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Ventilatory Chemoresponsiveness, Narcolepsy-Cataplexy, and HLA-DQB1*0602 Status

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Abstract

The hypothesis is that hypocretin (orexin) plays a role in the determination of ventilatory chemosensitivity. Patients with narcolepsy-cataplexy (n =130; 20±10 yrs; 69% male) and 117 controls (22±6.9 yrs; 62% male) were recruited and tested for HLA-DQB1*0602 status, hyperoxia hypercapnic ($\Delta\dot{V}E/\Delta PCO_2$, L/min/mmHg) and hypoxic ($\Delta\dot{V}E/\Delta SpO2$, L/min/%SpO2) responsiveness, and by spirometry. Hypocretin deficiency was determined either by measures of CSF hypocretin-1 (37 patients), or by positive HLA-DQB1*0602 status. All patients and 49% of controls underwent polysomnography and MSLT testing. Despite similar spirometric values, patients had higher apnea hypopnea index (2.8±5.4 vs. 0.8±1.6/hr, p=0.03) and lower minimal oxygen saturation during sleep (87%±7 vs. 91%±4, p=0.0002), independent of age, sex and body mass index (BMI). Patients had depressed hypoxic responsiveness (0.13±0.09 vs 0.19±0.13 L/min/%SpO2, p<0.0001), independent of AHI, but hypercapnic responsiveness did not differ. Examined by HLA status, positive (26 of 117) controls had lower hypoxic but similar hypercapnic responsiveness than those marker negative (0.13±0.08 vs. 0.20±0.14 L/min/%SpO2, p<0.0001). Thus, a lower hypoxic responsiveness in the narcolepsy-cataplexy group is a result of DQB1*0602 status rather than the clinical features of disease.

Key words: Narcolepsy, Chemoresponsiveness, hypoxia, hypercapnia, HLADQB1

Introduction

Hypoxic and hypercapnic sensing are processed through neuromuscular circuits resulting in an increase (or decrease) of tidal volume, respiratory frequency and minute ventilation. Such chemosensory reflexes in sleep apnea, COPD, heart failure, and acute adaptation to high altitude (1). A variation in chemosensitivity across various inbred rodent strains and familial clustering of ventilatory traits in humans provides a strong rationale for gene and protein isolation efforts to unravel molecular mechanisms for these traits operating in health and disease (2).

Hypocretin (orexin) is a hypothalamic neurotransmitter which when given intracerebroventricularly in mice, promotes both wakefulness and ventilation (3, 4). Both hypocretin knockout and hypocretin neuron-ablated mice show attenuation of respiratory excitation during fight-or-flight responses (5). Particular to chemoresponsiveness, hypocretin/orexin knockout mice have attenuated hypercapnic ventilatory responses (6); supplementation of hypocretin-1 or -2 partially restores an attenuated response to hypercapnia, while administration of hypocretin receptor-1 antagonist will reduce hypercapnic responsiveness in wild type mice (7). Post-hypoxic long-term facilitation, a physiologic feature that is presumed to reduce sleep apnea, is absent in hypocretin/orexin knockout mice (8).

The human model of hypocretin deficiency is narcolepsy with cataplexy (9, 10). This disorder is also associated with the gene marker, HLADQB1*0602 (11). This disease is also considered to be associated with a higher expression of sleep disordered breathing (12, 13). Our primary hypothesis was that an impaired chemoresponsiveness would be present in narcolepsy-cataplexy patients who are uniformly HLADQB1*0602 positive and have low hypocretin levels <110pg/ml in comparison to control subjects without disease or cataplexy or the HLA marker who have normal levels of hypocretin (over 99% >110pg/ml) (9). Results indicate an

unexpected finding that the mechanism for differences between patients and controls in hypoxic responsiveness could relate to HLA marker status rather than disease.

Methods

Human Subjects

Patients with narcolepsy/hypocretin deficiency and/or cataplexy (n=130) were prospectively identified from successive patients presenting to the Sleep Center, the Peking University People's Hospital, China, from 2006 to 2007. Diagnosis of narcolepsy with cataplexy was made using ICSD-II criteria (14). All patients completed a validated questionnaire predictive of cataplexy, the Center for Narcolepsy Sleep Inventory (15). Hypocretin deficiency was documented in 37 patients (all DQB1*0602 positive) through CSF hypocretin-1 evaluation, while the other 93 patients were selected based on DQB1*0602 positivity and the presence of cataplexy, as described in Hong et al (16). More than 95% of DQB1*0602 positive narcolepy-cataplexy patients have hypocretin deficiency (17). Demographic and polysomnography (PSG) results for subjects are reported in Table 1.

Controls (n=117) were willing local employees and college students, and were recruited and selected as age- and sex-match to the patient sample. All control subjects provided a blood sample for DQB1*0602 typing, completed a standard sleep questionnaire, and performed spirometry and ventilatory responses. Fifty seven of the 117 controls consented to nocturnal PSG and multiple sleep latency testing (MSLT) testing (Table 1).

Informed consent was obtained from patients and controls. The study was approved by the institutional review board of the People's Hospital of Beijing University.

Sleep study

Overnight nocturnal PSG was followed by a MSLT the following day. The overnight recording included EEG (C3/A2 and C4/A1), chin electromyography, anterior tibialis electromyography, microphone recording for snoring, electrooculography, ECG, nasal-oral airflow, thoracic and abdominal effort, and arterial oxygen saturation by pulse oximetry

(SpO_2). An apnea was defined as the cessation of airflow at the nose and mouth lasting for at least 10 seconds, and hypopnea was defined as a decrease in airflow, rib cage excursion, or abdominal excursion by > 50% that was associated with an oxygen desaturation of at least 4% below the preceding baseline or with an arousal. The absence of airflow in the upper airway with and without ribcage and abdominal movement was defined as obstructive (OSA) and central apnea (CSA), respectively; in a mixed apnea (MSA) there were features of both central and obstructive apneas/hypopneas in the same event. The AHI was calculated as the number of apneas and hypopneas per hour of total sleep time, and further examined as per hour of NREM and REM time. Mean SpO_2 and lowest SpO_2 during sleep were also calculated.

The MSLT was performed to determine sleep latency and probe for the presence of sleep onset REM sleep (SOREM). The sleep latency was defined as the elapsed time from lights-out to the first epoch scored as sleep. The REM sleep latency was defined as the time from the beginning of sleep onset to the first epoch of REM sleep. Sleep stages of both PSG and MSLT were scored in 30 second epochs following the Rechtschaffen and Kales criteria (18). In the (unlikely) event that patients or healthy subjects were on drugs known to affect the MSLT, recordings of the polysomnography or MSLT and ventilatory testing were performed off of these medications for at least 15 days.

Assessment of Ventilatory Responses

These studies were performed between 10:00 AM and noon. Responses to progressive hyperoxic hypercapnia were assessed using rebreathing technique of Read (19). Patients were in the seated posture connected to the circuit, wearing a facemask. Hypoxic testing was performed using an anesthesiology rebreathing circuit with a CO_2 absorbant was placed on the inspiratory line. This set-up resulted in a falling end-tidal CO2 value as ventilation increased. The hypoxic testing was concluded when oxygen saturation neared 70%. Minute ventilation ($\dot{V}E$) was regressed linearly against the PCO_2 values (hypercapnic testing) or

against the fall in SpO2 from 90% (hypoxic testing), and responsiveness reported as the slope of the linear regression: $\Delta\dot{V}E/\Delta PCO_2$ and $\Delta\dot{V}E/\Delta SpO2$ respectively.

$\Delta\dot{V}E/\Delta SpO2$ and $\Delta\dot{V}E/\Delta PCO_2$ values were analyzed both with and without correction by body surface area, so as to adjust to size differences due to the size range among subjects.

HLA-DQB1*0602 Typing and CSF hypocretin-1 evaluation

HLA DQB1*0602-specific codon 9 amino acid was determined at Beijing University using a previously described polymerase chain reaction sequence-specific primer method (20). The presence of DQB1*0602 is reported as positive and absence as negative. In a sub-set of 37 patients, CSF samples by lumbar puncture were collected between 10:00 AM and 1:00 PM. CSF samples were frozen immediately, and subsequently kept at -80°C until measurements were made. Hypocretin-1 was measured at Beijing University using a ^{125}I radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA) (9). All samples were measured in duplicate, averaged, and compared to standard CSF samples with known CSF hypocretin-1 levels.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Simple comparisons were performed using a chi square or student t-test, as appropriate. One way ANOVA for repeated measures was used to compare differences in regard to end-tidal PCO_2 measures over the course of hypoxic testing. Hypoxic and hypercapnic responses, as well as AHI, were log transformed to fit a normal distribution prior to statistical analysis using student tests or multivariate analysis models (actual values are reported, while p-values are derived from the transformed comparisons). Comparisons between measures before and after medication therapy were made using paired *t* tests. Differences were considered significant at p values of ≤ 0.05.

Results

Patients with narcolepsy/hypocretin deficiency have increased BMI and a tendency for increased sleep disordered breathing. Patients and age/sex matched controls were young and most often male (Table 1). As typically reported, BMI was slightly higher than in controls, although body surface area (BSA) values were similar. None of the patients were on medications for narcolepsy at the time of the study. In this sample of patients, the number of apnea and hypopnea was increased (Table 1). The prevalence of an AHI>5 was higher (16%) than in the those studied in the control group (5%); the latter prevalence is comparable to the reported value (4%-5%) in older (age>30) Chinese general population (21, 22); 5% of narcoleptic but no control subjects had an AHI >15. In regard to the type of sleep disordered breathing, hypopnea and obstructive apneas were commonly present, accounting for 61±37 % and 35±36 % of the total events, respectively, and CSA and MSA were less common (1.8 ±8.8% and 6.8 ±4.6%, respectively) (Table 1). While patients had lower lowest SpO2 sleep values than controls, the baseline and mean values of SpO2 during sleep did not differ.

AHI was not correlated to BMI. AHI was greater in those with narcolepsy before and after being adjusted for age, sex, and BMI (adjusted AHI: 2.53±4.23 versus 0.60±0.54, $p<0.01$). Similarly, the lowest SpO2 saturation in narcolepsy was still significantly lower before and after being adjusted for for age, sex, BMI and AHI (adjusted lowest SpO2: 87.9±4.7 vs. 90.6±4.7, $p<0.01$). In controls who had PSG and MSLT testing (n=57), there was no significant difference in sleep indices during sleep in regard to HLA status; however, those HLA-DQB1*0602 positive had a lower AHI (0.21± 0.42 vs. 1.10±1.83, $p<0.01$) and higher SpO2 (93.2±3.4 vs 90.1 ± 1.0%, $p<0.05$) values. These results provide evidence consistent with impaired control of breathing during sleep and of oxygen saturation in patients with narcolepsy-cataplexy.

Patients with narcolepsy/hypocretin deficiency have decreased ventilatory response to hypoxia but not hypercapnia. Spirometry values were within normal limits for controls and patients, and no different between groups. In contrast to findings suggested by animal KO models, we found no group differences in regard to hypercapnic responsiveness, but rather

found significant differences in hypoxic responsiveness (Table 2). Reduced hypoxic responsiveness in narcolepsy-cataplexy patients was unrelated to BMI, age and sex, but hypercapnic and hypoxic responsiveness were correlated with BSA ($p<0.001$). After adjustment for BSA, there remained no difference in hypercapnic responsiveness. However, hypoxic responsiveness was different between groups. During hypoxic rebreathing tests, end-tidal CO_2 fell on the average from 5.4±0.7% at 95% to 4.8±0.6% at 75% saturation ($p=0.005$), indicating that the hypoxic testing was poikilocapnic. Values were higher in narcolepsy-cataplexy vs. controls at most oxygen saturations ($p=0.035$). For instance, at a saturation of 90%, end-tidal values for narcolepsy-cataplexy patients was 5.3±0.5% vs. 5.1±0.7% for controls ($p=0.04$). Despite the higher value, the slope of the hypoxic response was lower in narcolepsy-cataplexy vs. controls (0.13±0.09 vs. 0.19±0.13 L/min/%SpO_2, $p=0.0007$).

*Ventilatory responses to hypoxia is decreased in relation to HLA-DQB1*0602 status, not hypocretin deficiency*. As all subjects with narcolepsy-cataplexy were also HLA-DQB1*0602 positive and 22% of the control population was also positive, we considered whether HLA status explained the differences between groups. No differences between these groups were present for hypercapnic responsiveness (1.07±0.75 vs. 1.03±0.74 L/min/mmHg), before or after controlling for BSA (0.64±0.42 vs. 0.60±0.42 L/min/mmHg). We considered whether carbon dioxide levels during hypoxic rebreathing explained the results since end-tidal CO_2 fell on the average from 5.4±0.6% at 95% to 4.9±0.5% at 75% saturation ($p=0.008$). However, end-tidal CO_2 during testing was similar in HLA positive vs. HLA negative controls ($p=0.65$). Therefore, the slope of hypoxic responsiveness was lower in HLA positive vs. negative controls (0.13±0.08 vs 0.20±0.14 L/min/%SpO_2, $p=0.00004$), an effect that remained significant after adjustment for BSA (0.078±0.04 vs 0.120±0.09 L/min/%SpO_2, $p=0.0001$).

Mulitivariate analysis was conducted to assess for significant covariates of hypoxia responses in the entire sample. Entered into a stepwise regression analyses were age, sex, BMI, HLA status, AHI, lowest oxygen saturation, hyperecapnic responsiveness, and hypocretin deficiency. Hypoxia responses were significantly correlated with HLA status (independent of hypocretin status, and without interaction by sex) ($p<0.00001$), and positively correlated hypercapnia response ($p<0.001$). In subjects with available sleep recordings, hypoxic responsiveness was strongly correlated with DQB1*0602 status ($p< 0.0001$) and weakly but significantly correlated with hypercapnic responsiveness ($p= 0.01$) and AHI ($p= 0.05$).

Discussion

The novel findings are: 1) a population difference between patients with narcolepsy-cataplexy and age-matched controls in regard to hypoxic chemosensitivity, and 2) an association between DQB1*0602 and acute ventilatory responsiveness to progressive hypoxia. While narcolepsy-cataplexy patients do have different sleep values, AHI and lowest SaO_2% were not a defining factor for the group differences in ventilatory chemosensitivity.

Contrary to results obtained in hypocretin gene knockout mice, hypercapnia responses were not affected by disease or marker state. Unlike knock-out mice where both the gene and protein are absent, in patients with narcolepsy hypocretin-1 is often detected at some level in the CSF (9). We suspect that the effects of hypocretin on chemoresponsiveness, if present, are below a threshold for detection.

Our findings implicate DQB1*0602 or a gene located nearby as a regulator of hypoxic responses. While DQB1*0602 is an allele present in all patients with narcolepsy and cataplexy, it is not a disease defining HLA maker, as it is present in the healthy populations (23). Few studies hint at potential relationships between immune markers and hypoxia. Microsatellite markers within the HLA region are reported to correlate with a blunted response to hypoxia in COPD with Type II respiratory failure (24) and with the development

of altitude-induced pulmonary oedema (25), although in these studies correlations specific for the DQB1*0602 allele were not reported. Intercross studies of C3H/HeJ (C3) and C57BL/6J (B6) mice have mapped potential loci for the hypoxic effect and its modulation by hypercapnia (26); however, these regions are not syntenic to human HLA gene regions. Glomus cell quantity differ across mice strains (27), and expression studies between high and low responder strains in carotid bodies suggest a number of potential candidates (28-30), but none reported have direct connections to immune function. With chronic hypoxic exposure, there is activation of the hypoxic inducible factor pathway in the carotid body (31), and HIF is upstream to many cellular pathways, including some which affect cell mediated immunity. An association of DQB1*0602 with hypoxic responsiveness might suggests a role for HLA class II molecules in the pathways for oxygen sensing and responses, but additional studies are needed to identify the culprit and its function. The region where this allele resides contains genes not only for immune related molecules, but also for peptide transporters, C450 oxygen binding metabolic enzymes, and transcription factors.

In regard to the impact of sleep disordered breathing, narcolepsy-cataplexy patients exhibited generally more occurrences, as measured by AHI, and severity, as measured by SaO_2%. These findings establish with greater confidence the contention that patients with narcolepsy could exhibit a higher incidence of sleep disordered breathing disorders than in the general population (32, 33). The prior literature reported on an older patient sample compared to expected norms and suggested the association might result from obesity, as hypocretin is a factor in metabolism (34). In this study patients were young, and the increased AHI and decreased minimal oxygen saturation were independent of the range of BMI. This correlation of more events with disease status may be independent of HLA status, as we found a decrease in indices for sleep disordered breathing in those positive for DQB1*0602. There may, however, be more complexity in the relations among sleep regulation, HLA status and sleep. Two prior reports with large numbers found significant associations between sleep onset REM (SOREM) during an MSLT and decreased lowest

nocturnal oxygen saturation during the prior nocturnal polysomnography- one clinic-based study in patients with sleep apnea (n= 1,145) and another in healthy controls (n= 556) (35, 36). The community study reported multiple SOREMPs to be associated with DQB1*0602 in men (36). Therefore, the presence of sleep disordered breathing could be the result of something more than HLA status.

The study was conducted in a Chinese population, and hence could be ethnically specific. As a limitation, one could consider that this might have reduced genetic heterogeneity, that likely influences chemosensitivity, and resulted in a haplotype inheritance that enhanced the likelihood of finding significant correlations. Nonetheless, this study is one of the numerically larger studies of human chemosensitivity, even in the healthy sample, so it is possible but unlikely that significance is false. We considered whether the poikilocapnic nature of the hypoxic testing resulted in group differences, but considered this unlikely for two reasons. First, poikilocapnic and isocapnic hypoxic responses are similarly informative when measured at the same time in previous studies in humans (37). Second, as end-tidal CO_2 values were generally higher in patients vs. controls, one would expect the interaction to enhance rather than blunt responses in the patient group. Third, the ventilatory response to CO_2 did not associate with disease or marker status, making it less likely that interactions resulted in a spurious finding. Studies in other ethnic groups and of related chemosensitivity traits, such as hypoxic short-term facilitation, appear indicated.

In conclusion, we found differences in hypoxic responsiveness between controls and HLA-DQB1*0602 narcolepsy patients with cataplexy, while hypercapnic responsiveness was unrelated to disease or hypocretin status. Hypoxic sensitivity correlated weakly with higher AHI values, but AHI values did not explain the results. An association with reduced hypoxic responsiveness was found to DQB1*0602 status in the healthy, unaffected subjects, and thus explains the difference in hypoxic responsiveness between groups. We speculate a novel

effect of HLA or of a gene polymorphism located nearby on the well-known interindividual variation in hypoxic responsiveness in the Chinese population.

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Figure legends

Figure 1. Comparisons between Controls and Patients

These figures illustrate the values (mean and SD) of hypercapnic response ($\Delta\dot{V}E/\Delta PCO_2$) and hypoxic ($\Delta\dot{V}E/\Delta$SpO2) of the controls and patients and are presented as box plot graphs (A,C) and as cumulative pencentage (B,D) Panels A and C indicate the median, quartiles, and the 90th and 10th percentiles The values graphed in Panels B and D represent the cumulative number of cases as a percentage of the total number of cases * $p<0.001$

Figure 2. Comparisons in Control Subjects in regard to HLA status

These figures present the values (mean and SD) and for hypercapnic response ($\Delta\dot{V}E/\Delta PCO_2$) in Panels A and B and hypoxic ($\Delta\dot{V}E/\Delta$SpO2) in Panels C and D between controls with HLADQB1*0602 positive (+) and negative (-) Data are presented as in Figure 1 * $p<0.01$

Contributions: HF was the principal investigator, He conceived and coordinated the study, wrote the initial manuscript, and edited the final paper EM and KPS contributed to the conception of the study, the manner of methods and analysis, interpretation of results, and manuscript content CYW, XSD, JL, LL, PA, HLW, SJW, ZMH,YHG, ZCG, and ML recruited study participants, collected study data, performed studies and organized sub-studies, contributed to the manuscript, and reviewed the final paper

Table 1. Demographic, clinical, sleep and ventilatory response characteristics in narcoleptic (NC) versus control subjects (Mean± SD)

	NC (n=130)	Controls (n=117)
Demographics		
Age (YR)	20.1±10.0	22.0±6.9
Sex (M)	69%	62%
BMI (kg/m^2)	23.6±4.3*	21.4±3.1
BSA (m^2)	1.73±0.34	1.70±0.24
Onset age (y)	12.3±6.5	NA
Illness duration (years)	7.8±6.9	NA
Symptoms at interview		
Daytime sleepiness	100%	0
Typical cataplexy	100%	0
Polysomnography study		
Sleep parameters		
Sleep efficiency (%)	91.4±9.3	87.1±9.9(n=57)
Sleep latency	8.0±10.0*	15.1±17.9(n=57)
REM latency	72.2±88.1†	124.2.±66.1(n=57)
%REM Latency <20 min	48% (63/130) *	7% (4/57)
MSLT		
Mean sleep latency	3.7±2.1*	16.0±3.4 (n=57)
Numbers of SOREMs	4.4±0.8*	0.5±0.9 (n=57)
% MSL≤8 min, SOREMP≥2	97.7% (127/130) *	0 (0/57)
Sleep disordered breathing		
AHI (events/h) overall	2.8±5.4 †	0.8±1.6 (n=57)

AHI>5	16% (21/130) *	5.3% (3/57)
AHI>15	5/130(4%)	0/57(0%)
Lowest O_2 %	86.7 ± 6.7 *	90.9 ± 4.1
Mean O_2 %	96.3±1.0	96.2±1.0
OSAI /AHI %	34.5±36.5‡	17.4±29.1
CSAI/AHI %	1.8±8.8	2.4±6.2
MSAI /AHI %	6.8±46.1	7.8±23.9
HI /AHI %	60.6±36.8	75.4±37.0
NREM AHI (events/h)	2.3±5.2‡	0.5±1.4
REMAHI (events/h)	5.1±11.9	1.6±4.1
HLADQB1*0602+	130/130 (100%)*	28/117(22%)
CSF Hcrt level pg/ml	18.8±1.70 (n= 28% fo the sample)	NA

BMI: body mass index; BSA: body surface area; REM: repid eye movement; MSLT: multiple sleep latency test; SOREM: sleep onset REM; MSL: mean sleep latency; AHI: apnea hypopnea index; SpO2: oxygen saturation; OSAI: obstrructive sleep apnea index; CSAI: central sleep apnea index; MSAI: mixed sleep apnea index; HI:hypopnea index; CSF: cerebrospinal fluid; Hcrt: hypocretin

*: p<0.001 †: p<0.01 ‡: P< 0 05

Table 2. Ventilatory Responses in Narcolepsy-Cataplexy versus Controls

(Mean and S D)

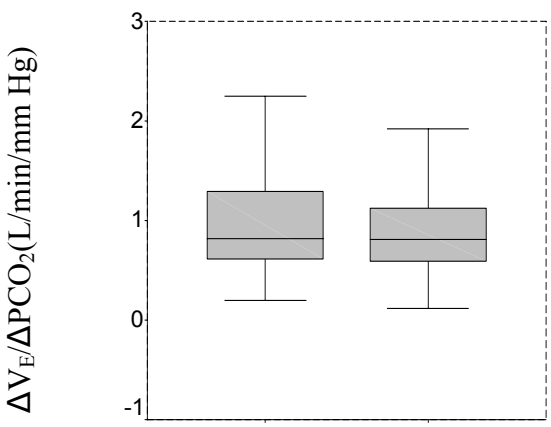
	Patients	Controls		
	(n=130)	Total (n=117)	DQ0602- (n=89)	DQ0602+ (n=28)
$\Delta\dot{V}E/\Delta PCO_2$ (L/min/mm Hg)	0.92±0.53	1.04±0.74	1.03±0.74	1.07±0.75
$\Delta\dot{V}E/\Delta PCO_2$/BSA (L/min/mmHg/$m^2$)	0.54±0.30	0.61±0.42	0.60±0.42	0.64±0.42
$\Delta\dot{V}E/\Delta$SpO2 (L/min/%SpO2)	0.13±0.09*	0.19±0.13	0.20±0.14	0.13±0.08†
$\Delta\dot{V}E/\Delta$SpO2 /BSA (L/min/%SpO2/m^2)	0.08±0.05*	0.11±0.08	0.12±0.09	0.08±0.04†

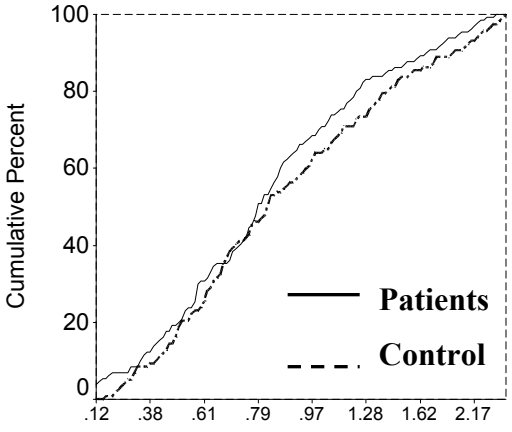
*: compared with the control, p<0.0001

†: compared with DQB1*0602-, p<0.0001

Figure 1

A B

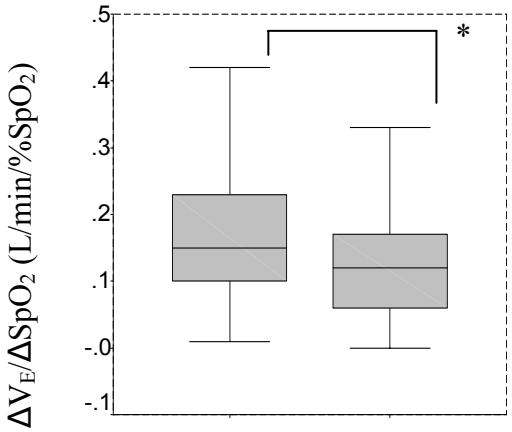


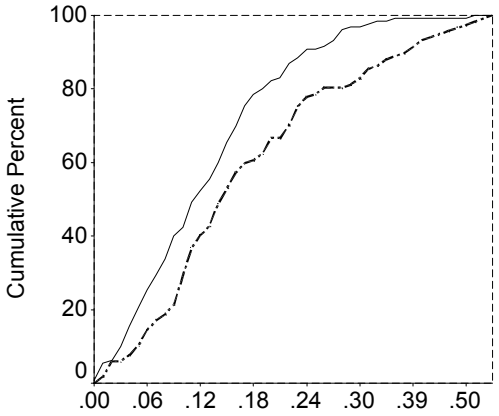


$\Delta V_E/\Delta PCO_2$(L/min/mm Hg)

C

D

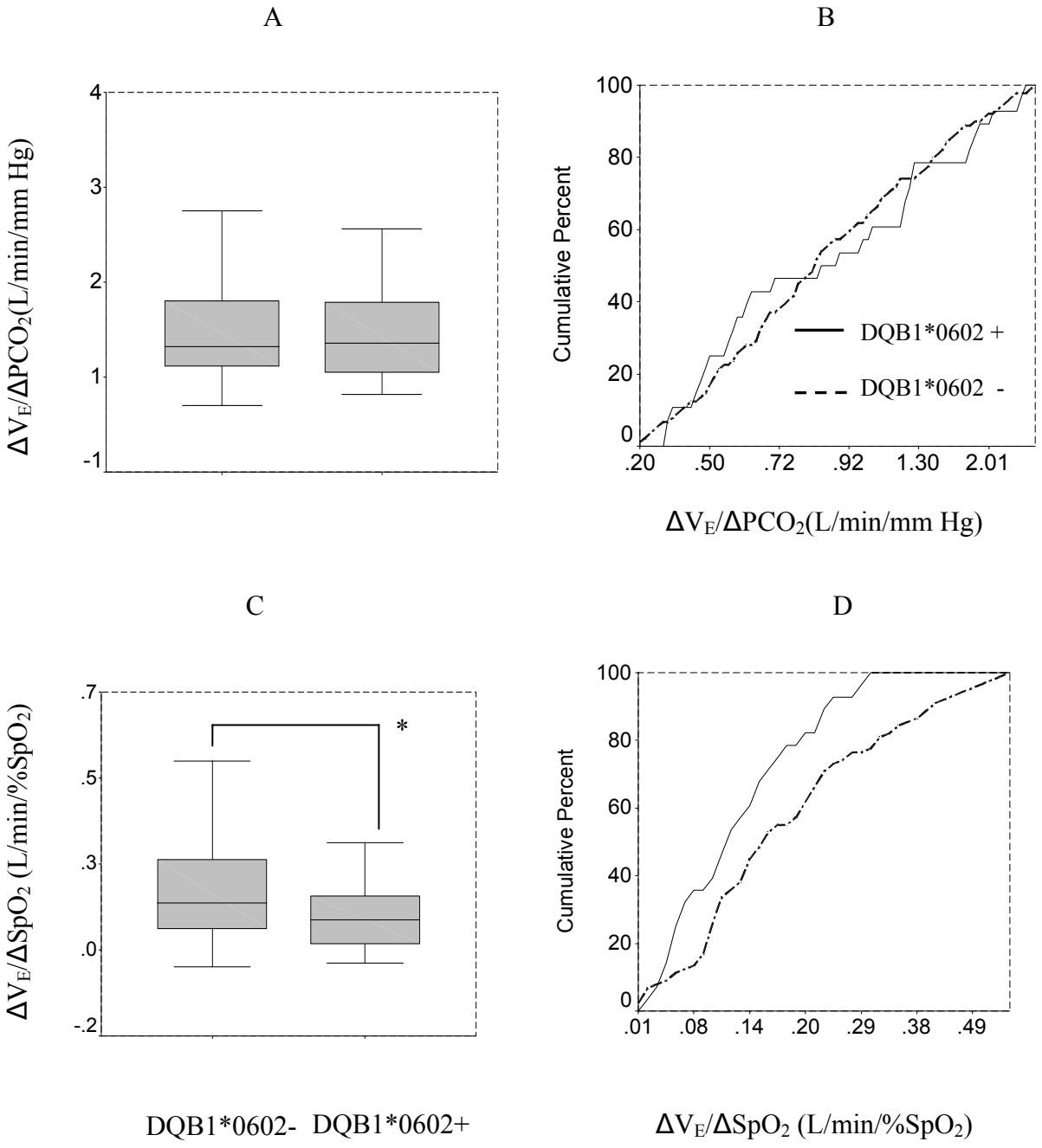




Controls Patients

$\Delta V_E/\Delta SpO_2$ (L/min/%SpO_2)

Figure 2


A
B
4
3
2
1
-1
$\Delta V_E/\Delta PCO_2$(L/min/mm Hg)
100
80
60
40
20
0
Cumulative Percent
DQB1*0602 +
DQB1*0602 -
.20
.50
.72
.92
1.30
2.01
$\Delta V_E/\Delta PCO_2$(L/min/mm Hg)
C
D
.7
.5
.3
.0
-.2
*
$\Delta V_E/\Delta SpO_2$ (L/min/%SpO_2)
100
80
60
40
20
0
Cumulative Percent
.01
.08
.14
.20
.29
.38
.49
DQB1*0602- DQB1*0602+
$\Delta V_E/\Delta SpO_2$ (L/min/%SpO_2)