memory subtest ($r = 0.52$, $p < 0.02$), and weakly with the total Mattis score ($r = 0.46$, $p < 0.05$). HIV RNA level correlated inversely with the memory subtest of the Mattis scale ($r = -0.47$, $p < 0.05$), but not with the total Mattis score.

Discussion. This study confirms reported metabolic abnormalities of HIV encephalopathy,[8-10] with decreased *N*-acetylaspartate and increased choline, and underscores their regional (frontal) or diffuse distribution. The longitudinal evaluation demonstrated that HAART partially reverses both clinical and metabolic abnormalities. Long-term follow-up will be needed to determine if patients with cognitive deficits recover.

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A *prepro-orexin* gene polymorphism is associated with narcolepsy

Article abstract—The orexin (hypocretin) neurotransmitter system was recently shown to be directly involved in the pathogenesis of narcolepsy in two animal models. Furthermore, decreased levels of orexin A in the CSF were shown in narcoleptic patients. To define any genetic contribution of orexin to the etiology of narcolepsy, the authors screened the entire *prepro-orexin* gene for mutations or polymorphisms in 133 patients suffering from narcolepsy. They report an association of a rare polymorphism in the *prepro-orexin* gene with narcolepsy in a cohort of 178 patients.

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M. Gencik, MD; N. Dahmen, MD; S. Wieczorek, Cand. med.; M. Kasten, Cand. med.; J. Bierbrauer, MD; I. Anghelescu, MD; A. Szegedi, MD; A.M. Menezes Saecker; and J.T. Epplen, MD

Narcolepsy is a debilitating disorder of the sleep/wake regulation characterized by excessive daytime sleepiness, short episodes of involuntary muscle relaxation (cataplexy), sleep paralysis, and hypnagogue and hypnopompic hallucinations. The etiopathogenesis of narcolepsy has remained unclear despite a strong human leukocyte antigen (HLA) association. Although the HLA-DR2 and the linked HLA-DQB1*0602 allele is found in >95% of narcoleptic patients but only in 8 to 35% of normal populations,[1] the pathogenetic role of the HLA system has remained unclear, especially because autoimmune phenomena are not obvious. Recently, abnormalities in the orexin neurotransmitter system have been shown to cause narcolepsy-related symptoms in an *orexin*-knockout mouse model,[2] as well as in narcoleptic breeds of Doberman pinschers and Labrador retrievers.[3] Finally, orexin A was not detected in seven of nine patients with narcolepsy, indicating disturbed orexin transmission (limit of the assay: 40 pg/mL). All nine healthy controls showed orexin A levels ranging from 250 to 285 pg/mL.[4]

Orexin A and orexin B are both encoded by the *prepro-*

From the Department of Molecular Human Genetics (Drs. Gencik and Epplen, S. Wieczorek and A.M. Menezes Saecker) and Neurologische Klinik (Dr. Bierbrauer), St. Josef-Hospital, Ruhr-University, Bochum; and Psytchiatrische Klinik der Johannes-Gutenberg-Universität Mainz (Drs. Dahmen, Anghelescu, Szegedi, and M. Kasten); Germany.

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J.B. is currently affiliated with Klinik für Psychiatrie und Psychotherapie II der Johann Wolfgang Goethe-Universität, Frankfurt, Germany.

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Address correspondence and reprint requests to Dr. med. Martin Gencik, Molecular Human Genetics, Ruhr-University, D-44780 Bochum, Germany; e-mail: martin.gencik@ruhr-uni-bochum.de

Table *Primers for amplification of the* prepro-orexin *gene*

Forward primer		Reverse primer		Length, bp	Restriction enzyme
PIF:	5′-CCTCATTAGTGCCCGGAGA	PIR:	5′-ATTGTGACCCACTCCCAGG	258	∅
PIIF:	5′-TAGTGGAAAGGGCAGAAG	PIIR:	5′-ATCACCCCCTTGTCTGTCTAT	338	Bsh1236I
PIIIF:	5′-AGAATCGCTTGAACCCAAA	PIIIR:	5′-TTCATGGAAAGGCTCCTTAG	262	∅
E1F:	5′-CGCCACCTCAGAGCACTA	E1R:	5′-TGAGTCACCCCTCCATCC	182	∅
E2F:	5′-AGGTCCTGGGCGTGGGAGCT	E2R:	5′-GGTGGGCAGAGGGCAGAGGC	497	EagI
IAF:	5′-TGAACCTTCCTTCCACAA	IAR:	5′-AGCAGGGAAACACATGGAAA	447	SacI
IBF:	5′-GGGGCAGGCACATGAGAGAGCA	IBF:	5′-ACGGCGGCCCAGGAGACCTA	472	DdeI

orexin gene on human chromosome 17q21–22. The gene consists of two exons and one intron. Exon 2 encodes a propeptide from which the orexin A and B are cleaved proteolytically.[5] To define any contributions of genetic variations in the *prepro-orexin* gene to the etiology of narcolepsy, 178 individuals with clinically proven narcolepsy and 189 matched healthy individuals were analyzed genetically for mutations in the *prepro-orexin* gene.

Methods. Patients were recruited from the University Hospital in Mainz and St. Josef Hospital in Bochum, Germany, and 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. All but three patients reported cataplectic attacks. In these three patients, the diagnosis was established by multiple sleep latency tests (MSLT) and polysomnographic findings in addition to the presence of other REM-associated clinical symptoms. All diagnoses were checked by an experienced physician (N.D.). Most narcoleptic patients visited our department to participate in a narcolepsy research program or were visited by a member of the research team at home. Narcolepsy symptoms, severity of symptoms, and the total duration of various aspects of the symptomatology were assessed by a semistructured clinical interview. The clinical interview included symptom checklists for narcolepsy, including MSLT and polysomnography. Patients who had not undergone these diagnostic procedures were only admitted to the study when unambiguous cataplexies, additional REM-associated symptoms, and severe daytime sleepiness were reported (n = 13). To exclude symptomatic narcolepsies, medical history was assessed and a neurologic examination was performed. The control group included 189 matched healthy individuals, 89 of whom were extensively neurologically investigated. All participants gave written, informed consent. The study design was approved by the local ethics committee.

A 770-bp region of the *prepro-orexin* promoter and both exons and the intron were amplified in five different PCR systems (table). One hundred thirty-three narcoleptic patients were screened by single-strand conformation polymorphism (SSCP) analysis with at least two different polyacrylamide gel electrophoretic conditions. PCR products longer than 270 bp were digested with restriction endonucleases (see the table). Shifted bands were eluted out of the gel, reamplified, and directly analyzed on a 377ABI automatic sequencer (PE Biosystems). For confirmatory purposes, the newly detected C3250T polymorphism in the 5′ untranslated region (UTR) was typed in 45 additional narcoleptic patients. HLA-DR2 typing was performed as described.[6] Allele frequencies of patients with narcolepsy and the control group were compared; significance was calculated by χ^2-test.

Results. The mutation analysis in the *prepro-orexin* revealed a sequence variation C3250T (reference accession number AF118885) in the 5′ UTR, 22 bp 5′ from the start codon. The 3250T allele was present in six of 178 narcoleptic patients, as well as in one healthy control subject out of 189 ($p < 0.05$, OR = 6.5).

Furthermore, an insertion of a single nucleotide A at position 2679 (reference sequence accession number AF118885) in a single patient was detected. This variation is situated in a tract of seven consecutive adenine residues in the promoter region. The functional relevance is not yet clear. No other polymorphisms could be detected in the investigated region of more than 2 kb of the *prepro-orexin* gene.

To define the HLA-DR2 status in $3250T^+$ patients, HLA-DR typing was performed. All six patients were HLA-$DR2^+$. These results are in agreement with the described HLA-DR distribution in narcoleptic patients. The only healthy individual carrying the 3250T allele was typed as HLA-DR2 negative.

Discussion. The data described above provide a suggestive evidence for a direct involvement of the *prepro-orexin* gene in the etiology of human narcolepsy. Yet, there are major differences between the animal models and the human association demonstrated. Firstly, human narcolepsy appears to be transmitted in most cases as a multifactorial trait, implying that the 3250T allele predisposes together with other genetic or environmental factors. Conversely, mutations in genes of the orexin system are causative in the aforementioned animal models. Whether the effects of the 3250T allele and the DR2/DQB1*0602 haplotype act synergistically is not yet known. The coexistence of the 3250T allele and HLA-DR2 in all six indicates an independent etiologic contribution. Furthermore, the effect of the 3250T allele influences only a small proportion of patients with narcolepsy (3%), supporting the concept of genetic heterogeneity. We need to clarify whether there are any $3250T^+$ healthy individuals who harbor the DR2/DQB1*0602 haplotype.

Peyron et al.[7] described a 18-year-old man who is carrier of a leucine-to-arginine mutation in the sig-

naling sequence of the orexin peptide. This patient was characterized by a very early onset of symptoms (cataplexies since the age of 6 months) and a particularly severe course of the narcolepsy, in addition to the presence of periodic leg movements and bulimia. This observation highlights the possibility of a genetic heterogeneity in narcolepsy, and prompted us to closely examine the symptomatology of our $3250T^{+}$ patients. However, no outstanding clinical features among $3250T^{+}$ patients were observed. The age at onset of symptoms was 7, 15, 15, 30, 32, and 36 years. All patients exhibited cataplexies, sleep attacks, automatic behavior, and sleep paralysis. Four of the six patients had hypnagogic hallucinations.

Finally, the traits in the *orexin*-knockout mouse and the narcoleptic dog breeds are both inherited in an autosomal recessive manner. In contrast, all $3250T^{+}$ patients were typed heterozygous, suggesting dominant influence. It is conceivable that the 3250T allele acts directly or via linked sequence variants to influence the expression of *orexin* negatively by diminished transcriptional activity or decreased RNA stability. However, the functional relevance has not been addressed directly in this study and remains to be established by independent means.

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