A population-based study suggested that the prevalence of narcolepsy was significantly lower in patients with normal sleep-onset REM periods, as do those with NA-CA, compared with controls (allele frequency: 16.6% vs. 7.8%, OR = 2.36; carrier frequency: 31.3% vs. 14.7%, OR = 2.64). Distributions of HLA-DQB1 alleles other than HLA-DQB1*06:02 were compared between patients with normal sleep and narcolepsy with cataplexy (NA-CA) to assess whether the genetic backgrounds of the two diseases have similarities. The distribution of the HLA-DQB1 alleles in DQB1*06:02-negative NA w/o CA was significantly different from that in NA-CA (P = 5.8 × 10^-7). On the other hand, the patterns of the HLA-DQB1 alleles were similar between DQB1*06:02-positive NA w/o CA and NA-CA. HLA-DQB1 analysis was also performed in 186 Japanese patients with idiopathic hypersomnia (IHS) with/without long sleep time, but no significant associations were observed.

Human Genome Variation (2015) 2, 15031; doi:10.1038/hgv.2015.31; published online 17 September 2015

The 2nd Edition of the International Classification of Sleep Disorders (ICSD-2), in the category of hypersomnia of central origin, subdivides narcolepsy into two groups: narcolepsy with cataplexy (NA-CA) and narcolepsy without cataplexy (NA w/o CA). NA w/o CA is characterized by excessive daytime sleepiness and abnormal manifestations of rapid eye movement (REM) sleep in common with NA-CA, but no cataplexy. Patients with NA w/o CA have frequent sleep-onset REM periods, as do those with NA-CA, as revealed by performance of the multiple sleep latency test. A population-based study suggested that the prevalence of NA w/o CA is 36% of the prevalence of narcolepsy as a whole, corresponding to a point prevalence of 0.02%. NA-CA is tightly associated with HLA-DQB1*06:02 and orexin (hypocretin) deficiency. Almost all patients with NA-CA in many populations consistently carry DQB1*06:02, while approximately 12% of Japanese, 25% of Caucasian and 38% of African American healthy individuals are DQB1*06:02-positive. Low levels of orexin A in cerebrospinal fluid (CSF) (< 110 pg/ml) are commonly observed in patients with NA-CA. Regarding NA w/o CA, positivity of HLA-DQB1*06:02 (30–50%) is also higher than that in the general population, but less than that in NA-CA. However, only approximately 20% of patients with NA w/o CA have low levels of CSF orexin A, indicating that the etiology of the majority of NA w/o CA is still unknown.
There have been a number of studies of HLA in NA-CA; results indicated that HLA-DQB1 alleles other than DQB1*06:02 modulate susceptibility or resistance to NA-CA. DQB1*06:01 and DQB1*05:01 in the Korean and Japanese populations and DQB1*06:03 in European populations are protective against NA-CA, whereas individuals with DQB1*03:01 and DQB1*03:02 are at an increased risk. In the present study, to test for associations of HLA-DQB1 alleles in NA w/o CA, we performed an association study for HLA-DQB1 in 160 Japanese patients with NA w/o CA and 1,418 control subjects.

Idiopathic hypersomnia (IHS) is a sleep disorder of presumed central nervous system origin that is associated with excessive daytime sleepiness consisting of prolonged non-REM sleep episodes. Daytime naps of IHS patients tend to be longer and less refreshing than those of NA-CA patients. IHS is a rare disease, representing 8:10 to 1:10 patients with NA-CA. This suggests that the prevalence of IHS approximates 0.005%. The ICSD-2 describes two clinical forms of IHS by the difference in nocturnal sleep time: IHS with long sleep time (IHS-LST) and IHS without long sleep time (IHS w/o LST). The nocturnal sleep time of IHS-LST is prolonged to at least 10 h, while that of IHS w/o LST is either normal or slightly prolonged (less than 10 h). CSF orexin A levels in IHS are normal. The cause and pathogenesis of IHS remain largely unknown. NA w/o CA and IHS w/o LST have several common characteristics except for REM-related symptoms. Distinguishing NA w/o CA and IHS w/o LST is impossible without the multiple sleep latency test to identify sleep onset REM periods. According to the ICSD-2, the diagnosis is based on the number of sleep-onset REM periods, two or more in the former and less than two in the latter. In the present study, we tested whether HLA-DQB1 alleles have an influence on susceptibility to IHS w/o LST and IHS-LST.

A total of 346 Japanese patients and 1,418 Japanese healthy controls were included in this study. NA w/o CA, IHS w/o LST and IHS-LST were diagnosed according to the ICSD-2 criteria. The patient groups consisted of NA w/o CA (n = 160), IHS w/o LST (n = 118) and IHS-LST (n = 68). We utilized HLA data of healthy individuals, who have been previously studied for disease association analyses. In addition, to assess genetic similarities between the above hypersonmia disorders and NA-CA, HLA-DQB1 data from 664 patients with NA-CA were utilized. All of the patients and controls were mainland Japanese and gave written informed consent. This study was approved by the local institutional review boards at participating institutions. Typing for the HLA-DQB1 locus was performed by a Luminox Multi-Analyte Profiling system (xMAP) with WAKFlow HLA typing kits (Wakunaga Pharmaceutical, Wakunaga, Hiroshima, Japan). Comparisons of frequencies were performed using the Chi-square test or Fisher’s Exact test as appropriate. To account for multiple testing, the significance level was adjusted by the number of HLA alleles with allele frequencies no less than 0.5% in controls (12 for HLA-DQB1 alleles). The significance level was set to be $P < 4.2 \times 10^{-3}$ (0.05/12). If any of the four cells was zero, the Woolf-Haldane correction was applied (adding 0.5 to all cells). An association analysis controlling for the effects of the other HLA-DQB1 alleles was performed by Woolf-Haldane correction. To determine whether there was a different allelic distribution between two groups, the overall frequency distribution of HLA-DQB1 alleles in one group was compared with the distribution in another group by using the global Chi-square test with 12 degrees-of-freedom.

Table 1. HLA-DQB1 allele frequencies of patients with NA w/o CA

| DQB1 | NA w/o CA (n = 320) | Control (n = 2836) | OR | P |
|------|---------------------|--------------------|----|---|
| 02:01 |  1                  |       0.3          | 11 | 0.4 | 0.81 | 1.00 |
| 03:01 |  29                 |       9.1          | 334 | 11.8 | 0.75 | 0.15 |
| 03:02 |  27                 |       8.4          | 264 | 9.3  | 0.90 | 0.61 |
| 03:03 |  46                 |       14.4         | 450 | 15.9 | 0.89 | 0.49 |
| 04:01 |  42                 |       13.1         | 374 | 13.2 | 0.99 | 0.97 |
| 04:02 |  12                 |       3.8          | 118 | 4.2  | 0.90 | 0.73 |
| 05:01 |  22                 |       6.9          | 191 | 6.7  | 1.02 | 0.92 |
| 05:02 |  3                  |       0.9          | 63  | 2.2  | 0.42 | 0.13 |
| 05:03 |  10                 |       3.1          | 106 | 3.7  | 0.83 | 0.58 |
| 06:01 |  45                 |       14.1         | 515 | 18.2 | 0.74 | 0.07 |
| 06:02 |  53                 |       16.6         | 220 | 7.8  | 2.36 | 1.1E-07 |
| 06:03 |  1                  |       0.3          | 16  | 0.6  | 0.55 | 1.00 |
| 06:04 |  28                 |       8.8          | 160 | 5.6  | 1.60 | 0.03 |
| 06:09 |  1                  |       0.3          | 14  | 0.5  | 0.91 | 1.00 |

Abbreviations: NA w/o CA, narcolepsy without cataplexy; OR, odds ratio.
reported to be different between $DQB1*06:02$-positive and -negative NA w/o CA groups. These findings correspond well to our result.

Patients with IHS w/o LST and IHS-LST were also typed for HLA-$DQB1$ in the present study. $DQB1$ allele and carrier frequencies are shown in Table 3 and Supplementary Table 2, respectively. $DQB1*06:02$, known to be associated with NA-CA and NA w/o CA, was not associated with IHS w/o LST or IHS-LST. Although $DQB1*05:02$ ($P=6.3 \times 10^{-3}$) and $DQB1*03:01$ ($P=0.04$) showed nominally significant associations with IHS-LST, there were no $DQB1$ alleles that reached the threshold after correction for multiple comparisons. The similarity of $DQB1$ allele distribution between NA-CA and IHS w/o LST or IHS-LST was tested after controlling for the effects of $DQB1*06:02$, and significant differences were found: for IHS w/o LST: $P=2.1 \times 10^{-4}$ and for IHS-LST: $P=2.2 \times 10^{-5}$. Taken together, these results indicate that IHS w/o LST and IHS-LST are caused by different etiological genetic factors than those that give rise to NA-CA.

The International Classification of Sleep Disorders was recently revised for the 3rd Edition (ICSD-3). When we had recruited patient samples, the 2nd edition (ICSD-2) was used. Main differences between the ICSD-2 and ICSD-3 regarding NA w/o CA, IHS w/o LST and IHS-LST are as follows. The terminology has been changed from 'narcolepsy without cataplexy (NA w/o CA)' to 'narcolepsy type 2'. The concept of NA w/o CA and narcolepsy type 2 is almost the same.

### Table 2. Frequencies of $DQB1$ alleles after removal of $DQB1*06:02$ effects

| $DQB1$ alleles | NA w/o CA | $DQB1*06:02 (+)$ | NA w/o CA | $DQB1*06:02 (-)$ | NA w/o CA | Control | NA-CA |
|----------------|-----------|-----------------|-----------|-----------------|-----------|---------|-------|
|                | No. | % | OR | P | No. | % | OR | P | No. | % | OR | P |
| 02:01          | 1   | 0.4 | 0.89 | 1.00 | 0 | 0.0 | 2.38 | 1.00 | 1 | 0.5 | 1.08 | 1.00 | 11 | 0.4 | 5 | 0.9 | 1.78 | 0.22 |
| 03:01          | 29  | 10.9 | 0.83 | 0.37 | 9 | 19.1 | 1.62 | 0.20 | 20 | 9.1 | 0.68 | 0.11 | 334 | 12.8 | 98 | 16.9 | 1.38 | 5.7E-03 |
| 03:02          | 27  | 10.1 | 1.00 | 0.99 | 6 | 12.8 | 1.30 | 0.47 | 21 | 9.5 | 0.94 | 0.80 | 264 | 10.1 | 107 | 18.4 | 1.97 | 2.5E-09 |
| 03:03          | 46  | 17.2 | 1.00 | 0.99 | 8 | 17.0 | 0.99 | 0.97 | 38 | 17.3 | 1.00 | 0.98 | 450 | 17.2 | 74 | 12.8 | 0.73 | 0.02 |
| 04:01          | 42  | 15.7 | 1.12 | 0.53 | 9 | 19.1 | 1.42 | 0.35 | 33 | 15.0 | 1.06 | 0.78 | 374 | 14.3 | 112 | 19.3 | 1.43 | 1.2E-03 |
| 04:02          | 12  | 4.5 | 1.00 | 0.99 | 1 | 2.1 | 0.46 | 0.72 | 11 | 5.0 | 1.11 | 0.74 | 118 | 4.5 | 20 | 3.4 | 0.77 | 0.27 |
| 05:01          | 22  | 8.2 | 1.14 | 0.58 | 2 | 4.3 | 0.56 | 0.58 | 20 | 9.1 | 1.27 | 0.33 | 191 | 7.3 | 20 | 3.4 | 0.48 | 0.26 |
| 05:02          | 3   | 1.1 | 0.46 | 0.18 | 1 | 2.1 | 0.88 | 1.00 | 2 | 0.9 | 0.37 | 0.15 | 63 | 2.4 | 28 | 4.8 | 1.80 | 4.4E-03 |
| 05:03          | 10  | 3.7 | 0.92 | 0.81 | 1 | 2.1 | 0.51 | 1.00 | 9 | 4.1 | 1.01 | 0.98 | 106 | 4.1 | 21 | 3.6 | 0.79 | 0.31 |
| 06:01          | 45  | 16.9 | 0.83 | 0.27 | 6 | 12.8 | 0.60 | 0.24 | 39 | 17.7 | 0.88 | 0.48 | 515 | 19.7 | 49 | 8.4 | 0.39 | 1.4E-10 |
| 06:03          | 1   | 0.4 | 0.61 | 1.00 | 0 | 0.0 | 1.66 | 1.00 | 1 | 0.5 | 0.74 | 1.00 | 16 | 0.6 | 1 | 0.2 | 0.25 | 0.26 |
| 06:04          | 28  | 10.5 | 1.80 | 5.9E-03 | 4 | 8.5 | 1.43 | 0.50 | 24 | 10.9 | 1.88 | 5.6E-03 | 160 | 6.1 | 42 | 7.2 | 1.10 | 0.58 |
| 06:09          | 1   | 0.4 | 0.70 | 1.00 | 0 | 0.0 | 1.89 | 1.00 | 1 | 0.5 | 0.85 | 1.00 | 14 | 0.5 | 3 | 0.5 | 1.50 | 0.46 |

Global P value versus NA-CA^b

### Table 3. HLA-$DQB1$ allele frequencies of patients with IHS w/o LST and IHS-LST

| $DQB1$ | IHS w/o LST (2n = 236) | IHS-LST (2n = 136) | Control (2n = 2836) |
|--------|------------------------|--------------------|---------------------|
| No.    | % | OR | P | No. | % | OR | P | No. | % |
| 02:01  | 0 | 0.0 | 1.00 | 0 | 0.0 | 0.90 | 1.00 | 11 | 0.4 |
| 03:01  | 33 | 14.0 | 1.22 | 0.32 | 8 | 5.9 | 0.47 | 0.04 | 334 | 11.8 |
| 03:03  | 26 | 11.0 | 1.21 | 0.39 | 10 | 7.4 | 0.77 | 0.44 | 264 | 9.3 |
| 03:04  | 34 | 14.4 | 0.89 | 0.55 | 19 | 14.0 | 0.86 | 0.55 | 450 | 15.9 |
| 04:01  | 35 | 14.8 | 1.15 | 0.48 | 13 | 9.6 | 0.70 | 0.22 | 374 | 13.2 |
| 04:02  | 9 | 3.8 | 0.91 | 0.80 | 4 | 2.9 | 0.70 | 0.48 | 118 | 4.2 |
| 05:01  | 19 | 8.1 | 1.21 | 0.44 | 11 | 8.1 | 1.22 | 0.54 | 191 | 6.7 |
| 05:03  | 8 | 3.4 | 0.90 | 0.79 | 8 | 5.9 | 2.75 | 0.03 | 334 | 11.8 |
| 06:01  | 39 | 16.5 | 0.89 | 0.53 | 33 | 24.3 | 1.44 | 0.07 | 515 | 18.2 |
| 06:02  | 16 | 6.8 | 0.86 | 0.59 | 12 | 8.8 | 1.15 | 0.65 | 220 | 7.8 |
| 06:03  | 2 | 0.8 | 1.51 | 0.64 | 1 | 0.7 | 1.31 | 0.55 | 16 | 0.6 |
| 06:04  | 9 | 3.8 | 0.66 | 0.24 | 8 | 5.9 | 1.05 | 0.91 | 160 | 5.6 |
| 06:09  | 1 | 0.4 | 0.86 | 1.00 | 1 | 0.7 | 1.49 | 0.51 | 14 | 0.5 |

Abbreviations: IHS-LST, idiopathic hypersomnia with long sleep time; IHS w/o LST, idiopathic hypersomnia without long sleep time; OR, odds ratio.
alleles in IHS was also significantly different from that of NA-CA, even after the effect of DQB1*06:02 was controlled (P = 8.6 × 10⁻⁶).

To conclude, the association of DQB1*06:02 in NA w/o CA was confirmed. Our results also suggested an immunological pathogenesis of DQB1*06:02-positive NA w/o CA, which is similar to that of NA-CA. DQB1*06:02-negative NA w/o CA, IHS w/o LST and IHS-LST may have a different etiology, which is not well understood.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.688.

ACKNOWLEDGEMENTS

The authors are deeply grateful to all participants in this study. This study was supported by Grants-in-Aid for Young Scientists (A) (23689022), Scientific Research (B) (15H04709) and Scientific Research on Innovative Areas (22133008) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Grants-in-Aid from the Takeda Science Foundation and the SENSHIN Medical Research Foundation.

COMPETING INTERESTS

Y.I. has a commercial research grant from Alphresa Pharma Corporation. The other authors declare no conflict of interest.

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