Novel genetic findings in an extended family pedigree with sleepwalking

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ABSTRACT

Background: Sleepwalking is a common and highly heritable sleep disorder. However, inheritance patterns of sleepwalking are poorly understood and there have been no prior reports of genes or chromosomal localization of genes responsible for this disorder.

Objective: To describe the inheritance pattern of sleepwalking in a 4-generation family and to identify the chromosomal location of a gene responsible for sleepwalking in this family.

Methods: Nine affected and 13 unaffected family members of a single large family were interviewed and DNA samples collected. Parametric linkage analysis was performed.

Results: Sleepwalking was inherited as an autosomal dominant disorder with reduced penetrance in this family. Genome-wide multipoint parametric linkage analysis for sleepwalking revealed a maximum logarithm of the odds score of 3.44 at chromosome 20q12-q13.12 between 55.6 and 61.4 cM.

Conclusion: Sleepwalking may be transmitted as an autosomal dominant trait with reduced penetrance. Here we describe the first genetic locus for sleepwalking at chromosome 20q12-q13.12. *Neurology*® 2011;76:49-52

Sleepwalking is a parasomnia that is defined by The International Classification of Sleep Disorders Diagnostic and Coding Manual as “a series of complex behaviors that are initiated during slow-wave sleep and result in walking during sleep.”¹ It is a common childhood sleep disorder that usually resolves by adolescence but can persist into adulthood.² The Finnish Twin Cohort study of 11,220 subjects found a sleepwalking prevalence rate of approximately 26% in childhood and 3% in adulthood.²

Previous studies suggested a genetic basis for sleepwalking. The Finnish Twin Cohort study revealed a concordance rate that was approximately 1.6 times greater in monozygotic vs dizygotic twins for childhood sleepwalking and approximately 5.3 times greater for adult sleepwalking.² In a separate study, first-degree relatives of sleepwalkers were shown to have at least a 10-fold increased likelihood of sleepwalking over that of the general population.³ In a small series, an association of sleepwalking with the HLA DQB1*0501 antigen was described.⁴

A mode of inheritance for sleepwalking has not been consistently demonstrated. A multifactorial method of inheritance was suggested based on segregation analysis of 25 families.³ However, a recessive model with incomplete penetrance has also been proposed.⁵ Here we describe a previously unreported inheritance pattern in a large family with a history of sleepwalking.

METHODS DNA was obtained from saliva samples (OraGene Self-Collection kit, DNAGenotek, Ottawa, Canada) from 22 Caucasian family members consisting of 9 affected and 13 unaffected family members. Human Subjects Committee approval was obtained for this study, and written informed consent was obtained from all participants. Diagnosis of sleepwalking followed the International Classification of Sleep Disorders criteria.¹ All family members were evaluated in person and a spouse or parent provided additional information about each individual's sleep patterns. Individuals were asked about the presence of sleepwalking, night terrors, confusional arousals, sleep apnea, nightmares, seizures, headaches, insomnia, daytime somnolence, cataplexy, restless legs, sleep paralysis, hypnopompic and hypnagogic hallucinations, and other medical problems.

A genome-wide search was undertaken using Affymetrix GeneChip Mapping 10K XbaI Array data, containing 10,055 single nucleotide polymorphisms. Call rates were >93%. Linkage programs were accessed through the easyLinkage package.⁶ All genotype

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Study funding: Supported in part by the Children's Discovery Institute.

Disclosure: Author disclosures are provided at the end of the article.

inconsistencies were zeroed out from the database. Linkage was performed assuming autosomal dominant inheritance with 70% penetrance, disease allele frequency of 0.1%, and 1% phenocopies. A 2-point parametric simulation analysis was performed to generate a maximal lod score using FastSLINK, using a total of 1,000 replicates for a biallelic marker (i.e., single nucleotide polymorphism). Multipoint analysis was performed with GENEHUNTER using sets of 100 markers, and repeated with sets of 60 markers. Haplotypes were created with GENEHUNTER and viewed on HaploPainter.⁷ The lod score graphs were generated with EasyLinkage software.

The coding sequence and 5' and 3' UTR regions of candidate genes were resequenced with the Big Dye Terminator Cycle Sequencing Ready Reaction sequencing kit (Life Technologies, Carlsbad, CA). Reactions were fractionated on an ABI PRISM 377 DNA sequencer and analyzed with Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI). To exclude the presence of a causative chromosomal copy number variant in the linkage region, a chromosomal microarray evaluation of the proband was performed using the Affymetrix 6.0 array.

RESULTS We identified a 4-generation Caucasian family in which sleepwalking segregates as an autosomal dominant condition with reduced penetrance (figure 1). The proband was a child who had been referred for sleepwalking since age 6. In addition, there were 8 other family members with an ongoing history of sleepwalking, including the proband's father. All adults had onset of sleepwalking from the ages of 4 to 10 years and continued to have sleepwalking episodes into their 30s, although with reduced frequency from childhood. Except for the proband, none had sought medical treatment for their sleep disorders.

Seven of the 9 affected individuals were male. All but 2 affected individuals were >18 years of age at the time of the study; the youngest individual was 7 years old. Three individuals recalled episodes of sleep paralysis. Only one of the affected individuals was overweight, and none endorsed symptoms of sleep apnea. Two of the carrier females endorsed daytime somnolence with no other symptoms of sleep disturbance. No family members had a history of seizures.

Linkage analysis for sleepwalking revealed a maximum multipoint logarithm of the odds (lod) score of 3.44 at chromosome 20q12-q13.12 between 55.6 and 61.4 cM (figure 2). For comparison, the maximal simulated 2-point lod score calculated by FastSLINK was 3.56. Chromosomal microarray evaluation of the proband detected no novel copy number variants within the linkage region.

Haplotype analysis demonstrated 7 contiguous single nucleotide polymorphisms at chromosome 20q12-q13.12 that were common to all individuals with sleepwalking in this family (figure 1). A critical centromeric recombination event occurred in individual 34 and a critical telomeric recombination occurred in individual 18 that delineated the linkage

interval to a 5.8-cM (4.4-Mb) region of chromosome 20q12-q13.12.

The linkage region at chromosome 20q12-q13.12 contains approximately 28 known or predicted genes. The coding sequences of 10 genes (*TOP1*, *PLCG1*, *PTPRT*, *L3MBTL*, *TOX2*, *JPH2*, *GDAP1L1*, *C20orf100*, *PKTG*, *ADA*) were sequenced for 2 affected individuals. No coding mutations were identified.

DISCUSSION Our identification of a family with a high prevalence of sleepwalking confirms previous reports that support a strong genetic basis for this condition.²⁻⁴ Various inheritance models have been proposed for sleepwalking, including multifactorial,³ autosomal recessive,⁵ and now autosomal dominant with reduced penetrance; it is possible that sleepwalking is genetically heterogeneous. Identification of the genes responsible for sleepwalking will be critical to improving our understanding the etiology, diagnosis, and treatment of this disorder. Optimizing treatment for sleepwalking is an important clinical objective due to its potential to cause injury. Our report of chromosome 20q12-q13.12 localization for a gene responsible for sleepwalking is a first step in the process of gene discovery.

The genetic bases of other sleep disorders have recently been described, including narcolepsy, restless legs syndrome, sleep apnea, and chronic primary insomnia.⁸ Symptoms of these sleep disorders were not endorsed by affected members of this family, and none of the genes identified for these disorders are located within our candidate region for sleepwalking on chromosome 20q12-q13.12.

The adenosine deaminase gene (*ADA*) is the most likely candidate gene in the chromosome 20q12-q13.12 linkage interval due to its association with slow-wave sleep during which sleepwalking occurs. Inhibition of adenosine metabolism has been shown to increase EEG slow wave activity, and caffeine, an A₁ and A_{2a} adenosine receptor antagonist, reduces slow-wave sleep when administered prior to sleep onset.⁹

Individuals with the G/A genotype at nucleotide 22 in the coding region of *ADA* demonstrated approximately 30 minutes more slow wave sleep on polysomnogram during an 8-hour sleep period than individuals with the G/G genotype.¹⁰ Slow-wave sleep duration may be a critical determinant of sleepwalking frequency, since it was found that longer duration of slow-wave sleep during recovery from sleep deprivation significantly increased the frequency of sleepwalking events.¹¹ However, the less frequent and potentially at risk G/A *ADA* genotype was present only once in this pedigree in an unaffected individual. Although we detected no coding mutations in sequencing the *ADA* gene, it is possible that insertions or deletions or

Figure 1 Pedigree of family with apparent autosomal dominant inheritance of sleepwalking with reduced penetrance

A haplotype consisting of 7 single nucleotide polymorphism markers on chromosome 20q12-q13.12 (red) segregates with all affected individuals with history of sleepwalking. Affected individuals are shaded black. The proband is indicated with an oblique arrow. Meiotic recombinants in individuals 34 and 18 delineate the critical linkage interval to 5.8 cM (4.4 Mb) between SNP_A-1514188 and SNP_A-1518796.

mutations in noncoding regulatory regions that alter *ADA* expression are responsible for a sleepwalking phenotype. The remaining 27 candidate genes in the linkage region at chromosome 20q12-q13.12 may also have a role in causing sleepwalking.

An increased prevalence of sleepwalking was noted in males in this family, with several nonmanifesting carrier females. Other studies have described sex-related variation in sleepwalking. In the Finnish Twin Cohort study of 11,220 participants, there was

Figure 2 Linkage of sleepwalking to chromosome 20q12-q13.12

Genome-wide parametric multipoint lod score profile for sleepwalking in family SW01 based on the Affymetrix 10K array data. Human chromosomes are concatenated from the p-terminal (left) to the q-terminal (right) on the x-axis. The parametric lod score is on the y-axis and is correlated to the physical location of human chromosome on the x-axis. lod scores >2 are seen on chromosomes 20q12-q13.12 and 7q.

a greater rate of childhood sleepwalking in girls but the opposite was true in adulthood.² Also, an increased male-to-female ratio, in which 70% of the 60-patient cohort presenting with sleepwalking were male, was described in another study.⁴

The sleepwalking severity in members of the family described in the current study was atypical in that it continued with regularity into adulthood.² Additional studies are needed to determine whether sleepwalking that persists into adulthood has different epidemiologic or genetic features than the more typical childhood-limited disorder.

ACKNOWLEDGMENT

The authors thank the NIH Neuroscience Microarray Consortium for performing the Affymetrix microarray analyses.

DISCLOSURE

Dr. Licis has received research support from Philips Respironics, the NIH (UL1RR024992 [postdoctoral scholar]), and a T32 research fellow training grant. Dr. Yamada may accrue revenue on a patent re: Intranasal leptin for treatment of seizures; receives research support from the NIH (NS32636 PPG [core PI] and NS57105 [core coinvestigator]); and his spouse has received speaker honoraria from Eli Lilly and Company, Pfizer Inc., Forest Laboratories, Inc., GlaxoSmithKline, and Teva Pharmaceutical Industries Ltd. Dr. Duntley has served on the speakers' bureau for Cephalon, Inc.; receives research support from Boehringer Ingelheim, XenonPort, Inc., Pfizer Inc., and Cephalon, Inc.; and has provided expert witness testimony in a legal proceeding. Dr. Gurnett has received research support from the NIH (K12 HD001459-08 [trainee]).

Received May 10, 2010. Accepted in final form August 16, 2010.

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