014; Holm et al., 2015; Toyoda et al., 2015). The frequencies of nine of these SNPs were significantly different based on age at onset, but only the frequencies of rs3181077 and rs9274477 remained significantly different after Bonferroni correction. Five GRSs were then constructed to represent the combined genetic contribution of various sets of risk-conferring SNPs, and narcolepsy risk was evaluated using logistic regression models. GRS1 and GRS5 were significantly associated with early-onset narcolepsy. Based on the area under ROC curves, both GRS1 and GRS5 demonstrated good discriminatory capacity for predicting early-onset narcolepsy. The GRS groups that included SNPs of the HLA region (GRS2, GRS3, and GRS4) were not associated with early-onset narcolepsy in our Chinese cohort. These results suggest that non-HLA genetic variants might play more important roles in early-onset narcolepsy than in late-onset narcolepsy. Moreover, our results indicate that the genetic contributions of SNPs to narcolepsy likely differ between early-onset and late-onset cases. It is generally believed that narcolepsy is caused by damage to hypothalamic neurons induced by the immune response

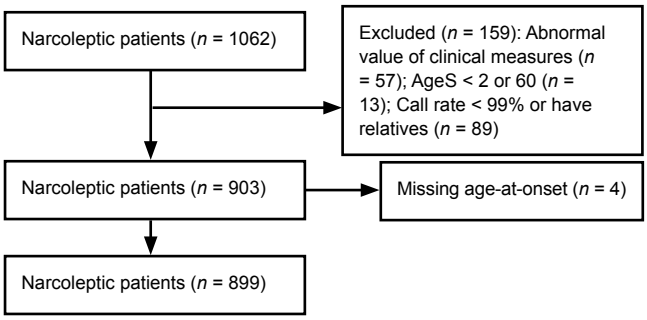

Figure 1 Test flow chart.

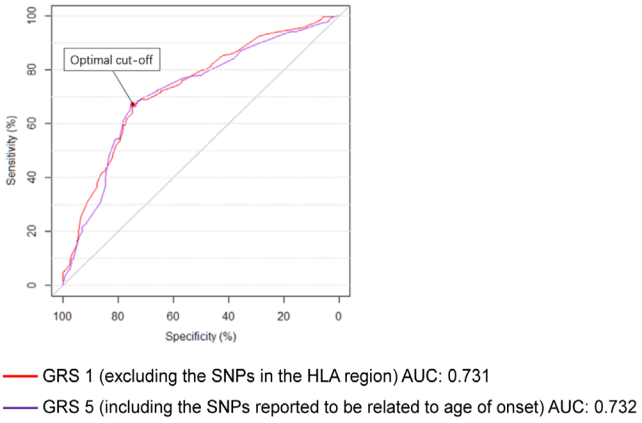

Figure 2 ROC curves of GRSs predicting age-at-onset.
Red line: GRS1, the GRS excluding the SNPs in the HLA region, AUC = 0.731. Blue line: GRS5, the GRS including the SNPs reported to be related to age of onset, AUC = 0.732. AUC: Area under the receiver operating characteristic curve; GRS: genetic risk score; ROC: receiver operating characteristic.

to pathogens (such as H1N1 virus). However, different immune responses to pathogens and different levels of immune activation may all be associated with genetic polymorphisms in different individuals. Therefore, patients with higher genetic risk scores carry more narcoleptic-associated genes, and they are more likely to develop narcolepsy at an earlier age in response to environmental factors (such as virus infection). In addition, patients with higher genetic risk scores carry more SNPs associated with early-onset. The immune system response to pathogens and the type of virus to which people are susceptible differ between young people and adults. The more early-onset-associated SNPs an individual carries, the more likely they are to be infected by pathogens that young people are susceptible to, which induces a specific immune response and leads to damage of hypothalamic neurons. GRSs have been employed previously to increase statistical power when investigating the association with disease-related phenotypes. Our results justify the use of GRSs to increase statistical power.

Our findings have certain limitations. Our data were collected at a single study center, which cannot represent a nationwide cohort. However, our institution receives patients from all regions of China, so our cases can reflect the characteristics of narcolepsy patients throughout China to some degree. During the 10-year study period (2008–2018), much has been learned regarding the effects of inflammation on

narcolepsy risk (Partinen et al., 2014; Sturkenboom, 2015). In our analysis, pre-onset inflammation consisted of a combined index of self-reported histories of various inflammatory conditions. Future studies would benefit from a more detailed analysis stratified by the types of immune-related conditions, such as influenza virus infection, inflammatory disorders, and autoimmunity, which might provide further insight into the specific types of immune processes that influence narcolepsy age at onset. In addition, inflammatory factors should be examined. An analysis of the impact of environmental factors on age at narcolepsy diagnosis might provide further insight into the biological mechanisms that influence the development of clinical symptoms. Furthermore, only Chinese narcoleptic patients were included in the study; the associations of GRS1 and GRS5 with younger age at onset in Chinese patients might differ from those in patients of other ethnicities. Similar large-scale studies in other populations are required to explore whether the genetic contributions to age of narcolepsy onset differ based on ethnicity or race. When evaluating the contributions of different sets of SNPs to age of narcolepsy onset, only when the difference of the minimum allele frequency between the two groups was > 0.12, would the statistical power be > 0.8. This can affect the extrapolation of the conclusion to some degree, and we need to enlarge the sample size to improve the statistical power in our future work. Fortunately, the statistical power of GRS was satisfactory.

In summary, this study compared clinical features between early- and late-onset narcolepsy patients in a large Chinese cohort. While early-onset cases had significantly shorter mean sleep latency and rapid eye movement latency and a significantly greater rate of pre-onset inflammation, the symptoms of narcolepsy were more severe in late-onset cases, as evidenced by significantly higher rates of sleep paralysis, hypnagogic hallucinations, health-related quality of life impairment, and presentation with four or more symptoms concurrently in patients who were diagnosed with narcolepsy as adults. The present study also compared the frequencies of previously reported risk-conferring SNPs between early- and late-onset narcolepsy groups, and the results found that only two of these SNPs, rs3181077 and rs9274477, were significantly associated with age at onset. Using GRS to represent the genetic contributions of various sets of SNPs, we found that one set of 26 of SNPs (GRS1) and another set consisting of six SNPs were significantly associated with early-onset narcolepsy. These results suggest that symptomology and genetic risk factors differ between early- and late-onset narcolepsies, which are two narcolepsy subtypes with different pathogenesis and that may have different mechanisms of hypothalamic neuron degeneration. Large-scale studies in other populations and brain biopsies of narcoleptic patients are required to confirm our findings.

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Declaration of participant consent: *The authors certify that they have obtained all appropriate participant consent forms. In the form the participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity.*

Reporting statement: *This study followed the STrengthening the Reporting of OBservational Studies in Epidemiology (STROBE) statement.*

Biostatistics statement: *The statistical methods of this study were reviewed by the biostatistician of Peking University People's Hospital, Beijing, China.*

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Data sharing statement: *Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices) will be in particular shared. Study protocol form will be available. The data will be available immediately following publication without end date. Anonymized trial data will be available indefinitely at www.figshare.com.*

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Peer review: *Externally peer reviewed.*

Open peer reviewer: *Xin Li, Guangdong General Hospital, China.*

Additional files:

Additional file 1: *Open peer review report 1.*

Additional Table 1: *Selected narcolepsy-risk-conferring SNPs.*

Additional Table 2: *Demographic and clinical variables of the narcolepsy patients.*

Additional Table 3: *Association of individual SNP with age-at-onset of narcolepsy.*

Additional Table 4: *Association of GRS with the risk of early-onset narcolepsy.*

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Additional Table 1 Selected narcolepsy-risk-conferring SNPs

Chromosome	Gene	SNP	Tag SNP	r^2	LD	Allele	Risk allele	Missing haplotype (%)	MAF	HWE *P*-value
1	TNFSF4	rs7553711	rs4090391	1	1	C/T	T	0.5	0.08	0.057
1	MIR-552	rs10915020	rs12026171	0.928	1	A/G	G	0.2	0.06	0.681
3	CCR1/CCR3	rs3181077	–	–	–	C/T	C	0.1	0.11	0.246
4	BTBD9	rs3923809	–	–	–	A/G	G	0.5	0.4	0.999
7	TRB	rs2854536*	–	–	–	T/C	T	0.3	0.24	0.48
8	UBXN2B	rs2859998†	rs6993992	1	1	T/C	T	0.3	0.47	0.999
10	ZNF365	rs10995245*	–	–	–	G/A	A	0.2	0.31	0.507
10	A1CF	rs4290173	rs16911668	0.956	1	A/G	A	0.1	0.12	0.172
12	TEAD4	rs12425451†	rs12322530	0.947	1	G/A	G	0.3	0.09	0.283
14	TRA	rs1154155*†	–	–	–	T/G	T	0.8	0.45	0.682
14		rs12587781	rs1154153	1	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	CTSH	rs12148472	–	–	–	T/C	C	0.1	0.05	1
15		rs3825932	–	–	–	T/C	T	0.1	0.11	0.745
19	DNMT1	rs2290684	rs2114724	1	1	C/T	T	0	0.29	0.406
19		rs6511570	rs7253062	1	1	G/A	G	0.2	0.25	0.999
20	NFATC2	rs8119787	–	–	–	A/G	G	0.1	0.37	0.841
21	IL10RB-IFNAR1	rs2834118*	rs2834113	0.934	0.966	T/G	G	0.6	0.16	0.176
21		rs2252931	rs2834188	1	1	A/G	A	0.3	0.28	0.625
22	CPT1B-CHKB	rs5770917†	rs5770911	1	1	C/T	C	0.2	0.23	0.237
1	DHS	rs213038	rs213026	0.961	1	C/T	T	0	0.39	0.847
6		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	HLA- DQB1	rs9274477*†	–	–	–	A/G	A	0.1	0.3	0.243
6		rs9271117	–	–	–	T/C	C	0.42%	0.34	0.899
6		rs2858884	rs2859090	1	1	G/A	G	0.10%	0.06	0.209
6		rs3117242	rs3117221	1	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	1	1	C/G	G	0.76%	0.14	0.093

*SNPs previously reported in the Chinese population. †SNPs previously reported to be associated with age at onset. HWE: Hardy-Weinberg equilibrium; LD: linkage disequilibrium; SNP: single-nucleotide polymorphism.

Additional Table 2 Demographic and clinical variables of the narcolepsy patients

Variables	Early-onset (n = 773)	Late-onset (n = 126)	P-value
Men	522 (67.5)	93 (73.8)	0.160
Han ethnicity	737 (95.3)	119 (94.4)	0.661
Diagnosis delay (yr)	1 (0–7)	6 (2–12)	< 0.001
Age at onset (yr)	8 (6–11)	22 (17–30)	< 0.001
Pre-onset inflammation	39 (5.0)	0 (0)	0.004
Cataplexy	773 (100)	126 (100)	0.999
Sleep paralysis	228 (30.3)	103 (81.7)	< 0.001
Hypnagogic hallucinations	430 (55.7)	94 (74.6)	< 0.001
Total symptoms			
2	271 (36.0)	10 (7.9)	< 0.001
3	321 (42.6)	35 (27.8)	
4	161 (21.4)	81 (64.3)	
Family history	15 (1.9)	2 (1.6)	0.999
HRQL impairment			0.011
None	85 (17.1)	14 (14.6)	
Small	213 (42.9)	28 (29.2)	
Great	199 (40.0)	54 (56.3)	
BMI (kg)	21.7 (18.5–25.4)	26.7 (24.0–28.7)	< 0.001
Hypocretin (pg/mL)	20.3 (12.7–30.5)	15.2 (12.7–22.9)	0.082
MSL (min)	2.6 (1.7–3.8)	3.2 (2.1–4.7)	< 0.001
REML (min)	1.6 (1.1–2.5)	2.4 (1.6–4.0)	< 0.001
SOREMP	5 (5–5)	5 (5–5)	0.838

Data are presented as the number (percentage) of patients or the mean (range). Categorical variables are presented as the number and percentage of patients, and continuous variables are presented as the mean and range. A chi-squared test or Wilcoxon rank-sum test was used to compare values of quantitative traits between groups for data with or without normal distributions, respectively. BMI: Body mass index; HRQL: health care related quality of life; MSL: mean sleep latency; REML: rapid eye movement latency; SOREMP: sleep-onset rapid eye movement periods.

Additional Table 3 Association of individual SNP with age-at-onset of narcolepsy

Gene	Full name	SNP	Allele	Early-onset [*n*(%)]	Late-onset [*n*(%)]	*P*-value*
TNFSF4	Tumor necrosis factor superfamily member 4	rs4090391	CC	646 (84.2)	99 (78.6)	0.285
			CT	116 (15.1)	26 (20.6)	
			TT	5 (0.7)	1 (0.8)	
			C	1048 (89.3)	224 (88.9)	0.949
			T	126 (10.7)	28 (11.1)	
MIR-552	Micro RNA-552	rs12026171	AA	683 (88.5)	114 (88.1)	0.628
			AG	87 (11.3)	14 (11.1)	
			GG	2 (0.3)	1 (0.8)	
			A	1453 (94.1)	236 (93.7)	0.889
			G	91 (5.9)	16 (6.3)	
CCR1/CCR3	Chemokine receptor 1/chemokine receptor 3	rs3181077	CC	643 (83.2)	96 (76.2)	0.117
			CT	121 (15.7)	29 (23.0)	
			TT	9 (1.2)	1 (0.8)	
			C	1047 (91.0)	96 (76.8)	< 0.001†
			T	139 (9.0)	29 (23.2)	
BTBD9	BTB domain containing 9	rs3923809	AA	281 (36.4)	42 (33.3)	0.256
			AG	347 (44.9)	66 (52.4)	
			GG	144 (18.7)	18 (14.3)	
			A	909 (58.9)	150 (59.5)	0.900
			G	635 (41.1)	102 (40.5)	
TRB	T cell receptor beta	rs2854536	TT	477 (61.9)	75 (59.5)	0.706
			TC	268 (34.8)	48 (38.1)	
			CC	25 (3.2)	3 (2.4)	
			T	1222 (79.4)	198 (78.6)	0.842
			C	318 (20.6)	54 (21.4)	
UBXN2B	UBX domain protein 2B	rs6993992	TT	157 (20.4)	41 (32.8)	0.008
			TC	391 (50.8)	53 (42.4)	
			CC	222 (28.8)	31 (24.8)	
			T	835 (54.2)	115 (46.0)	0.019
			C	705 (45.8)	135 (54.0)	
ZNF365	Zinc finger protein 365	rs10995245	GG	319 (41.3)	53 (42.1)	0.827
			GA	366 (47.3)	61 (48.4)	
			AA	88 (11.4)	12 (9.5)	
			G	1004 (64.9)	167 (66.3)	0.735
			A	542 (35.1)	85 (33.7)	
A1CF	APOBEC1 complementation factor	rs16911668	AA	620 (80.2)	97 (77.0)	0.531
			AG	147 (19.0)	27 (21.4)	
			GG	6 (0.8)	2 (1.6)	
			A	1387 (89.7)	221 (87.7)	0.392
			G	159 (10.3)	31 (12.3)	
TEAD4	TEA domain transcription factor 4	rs12322530	GG	635 (82.4)	98 (79.0)	0.385
			GA	131 (17.0)	26 (21.0)	
			AA	5 (0.6)	0 (0)	
			G	1401 (90.9)	222 (89.5)	0.578
			A	141 (9.1)	26 (10.5)	
TRA	T cell receptor alpha	rs1154155	TT	278 (36.3)	37 (30.1)	0.134
			TG	410 (53.6)	67 (54.5)	
			GG	77 (10.1)	19 (15.4)	
			T	966 (63.1)	141 (57.3)	0.093
			G	564 (36.9)	105 (42.7)	
		rs1154153	TT	378 (48.9)	50 (39.7)	0.095
			TC	339 (43.9)	62 (49.2)	
			CC	56 (7.2)	14 (11.1)	
			T	1095 (70.8)	162 (64.3)	0.043
			C	451 (29.2)	90 (35.7)	
		rs1263647	AA	274 (35.4)	35 (27.8)	0.009
			AG	403 (52.1)	63 (50.0)	
			GG	96 (12.4)	28 (22.2)	
			A	951 (61.5)	133 (52.8)	0.011
			G	595 (38.5)	119 (47.2)	

Additional Table 3 Continued

Gene	Full name	SNP	Allele	Early-onset [*n*(%)]	Late-onset [*n*(%)]	*P*-value*
CTSH	Cathepsin H	rs12148472	TT	683 (88.5)	119 (95.2)	0.074
			TC	87 (11.3)	6 (4.8)	
			CC	2 (0.3)	0 (0)	
			T	1453 (94.1)	244 (97.6)	0.034
			C	91 (5.9)	6 (2.4)	
		rs3825932	TT	618 (80.1)	107 (84.9)	0.403
			TC	149 (19.3)	18 (14.3)	
			CC	5 (0.6)	1 (0.8)	
			T	1385 (89.7)	232 (92.1)	0.295
			C	159 (10.3)	20 (7.9)	
DNMT1	DNA methyltransferase 1	rs2114724	CC	363 (47.0)	61 (48.4)	0.535
			CT	330 (42.7)	56 (44.4)	
			TT	80 (10.3)	9 (7.1)	
			C	1056 (68.3)	178 (70.6)	0.506
			T	490 (31.7)	74 (29.4)	
		rs7253062	GG	463 (60.0)	74 (59.2)	0.543
			GA	268 (34.7)	47 (37.6)	
			AA	41 (5.3)	4 (3.2)	
			G	1194 (77.3)	195 (78.0)	0.878
			A	350 (22.7)	55 (22.0)	
NFATC2	Nuclear factor of activated T cells 2	rs8119787	AA	289 (37.4)	52 (41.3)	0.705
			AG	381 (49.3)	58 (46.0)	
			GG	103 (13.3)	16 (12.7)	
			A	959 (62.0)	162 (64.3)	0.539
			G	587 (38.0)	90 (35.7)	
IL10RB-IFNAR1	Interleukin 10 receptor, beta -interferon alpha and beta receptor subunit 1	rs2834113	TT	539 (70.3)	86 (68.8)	0.830
			TG	198 (25.8)	35 (28.0)	
			GG	30 (3.9)	4 (3.2)	
			T	1276 (83.2)	207 (82.8)	0.954
			G	258 (16.8)	43 (17.2)	
		rs2834188	AA	431 (55.8)	63 (50.0)	0.291
			AG	294 (38.0)	57 (45.2)	
			GG	48 (6.2)	6 (4.8)	
			A	1156 (74.8)	183 (72.6)	0.516
			G	390 (25.2)	69 (27.4)	
CPT1B-CHKB	Carnitine palmitoyltransferase 1B, choline kinase-β	rs5770911	CC	461 (59.6)	72 (57.1)	0.176
			CT	271 (35.1)	42 (33.3)	
			TT	41 (5.3)	12 (9.5)	
			C	1193 (77.2)	186 (73.8)	0.276
			T	353 (22.8)	66 (26.2)	
DHS	DNase hypersensitive site	rs213026	CC	291 (37.6)	50 (39.7)	0.892
			CT	350 (45.3)	56 (44.4)	
			TT	132 (17.1)	20 (15.9)	
			C	932 (60.3)	156 (61.9)	0.676
			T	614 (39.7)	96 (38.1)	
		rs9444828	CC	205 (26.6)	37 (29.4)	0.578
			CT	382 (49.5)	64 (50.8)	
			TT	184 (23.9)	25 (19.8)	
			C	792 (51.4)	138 (54.8)	0.351
			T	750 (48.6)	114 (45.2)	
		rs473267	CC	351 (45.5)	47 (37.3)	0.116
			CT	327 (42.4)	57 (45.2)	
			TT	93 (12.1)	22 (17.5)	
			C	1029 (66.7)	151 (59.9)	0.041
			T	513 (33.3)	101 (40.1)	
		rs7122887	CC	328 (42.4)	50 (39.7)	0.206
			CT	343 (44.4)	65 (51.6)	
			TT	102 (13.2)	11 (8.7)	
			C	999 (64.6)	165 (65.5)	0.847
			T	547 (35.4)	87 (34.5)	

Additional Table 3 Continued

Gene	Full name	SNP	Allele	Early-onset [*n*(%)]	Late-onset [*n*(%)]	*P*-value*
		rs524513	CC	642 (83.2)	106 (84.1)	0.160
			CT	121 (15.7)	16 (12.7)	
			TT	9 (1.2)	4 (3.2)	
			C	1405 (91.0)	228 (90.5)	0.882
			T	139 (9.0)	24 (9.5)	
		rs169160	TT	306 (39.8)	64 (50.8)	0.051
			TC	364 (47.4)	46 (36.5)	
			CC	98 (12.8)	16 (12.7)	
			T	976 (63.5)	174 (69.0)	0.105
			C	560 (36.5)	78 (31.0)	
HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	rs9274477	AA	420 (29.4)	89 (70.6)	< 0.001†
			AG	353 (45.7)	37 (54.3)	
			GG	0 (0)	0 (0)	
			A	1193 (77.2)	215 (85.3)	0.005
			G	353 (22.8)	37 (14.7)	
		rs9271117	TT	176 (22.9)	42 (33.6)	0.033
			TC	561 (72.9)	79 (63.2)	
			CC	33 (4.3)	4 (3.2)	
			T	913 (59.3)	163 (65.2)	0.089
			C	627 (40.7)	87 (34.8)	
		rs2859090	GG	522 (93.4)	110 (87.3)	0.025
			GA	51 (6.6)	16 (12.7)	
			AA	0 (0)	0 (0)	
			G	1495 (96.7)	236 (93.7)	0.028
			A	51 (3.3)	16 (6.3)	
		rs3117221	CC	217 (28.2)	37 (29.4)	0.955
			CT	383 (49.7)	61 (48.4)	
			TT	170 (22.1)	28 (22.2)	
			C	817 (53.1)	135 (53.6)	0.932
			T	723 (46.9)	117 (46.4)	
		rs2517455	CC	260 (33.6)	38 (30.2)	0.709
			CT	381 (49.3)	64 (50.8)	
			TT	132 (17.1)	24 (19.0)	
			C	901 (58.3)	140 (55.6)	0.457
			T	645 (41.7)	112 (44.4)	
		rs3129932	CC	502 (65.5)	89 (70.6)	0.530
			CG	237 (30.9)	33 (26.2)	
			GG	27 (3.5)	4 (3.2)	
			C	1241 (81.0)	211 (83.7)	0.346
			G	291 (19.0)	41 (16.3)	

*By chi-squared analysis followed by Bonferroni correction. †Significantly different. Categorical variables are presented as the number and percentage of patients; continuous variables are presented as the mean and range. A chi-squared test was used to assess Hardy-Weinberg equilibrium and to determine differences in genotype frequencies between early- and late-onset narcolepsy patients. A chi-squared test or Wilcoxon rank-sum test was used to compare values of quantitative traits between groups for data with or without normal distributions, respectively. SNP: Single-nucleotide polymorphism.

Additional Table 4 Association of GRS with the risk of early-onset narcolepsy

GRS set and risk category	Control (%)	Case (%)	Crude model [OR (95%CI)]	*P*-value	Adjusted model* [OR (95%CI)]	*P*-value
GRS1†	126	773	1.143 (1.049–1.248)	0.003	1.153 (1.052–1.265)	0.002
Low risk (GRS < 16)	49 (38.9)	198 (25.6)	1		1	–
Intermediate risk (16 ≤ GRS < 18)	37 (29.4)	269 (34.8)	1.799 (1.133–2.878)	0.013	1.974 (1.211–3.244)	0.007
High risk (GRS ≥ 18)	40 (31.7)	306 (39.6)	1.893 (1.204–2.993)	0.006	1.961 (1.216–3.182)	0.006
GRS2‡	126	773	0.969 (0.837–1.124)	0.68	0.987 (0.847–1.151)	0.864
Low risk (GRS < 4)	42 (33.3)	264 (34.2)	1		1	–
Intermediate risk (4 ≤ GRS < 5)	41 (32.5)	243 (31.4)	0.943 (0.592–1.502)	0.804	0.906 (0.554–1.482)	0.694
High risk (GRS ≥ 5)	43 (34.1)	266 (34.4)	0.984 (0.622–1.557)	0.945	1.070 (0.661–1.734)	0.782
GRS3§	126	773	1.046 (0.983–1.114)	0.157	1.055 (0.988–1.127)	0.113
Low risk (GRS < 25)	44 (34.9)	232 (30.0)	1		1	–
Intermediate risk (25 ≤ GRS < 28)	49 (38.9)	288 (37.3)	1.115 (0.715–1.735)	0.630	1.063 (0.664–1.695)	0.799
High risk (GRS ≥ 28)	33 (26.2)	253 (32.7)	1.454 (0.897–2.376)	0.131	1.466 (0.885–2.449)	0.139
GRS4‖	126	773	1.024 (0.894–1.171)	0.727	1.054 (0.913–1.215)	0.474
Low risk (GRS < 6)	26 (20.6)	165 (21.3)	1		1	–
Intermediate risk (6 ≤ GRS < 8)	73 (57.9)	408 (52.8)	0.881 (0.535–1.411)	0.606	0.931 (0.554–1.529)	0.783
High risk (GRS ≥ 8)	27 (21.4)	200 (25.9)	1.167 (0.653–2.082)	0.599	1.236 (0.675–2.259)	0.490
GRS5¶	126	773	1.156 (1.015–1.318)	0.029	1.196 (1.044–1.374)	0.010
Low risk (GRS < 7)	39 (25.0)	169 (22.3)	1		1	–
Intermediate risk (7 ≤ GRS < 9)	78 (50.0)	398 (51.3)	1.390 (0.880–2.169	0.151	1.588 (0.980–2.550)	0.057
High risk (GRS ≥ 9)	39 (25.0)	206 (26.4)	1.513 (0.893–2.585)	0.125	1.802 (1.034–3.168)	0.038

P-values for GRS groups are comparisons between all cases (early-onset) and controls (late-onset) for the same GRS group, and *P*-values for risk categories represent comparisons with the low risk category for the same GRS group. *Adjusted for sex, ethnicity, pre-onset inflammation, and body-mass index (These variables are associated with age-on-set of narcolepsy, selected from literature review and clinical experience). †SNPs outside HLA, ‡SNPs in HLA, §all selected SNPs, ‖SNPs previously reported in Chinese population, ¶SNPs previously reported to be associated with age at onset. The selected SNPs were grouped into GRS sets 1 to 5. These groupings were based on the results of SNP screening and the findings of previous studies. We used unweighted GRSs; the association of the GRSs with early- and late-onset narcolepsy was evaluated using logistic regression models. Treating the GRSs as continuous variables, according to the distribution of scores in the population, the risk tertiles were stratified as low-, intermediate-, and high-risk categories. The models were adjusted for potential confounders, which included sex, ethnicity, pre-onset inflammation, and body mass index. Crude and adjusted odds ratios (ORs), 95% confidence intervals (CIs). GRS: genetic risk score; SNP: single-nucleotide polymorphism.