ApoE polymorphisms in narcolepsy
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Summary

Background: Narcolepsy is a common neuropsychiatric disorder characterized by increased daytime sleepiness, cataplexy and hypnagogic hallucinations. Deficiency of the hypocretin neurotransmitter system was shown to be involved in the pathogenesis of narcolepsy in animals and men. There are several hints that neurodegeneration of hypocretin producing neurons in the hypothalamus is the pathological correlate of narcolepsy. The ApoE4 allele is a major contributing factor to early-onset neuronal degeneration in Alzheimer disease and other neurodegenerative diseases as well.

Methods: To clarify whether the ApoE4 phenotype predisposes to narcolepsy or associates with an earlier disease onset, we have genotyped the ApoE gene in 103 patients with narcolepsy and 101 healthy controls.

Results: The frequency of the E4 allele of the ApoE gene was 11% in the patient and 15% in the control groups. Furthermore, the mean age of onset did not differ between the ApoE4+ and ApoE4- patient groups.

Conclusion: Our results exclude the ApoE4 allele as a major risk factor for narcolepsy.

Background
Narcolepsy is a frequent debilitating neuropsychiatric disorder characterized by increased daytime sleepiness, cataplectic episodes and hypnopompic and hypnagogic hallucinations. The occurrence of narcolepsy is sporadic; however, a proportion of cases is familial with an autosomal-dominant type of inheritance. In contrast to the normal population with an HLA-DR2 allele frequency of ~30%, over 90% of narcoleptics type HLA-DR2++ and HLA-DQB1*0602++. The biological significance of this association remains elusive implicating autoimmune aspects in the etiology [3]. In two animal models the involvement of the hypocretin (orexin) neurotransmitter system was demonstrated. Murine narcolepsy induced by knocking out the hypocretin gene shows symptoms corresponding to human narcolepsy [4]. Dobermann pincher and Labrador breeds with autosomal recessively inherited narcolepsy each share a splice-site mutation in the hypocretin-receptor 2 gene [5]. Although hypocretin levels in CSF of most narcoleptics is decreased or not detectable [6], no causative mutations in both hypocretin receptor genes were found in humans. A single patient with atypical early-onset narcolepsy carries a dominant signal peptide mutation in the prepro-hypocretin gene [7]. Furthermore a rare sequence variant in the 5’UTR of preprohypocretin gene has been...
shown to be a risk factor for narcolepsy [8]. Recent reports describe a nearly complete loss of hypocretinergic neurons in brains of narcoleptic patients as well as scar tissue normally occupied by the hypocretin-producing cells [7,9].

Among several neurodegenerative diseases the E4 allele of the ApoE gene has been recognized as a predisposing genetic risk factor mainly influencing the age of manifestation of M. Alzheimer. The ApoE protein is a component of the VLDL particles and chylomicrons and its primary role is lipid transport [10,11]. The pathophysiological effect of ApoE4 in neurodegeneration is not clarified yet and may possibly involve diminished neuroprotection against amyloid depositions, reactive oxygen species or exitotoxins [12]. We have tested the hypothesis of the involvement of the E4 allele of ApoE in the etiology of narcolepsy.

**Methods**

Patients were recruited from the University Hospital in Mainz and St. Josef Hospital in Bochum, Germany. All but two patients suffered from cataplexies. All patients fulfilled diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM IV) and the International Classification of Sleep Disease of the American Sleep Disorders Association for narcolepsy. For further details see Gencik et al. 2000 [8]. The control group was composed of neurologically investigated 101 healthy individuals. All participants gave written, informed consent.

ApoE genotyping was performed as described [13]. The HLA-DR2 status of the patients was determined previously. 94 patients typed HLA-DR2+ and 9 patients HLA-DR2−[8]. Genotype and allele frequencies were compared with the X²–significance test. The age of onset was known in 60 patients with E4−genotypes and 13 patients with E4+ genotypes, these data were compared by the Mann Whitney test.

**Results and discussion**

Until now, only a few factors were recognized to predispose to narcolepsy. It is the major association with the HLA-DR2 allele on the one hand. Specific TNFα alleles [14] as well as the 3250T allele of the preprohypocretin gene [8] are minor contributors to the etiology on the other hand. No exogenous risk factors for narcolepsy have been recognized so far.

Recently, a novel neurotransmitter system was shown to be involved in narcolepsy in the canine disease model and in the orexin knock-out mouse. Autopsy reports of narcoleptic dogs and patients with narcolepsy pointed out possible neurodegenerative processes in areas with hypocretin-producing neurons. Taken together, the pathophysiology of narcolepsy seems to involve an autoimmune driven neurodegeneration of yet unkown cause [3,15].

In order to specify the role of ApoE isoforms in narcolepsy, we have determined the allele and genotype frequencies of the E2, E3 and E4 alleles in patients with narcolepsy and healthy controls. Allelic and genotypic frequencies are shown in table 1. No statistically significant differences were detected between nacoleptics, the DR2 subgroups and the controls. Although not significant, a tendentially increased E3 frequency was seen among the DR2+ subgroup of narcoleptics. Furthermore, in 73 narcoleptics exact age of onset could be determined. 60 patients had an non-E4, 13 patients had an E4 phenotype. The manifestation ages were 19.6 ± 9.9 years (mean ± SD) and 21.4 ± 8.6 years for the non-E4 group and E4 group, respectively. The mean difference of 1.8 years were not statistically significant (p = 0.44).

**Table 1: Allele and genotype frequencies of the ApoE gene in narcolepsy patients and controls.**

| Alleles | Patients (n = 103) | DR2+ (n = 94) | DR2− (n = 9) | Controls (n = 91) |
|---------|-------------------|---------------|--------------|------------------|
| E2      | 8% (16)           | 7.5% (14)     | 11% (2)      | 10% (18)         |
| E3      | 82% (168)         | 83% (155)     | 72% (13)     | 75% (136)        |
| E4      | 11% (22)          | 10% (19)      | 17% (3)      | 15% (28)         |
| Genotypes |                  |               |              |                  |
| E2/E3   | 14% (14)          | 14% (13)      | 11% (1)      | 18% (16)         |
| E2/E4   | 2% (2)            | 1% (1)        | 11% (1)      | 2% (2)           |
| E3/E3   | 66% (68)          | 67% (63)      | 56% (5)      | 55% (50)         |
| E3/E4   | 18% (18)          | 17% (16)      | 22% (2)      | 22% (20)         |
| E4/E4   | 1% (1)            | 1% (1)        | /            | 3% (3)           |
Conclusion
The presented results indicate, that the E4 isotype of the ApoE protein, which is an important risk factor for complex traits like alzheimer disease, parkinsonism and other neurodegenerative disorders is not involved in pathophysiologic processes in narcolepsy.

Declaration of competing interests
None declared.

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