Polymorphism Located between CPT1B and CHKB, and HLA-DRB1*1501-DQB1*0602 Haplotype Confer Susceptibility to CNS Hypersomnias (Essential Hypersomnia)

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Abstract

Background: SNP rs5770917 located between CPT1B and CHKB, and HLA-DRB1*1501-DQB1*0602 haplotype were previously identified as susceptibility loci for narcolepsy with cataplexy. This study was conducted in order to investigate whether these genetic markers are associated with Japanese CNS hypersomnias (essential hypersomnia: EHS) other than narcolepsy with cataplexy.

Principal Findings: EHS was significantly associated with SNP rs5770917 (\(P_{\text{alleles}} = 3.6 \times 10^{-3} \); OR = 1.56; 95% c.i.: 1.12–2.15) and HLA-DRB1*1501-DQB1*0602 haplotype (\(P_{\text{positivity}} = 9.2 \times 10^{-11} \); OR = 3.97; 95% c.i.: 2.55–6.19). No interaction between the two markers (SNP rs5770917 and HLA-DRB1*1501-DQB1*0602 haplotype) was observed in EHS.

Conclusion: CPT1B, CHKB and HLA are candidates for susceptibility to CNS hypersomnias (EHS), as well as narcolepsy with cataplexy.

Introduction

We previously reported that SNP rs5770917 located between the carnitine palmitoyltransferase 1B (CPT1B) and choline kinase beta (CHKB) genes was associated with susceptibility to narcolepsy with cataplexy after performing a genome-wide association study in Japanese and Korean populations (\(P = 1.4 \times 10^{-7} \); odds ratio = 1.68) [1]. In addition, significantly lower levels of both CPT1B and CHKB mRNA expression were observed in heterozygotes (TG) with the risk allele, as compared to homozygotes (TT) with the major allele [1]. Moreover, it is noteworthy that all narcoleptic patients with cataplexy in Japan carry a human leukocyte antigen (HLA)-DRB1*1501-DQB1*0602 haplotype [2,3]. Similar findings have been confirmed in individuals of European and African decent for whom the association is with DQB1*0602 [3,4].

Central nervous system (CNS) hypersomnias other than narcolepsy with cataplexy are also complex disorders. Both genetic and environmental factors may contribute to the development of CNS hypersomnias, as in the case of narcolepsy with cataplexy [5,6]. Identification of the genetic factors has been challenging because of the low prevalence and the difficulty of diagnosis [5,6]. Although several reports have confirmed the association between some CNS hypersomnias and HLA, HLA typing was performed at the low-resolution serological level, and the association showed marginally statistical significance (0.01 < \(P < 0.05 \)) because the number of cases was small [6–9]. Thus, in this study, we extended previous association studies to essential hypersomnia (EHS), which is a group of other CNS hypersomnias similar to narcolepsy with cataplexy in the symptom of excessive daytime sleepiness. Our diagnostic criteria for EHS comprised three clinical items: 1) recurrent daytime sleep episodes that occur basically every day over a period of at least 6 months; 2) absence of cataplexy; 3) the condition does not meet the diagnostic criteria of any other disorder causing excessive daytime sleepiness, such as sleep-apnea syndrome [9,10]. EHS is heterogeneous and consists of several CNS hypersomnias from aborted form of narcolepsy with cataplexy, narcolepsy without cataplexy and a part of idiopathic hypersomnia without long sleep time if we employed International Classification of Sleep Disorders 2nd edition criteria (AASM 2005). Our diagnostic criteria for EHS are focused on the daytime clinical characteristics of CNS hypersomnias [9,10]. A case-control

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association study was conducted in order to examine whether SNP rs5770917 and HLA-DRB1*1501-DQB1*0602 haplotype are associated with EHS.

**Methods**

Cases of EHS (n = 137) and Controls (n = 569) were unrelated Japanese living in Tokyo or neighboring areas. Written informed consent was obtained from participants, and the study was approved by the local institutional review boards of all collaborative organizations. Our diagnostic criteria for EHS comprised three clinical items: 1) recurrent daytime sleep episodes that occur basically every day over a period of at least 6 months; 2) absence of cataplexy; 3) the condition does not meet the diagnostic criteria of any other disorder causing excessive daytime sleepiness, such as sleep-apnea syndrome [9,10].

Genotyping for SNP rs5770917 in cases was performed using Taqman genotyping assays. For controls, we used the genotypic data from the previous genome-wide association study for narcolepsy with cataplexy [1]. Genotyping for HLA-DRB1 and HLA-DQB1 in cases was performed by Luminex Multi-Analyte Profiling system (xMAP) with a WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan). Briefly, target DNA was amplified by polymerase chain reaction (PCR) with biotinylated primers. The PCR amplicon was then denatured and hybridized to complementary oligonucleotide probes immobilized on fluorescent coded microsphere beads. At the same time, biotinylated PCR products were labeled with phycoerythrin-conjugated streptavidin and immediately examined with Luminex 100 (Luminex, Austin, TX). We did not perform HLA-typing in controls, instead we utilized HLA-DRB1 and HLA-DQB1 frequency data obtained from Japanese Society for Histocompatibility and Immunogenetics databank (a total of 516 Japanese general population were genotyped [http://jshi.umin.ac.jp/mhc/mhc_vol06-10/v08naka-jima_all.pdf]).

The observed associations of SNP rs5770917 and HLA-DRB1*1501-DQB1*0602 haplotype were assessed by comparing frequency differences between cases and controls using chi-squared test. Population attributable risk percentage (PAR) for the risk genotypes (SNP rs5770917 C/C and T/C) was evaluated using the following formula, \( PAR = \frac{P(RR-1)}{P(RR-1) + 1} \), where \( p \) is calculated based on genotype frequencies in healthy controls and RR indicates relative risk of the risk genotypes. Odds ratio (OR) of the risk genotypes is similar to RR because of the low prevalence of EHS and narcolepsy with cataplexy. Interactions between the two markers (SNP rs5770917 and HLA-DRB1*1501-DQB1*0602 haplotype) were calculated based on the positivity of each risk allele in EHS using the correlation coefficient.

**Results**

In order to investigate whether SNP rs5770917 is also a genetic risk marker for the development of EHS, the SNP was genotyped in 137 cases and 569 controls. We found a significant difference between cases and controls \( (P_{\text{allele}} = 3.6 \times 10^{-5}; \text{OR} = 1.56; 95\% \text{ c.i.:} 1.12-2.15) \) (Table 1). Significant deviation from Hardy-Weinberg equilibrium (HWE) was not observed in either cases or controls. To estimate the epidemiological significance of SNP rs5770917 for EHS development in the Japanese population, PAR was found to be 14.2% in our Japanese samples (Table 2).

We also conducted an association study for HLA-DRB1*1501-DQB1*0602 haplotype. Cases carried a significantly higher frequency of HLA-DRB1*1501-DQB1*0602 haplotype than the Japanese general population \( (P_{\text{positivity}} = 9.2 \times 10^{-11}; \text{OR} = 3.97; 95\% \text{ c.i.:} 2.55-6.19) \) (Table 3).

The interaction between these two markers (SNP rs5770917 and HLA-DRB1*1501-DQB1*0602 haplotype) was then calculated in EHS. No significant interaction was found \( (P = 0.20; r^2 = 0.01) \), thus suggesting that these markers independently affect susceptibility to EHS.

**Discussion**

In the present study, SNP rs5770917, which was identified in our previous genome-wide association study of narcolepsy with cataplexy [1], was also found to confer susceptibility to EHS, a group of other CNS hypersomnias similar to narcolepsy with cataplexy. Thus, the SNP is considered to be a common risk marker for CNS hypersomnias in general. Nevertheless, the OR for narcolepsy with cataplexy [1.92] was greater than that for EHS (1.57) (Table 2). Moreover, PAR for narcolepsy with cataplexy
mouse models of narcolepsy [14,15]. Moreover, fasted acetylcholine release [21]. Acetylcholine is a known REM- and treatment of disorders of a cerebrovascular nature [20], increases cells [18] and inhibition of phosphatidylcholine synthesis triggers tidylcholine is the major membrane phospholipid in mammalian mitochondria, significantly recovers slow theta frequency in catalyzing the first step of acyl-CoA dehydrogenase (encoded by Acads), an enzyme human narcolepsy [12]. Second, mice deficient in short-chain are activated by modafinil, which is used for the treatment of narcolepsy [12,13]. These phenotypes in fasted mice are similar to those in mouse models of narcolepsy [14,15]. Moreover, fasted mice are activated by modafinil, which is used for the treatment of human narcolepsy [12].

CHKB catalyzes the first phosphorylation reaction in the CDP-choline pathway for phosphatidylcholine synthesis [17]. Phosphatidylcholine is the major membrane phospholipid in mammalian cells [18] and inhibition of phosphatidylcholine synthesis triggers apoptosis in the brain [19]. CDP-choline, which is used in the treatment of disorders of a cerebrovascular nature [20], increases acetylcholine release [21]. Acetylcholine is a known REM- and wake-promoting neurotransmitter that increases narcolepsy symptoms [22]. Thus, either of these two genes (CHTB and CHKB) is a plausible candidate for susceptibility to CNS hypersomnias (EHS), as well as narcolepsy with cataplexy.

To date, reports on the association between HLA and CNS hypersomnias other than narcolepsy with cataplexy have been based on serological typing and small sample size, thus yielding only marginal statistical significance [6–9]. In this study, we found a highly significant association between HLA and EHS using DNA-based high-resolution typing (P< 9.2 × 10−11), suggesting an immunological pathogenesis for EHS.

It is important to examine the interaction between SNP rs5770917 and HLA with regard to susceptibility to CNS hypersomnias. However, it is impossible to calculate the interaction between SNP rs5770917 and HLA in narcolepsy with cataplexy, because almost all patients possess HLA-DRB1*1501-DQB1*0602 haplotype. No interaction was observed between SNP rs5770917 and HLA-DRB1*1501-DQB1*0602 haplotype, suggesting that they are independent risk factors for not only EHS but also narcolepsy with cataplexy.

EHS analyzed in this study have recurrent daytime sleep episodes but do not have cataplexy. Thus, SNP rs5770917 located between CPT1B and CHKB might be involved in the pathogenesis of excessive daytime sleepiness, not cataplexy. Although our cases are heterogeneous, as exemplified by the findings that not all patients with EHS carry HLA-DRB1*1501-DQB1*0602 haplotype, SNP rs5770917 is considered to be a common risk marker for both HLA positive and negative EHS, because no interaction was observed between SNP rs5770917 and HLA-DRB1*1501-DQB1*0602 haplotype.

Our data suggest that EHS, a group of heterogeneous CNS hypersomnias similar to narcolepsy with cataplexy, might be in continuity with narcolepsy with cataplexy in common genetic background.

**Author Contributions**

Conceived and designed the experiments: TM MH MK ST KT. Performed the experiments: TM MS. Analyzed the data: TM. Contributed reagents/materials/analysis tools: TM MH MK YH KT. Wrote the paper: TM MH KT.

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