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"No Thanks, Coffee Keeps Me Awake": Individual Caffeine Sensitivity Depends on *ADORA2A* Genotype

Commentary on Byrne et al. A genome-wide association study of caffeine-related sleep disturbance: confirmation of a role for a common variant in the adenosine receptor. SLEEP 2012;35:967-975.

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Each aspect of sleep is a highly complex phenotype and little is currently known about their molecular bases. Nevertheless, genetic studies in model organisms have uncovered sleep regulatory mechanisms and distinct neurochemical processes that are conserved from *Drosophila* to rodents to humans.[1] Although no dedicated "sleep genes" have been identified (if they exist), the adenosine neuromodulator/receptor system is believed to play an important role in sleep and sleep-wake regulation. Support for this hypothesis comes from the finding that the wake-promoting effects of caffeine, the major stimulant in the world, are mediated by antagonistic interaction with adenosine A_1 and A_{2A} receptors.[2,3]

Since people drink coffee, it is well-known that some individuals are sensitive to its stimulant effects whereas some others are not. The behavioral actions of caffeine in humans were first studied scientifically 100 years ago by Harry Levi Hollingworth.[4] Hollingworth's studies set a new standard in psychopharmacological research because for the first time, they included double-blind and placebo-controlled experimental design. With respect to sleep disturbances, he concluded that "a few individuals show complete resistance to the effects of small doses of caffeine" (p. 100). Until recently, the biological reasons for these inter-individual differences remained unknown. Now we know they have a basis in genetics.

Because no consistent differences in caffeine pharmacokinetics were found between caffeine sensitive and insensitive subjects, Goldstein and colleagues proposed that endogenous diversity at the site of action of caffeine could influence its effects on sleep.[5] Work in mice provided strong evidence that the stimulant promotes wakefulness primarily by blocking the A_{2A} subtype of adenosine receptors.[6] Thus, a moderate dose of caffeine (15 mg/kg) failed to disrupt sleep in mice with genetically abolished A_{2A} receptor function. Conversely, the stimulant potently promoted wakefulness by 3-4 hours in wild-type animals, as well as in transgenic mice without functional A_1 receptors. In accordance with these findings, a pharmacogenetic study in humans also suggested that common variation of the A_{2A} receptor gene (*ADORA2A*) contributes to individual sensitivity to caffeine effects on sleep.[7] More than 20,000 individuals were addressed with a brief questionnaire about self-rated caffeine sensitivity and sleep, and 4,329 people responded. Caffeine consumption was associated with subjectively reduced sleep quality in caffeine-sensitive respondents, but not in caffeine-insensitive respondents. Moreover, the distribution of individuals carrying C/C and T/T alleles of the c.1976T > C single nucleotide polymorphism (SNP) of *ADORA2A* (SNP-ID: rs5751876) differed between caffeine-sensitive and caffeine-insensitive individuals. Double-blind administration of the stimulant (2 × 200 mg) confirmed the classification of caffeine sensitivity based on questionnaire, whereas caffeine concentration in saliva did not differ. Intriguingly, the stimulant induced sleep EEG characteristics of insomnia in genotype-dependent manner. The results strongly suggested that genetic variation of *ADORA2A* is a determinant of individual sensitivity to subjective and objective effects of caffeine on sleep.

Independent replication is essential for establishing a credible genotype-phenotype association.[8,9] In this issue of *SLEEP*, Byrne and colleagues[10] report the independent confirmation of a role for *ADORA2A* in caffeine-related sleep disturbances. These authors conducted a genome-wide association study (GWAS) in a large number of twins and their families of the Australian Twin Registry (n = 2,402). More than 2 million common SNPs were examined. Caffeine-associated sleep disturbance was based on the participants' report of whether or not they have ever experienced caffeine-induced insomnia, statistically corrected by a "general insomnia factor score" derived from a questionnaire. Although no single SNP reached the stringent threshold of genome-wide significance ($P < 7.2 \times 10^{-8}$), a few genes showed evidence for meaningful association with caffeine-induced insomnia.

Notably, the previously suggested association between genetic variation of *ADORA2A* and disturbed sleep after caffeine was successfully replicated. This finding is remarkable in the genetics of complex traits because only a small minority of candidate genes has typically been confirmed by GWAS.[9] Although the original SNP (rs5751876) was not typed in the present sample, it forms a perfect linkage-disequilibrium with several SNPs of *ADORA2A* that significantly affect caffeine-induced sleep disturbance.[10] Apart from *ADORA2A*, some other "suggestive hits" may be targeted for future replication. Among the most interesting of them is *MTNR1B*, which codes for high-affinity melatonin MT_2 receptors. Genetic variants of *MTNR1B* modulate fasting blood glucose concentrations[11] and may contribute to the intriguing yet poorly understood relationships

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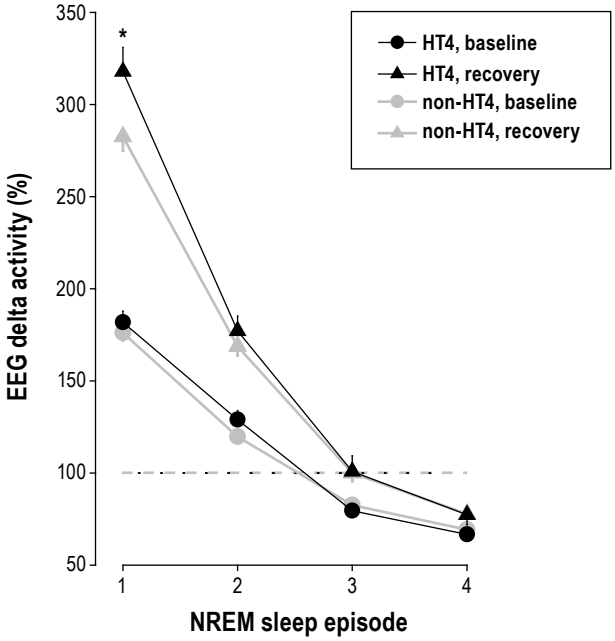

Figure 1—Genetic variation of *ADORA2A* modulates the rebound of EEG delta oscillations (0.75-4.5 Hz) after sleep loss. Delta activity in baseline (circles) and recovery (triangles) nights across consecutive NREM sleep episodes was expressed as a percentage of the all-night value in baseline (NREM sleep stages 1-4, horizontal dashed line). Data represent means ± SEM in carriers of HT4 (black symbols, n = 14) and non-HT4 haplotype (gray symbols, n = 31) alleles of *ADORA2A*. Forty hours prolonged wakefulness induced a larger relative rebound in delta activity in HT4 haplotype than in non-HT4 haplotype ("haplotype" × "night" × "NREM sleep episode": $F_{6,125} = 68.95$, $P < 0.0001$). *$P < 0.02$ (HT4 *vs.* non-HT4, unpaired 2-tailed *t*-test). Figure corresponds to supplementary Figure S3 in[15] (re-plotted with permission).

among caffeine consumption, short duration and poor quality of sleep, and the risk to develop type-2 diabetes.[12]

Rétey et al.[7] combined self-reports and polysomnography after double-blind caffeine administration, to document individual differences in the effects of caffeine on sleep. By contrast, the present work was restricted to self-classification of caffeine sensitivity. The successful replication of the previously reported association with this less accurate and less reliable (i.e., subjective) phenotype indicates that questionnaires are useful as an initial step in large-scale epidemiological studies, which are followed-up by physiological studies aimed to provide insights into the molecular bases of sleep-wake regulation (e.g., pharmacogenetics). For example, such an approach may lead to a better understanding of individual vulnerability to sleep deprivation, which is intensively investigated in sleep research. Interestingly, caffeine sensitive and insensitive individuals appear to be differently affected by sleep loss.[13] Together with the present results, this observation suggests that genetic variants of *ADORA2A* may alter the accumulation of homeostatically regulated sleep propensity during prolonged wakefulness. Convergent findings in mice[14] and humans[15] are consistent with this notion. They indicate that the sleep deprivation-induced rebound of EEG delta activity in NREM sleep, the most reliable marker of sleep homeostasis, depends on the functional state of A_{2A} receptors (Figure 1).

One century after Hollingworth, pharmacogenetic studies of caffeine not only revealed insights into a distinct molecular contribution to individual caffeine sensitivity, but also indicate that A_{2A} receptors are part of a biological pathway that regulates sleep in mammals. These findings could have important implications for the pathophysiology and the rational treatment of insomnia, as well as for recommendations for the critical use of caffeine, which is consumed on a daily basis by up to 90% of adults in western societies.

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DISCLOSURE STATEMENT

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