Two Pedigrees of Familial Advanced Sleep Phase Syndrome in Japan

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Study Objectives: To determine whether a known missense mutation (bp2106 A/G) in *hPer2* (a human homolog of the Drosophila period gene) for familial advanced sleep phase syndrome in a Caucasian family is involved in Japanese familial advanced sleep phase syndrome pedigrees.
Measurements and Results: We identified 2 new Japanese families with advanced sleep phase syndrome, and a systematic survey was carried out in 28 relatives of theses 2 families. A total of 9 affected subjects were identified. The affected members showed significantly strong morningness tendencies compared with the unaffected members in various circadian parameters including the Horne-Ostberg Morningness-Eveningness Questionnaire score (77.3 ± 4.8 vs 57.5 ± 7.6, $p < 0.001$), average sleep-onset time (20:45 ± 75 min vs 23:16 ± 64 min, $p < 0.02$), and average wake time (4:55 ± 38 min vs 6:13 ± 25 min, $p < 0.01$), as well as saliva dim-light melatonin-onset time (20:15 ± 21 min vs 22:25 ± 65 min, $p < 0.02$). DNA samples were obtained from 7 affected and 7 unaffected subjects. None of the tested subjects possessed the missense mutation (bp2106 A/G) in *hPer2*. Furthermore, there is no significant linkage between affected subjects with *hPer2* region by 2-point mapping and by direct sequencing of 23 exons of *hPer2*.
Conclusion: These findings support the notion of genetic heterogeneity of familial advanced sleep phase syndrome cases in humans. The search for more familial advanced sleep phase syndrome cases and for loci other than *hPer2* are necessary to further examine the roles of circadian-related genes in genetically determined human circadian rhythm disorders.
Key Words: familial advanced sleep phase syndrome, *hPer2*, phenocopy, circadian rhythm
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INTRODUCTION

ADVANCED SLEEP PHASE SYNDROME (ASPS) IS A DISORDER IN WHICH THE MAJOR SLEEP EPISODE IS ADVANCED IN RELATION TO THE DESIRED CLOCK TIME, resulting in symptoms of compelling evening sleepiness, early sleep onset, and awakening earlier than desired. ASPS has been assumed to be a very rare sleep disorder, and there are only a small number of reports about this syndrome.[1-5] ASPS has received recent attention from the standpoint of molecular genetics because of the discovery of 3 Caucasian kindreds with ASPS, segregated as an autosomal dominant trait with high penetrance.[6] Linkage analysis of familial ASPS (FASPS) revealed an involvement of a missense mutation in a circadian clock-related gene (h*Per2*, a human homolog of the Drosophila period gene) among 1 of the pedigrees[7]: a serine to glycine mutation (bp2106 A/G) was segregating with the affected phenotype, although some affected members in a branch of the family do not have this mutation. This single base-pair mutation has been shown to diminish the phosphorylation of this amino acid site on the h*Per2* protein and is thought to accelerate the circadian cycle through earlier accumulation of h*Per2*, which triggers the negative feedback loop for clock genes, including h*Per2*. This suggests the direct involvement of this mutation in the pathophysiology of these FASPS cases. However, Reid et al recently reported another FASPS pedigree,[8] in which the affected family members possessed no h*Per1*, h*Per2*, and h*Per3* gene mutations.[9] These findings suggest the possibility of a genetic heterogeneity in the FASPS phenotype, but more extensive mutation screenings (especially for h*Per2*) may also be needed.

Here, we are reporting 2 Japanese families with FASPS who have neither linkage with h*Per2* nor with h*Per2* gene missense (bp2106 A/G) in affected subjects. The proband in Family A exhibited a severe advanced phase of the sleep-wake pattern (a 37-year-old man, Figure 1). His wife consulted with sleep clinics because their marriage was at risk from the incompatibility of their sleep-wake times. He suffered from an inability to remain alert during the early evening hours, even when he was required to work overtime. (He usually slept just after dinner, around 7 PM, and awoke before 4 AM). The proband in Family B (32-year-old woman, Figure 1) arrived at our outpatient clinic, also complaining of the early timing of her sleepiness in the evening. Usually, she slept before 6 PM and woke up before 2 AM. Since some of the family members of these probands exhibited similar symptoms, we conducted a systematic survey of 28 relatives in these 2 families (all of whom gave written informed consent). Identification of the ASPS phenotype was by psychiatrists and physicians specializing in sleep on the basis of a combination of the following: International Classification of Sleep Disorders (ICSD) criteria, a structured diagnostic psychiatric interview for DSM-IV disorders, a sleep diary maintained by the subject for over 4 weeks, an actigraph recording, the Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ),[10] and the determination of the salivary dim-light melatonin-onset time (sDLMO). The time of the first saliva sample in which the level of melatonin exceeded the mean level of all prior samples by at least 2 standard deviations was used as a circadian phase marker. Diagnosis of ASPS was based on the criteria of Reid et al.[8] Subjects who met the ICSD criteria for ASPS with a morning-type MEQ score (70 - 86 points) or had an advanced shift in the sDLMO of more than 2 hours beyond normal were considered to exhibit the ASPS phenotype.

A total of 9 affected subjects were identified: 5 subjects were identified as *affected/confirmed*, 1 as *possibly affected*, 3 as *said to be affected*, and 19 as *unaffected* (Figure 1). The Family A proband's 10-year-old daughter was diagnosed as being possibly affected because her MEQ score was less than 70 (60 points) and sDLMO data were lacking (in spite of her advanced sleep phase and distinct impression of remarkable morningness by herself and her family members). Each of 3 said-to-be-affected subjects were diagnosed by possessing a life history that strongly suggested the affliction of ASPS but lacking objective data due to death, dementia, or personal matters.

The average MEQ score of affected subjects was 77.3 ± 4.8, which was significantly higher than the 57.5 ± 7.6 score of unaffected members

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($p < 0.001$). Morningness was recognized in all affected members who were at least 20 years of age. The earliest confirmed onset was at age 7 years. Only 4 out of 421 people in a survey of the general Japanese population (data not shown) showed MEQ scores more than 70 (73 point at highest), suggesting the remarkable morning-lark trait for the affected members in the present FASPS families. Sleep and melatonin-phase parameters for affected and unaffected members were as follows: sleep-onset time, 20:45 ± 75 minutes versus 23:16 ± 64 minutes ($p < 0.02$); wake time, 4:55 ± 38 minutes versus 6:13 ± 25 minutes ($p < 0.01$); total sleep time, 8.18 ± 1.78 hours versus 6.95 ± 1.00 hours (NS); and sDLMO 20:15 ± 21 minutes versus 22:25 ± 65 minutes ($p < 0.02$), respectively.

In the 14 members of the 2 Japanese FASPS families we studied (7 affected and 7 unaffected), 2-point mapping of the 2qter region of the h*Per2* showed no significant linkage with h*Per2*. Direct sequencing of the total 23 exons (GenBank accession number, NM_022817.1) of affected and unaffected family members revealed a wild type in bp2106 (A) for all subjects tested. We also found known silent single nucleotide polymorphisms (SNPs) bp2087 A/G, bp2114 A/G, and bp2117 A/G and a new silent SNP, bp3563 C/G, in the coding region. However, none of these SNPs was linked with FASPS phenotype. In addition, we observed SNP bp111 C/G (IMS-JST accession number, 061739) in the 5' untranslated region of Ex2. Interestingly, this SNP was observed only in 2 affected and 1 said to be affected member of kindred B. The meaning of this finding remains unclear at this time.

In FASPS subjects in these 2 families, the differences in sleep and melatonin-phase parameters between affected and unaffected members were not as dramatic as we would have expected from their MEQ scores. More specifically, an affected subject had later sleep-onset time (21:53 on average) than the earliest sleep-onset time (21:22) in a 61-year-old unaffected family member (whose sleep-onset time has started earlier with advancing age). Also, 2 affected subjects had a later wake time (6:05 and 6:17) than the earliest (5:39) and the second earliest wake time (6:01) in unaffected family members (one of whom had to wake up early in the morning due to her living needs and the other was the above-mentioned 61-year-old unaffected member). However, these affected subjects who had a sleep phase that overlapped with unaffected subjects were diagnosed as suffering from ASPS based on their distinct and long-lasting early timing of sleepiness and morning-type MEQ scores. Diagnostic interviews with these subjects suggested that changes in their sleep parameters might be offset by the desire to adapt socially to the schedules of other family members and coworkers. In fact, sleep-onset time (which could be fictitiously modified) generally varied more than did the wake time (which depends more on the circadian system) in the affected subjects, resulting in sleeping less on weekdays than on weekends. As Jones suggested,[6] we also observed that affected members tended to fall asleep and wake up even earlier during vacation times when they did not have social obligations.

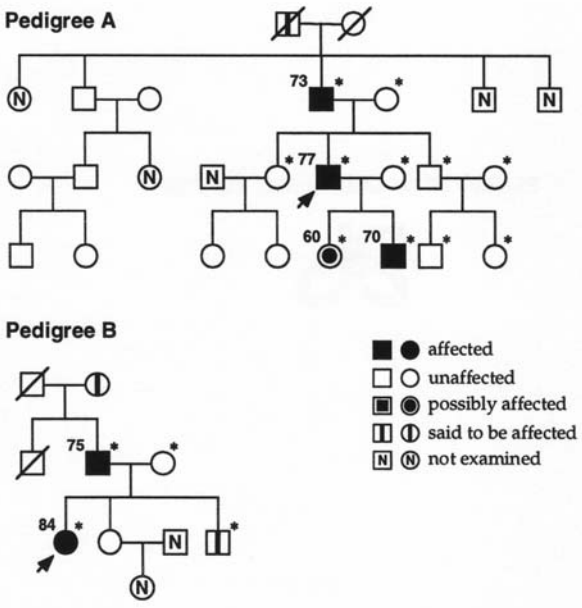

Figure 1—Pedigrees of 2 kindreds with familial advanced sleep phase syndrome. Arrows indicate probands. Numbers are Morningness-Eveningness Questionnaire scores of affected and possibly affected members. * indicates blood donor for genotyping.

These findings suggest the difficulty in diagnosing ASPS cases with accuracy by the sleep phase under their usual living conditions and with the currently available criteria. Measuring the free-running period in affected subjects may offer another reliable source of information; however, it often imposes a burden on test subjects, and an adequate isolation facility is required to conduct the measures. We, therefore, used sDLMO measures to determine the circadian phase of the subjects. While all of the present affected subjects had MEQ scores of higher than 70 (which met with the ASPS criteria by Reid et al), the degree of advanced shift of sDLMO in the affected subjects was not as robust as was their sleep-phase shift. Since normative data of sDLMO in Japanese is not yet available, the sensitivity and specificity of this measure to detect ASPS are not yet known, and, thus, further evaluations are needed.

We observed that none of the affected subjects in the 2 Japanese families with FASPS had the previously reported missense mutation in h*Per2*, and we did not identify any significant linkage with other loci in h*Per2*, suggesting the genetic heterogeneity of FASPS in humans. This finding is not surprising considering the fact that mutation or SNPs of alternative clock genes could also change the translation or transcription rate of the circadian feedback loop or loops, and the search for more FASPS cases are necessary. Our results also suggest that establishment of a more uniform criteria, including the sleep-phase determination, MEQ scoring for persons of younger age, and estimating the influence of aging, as well as a identifying a cutoff point for circadian phase markers, is necessary in order to integrate the results found in each FASPS family.

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