Supplemental Material

In addition to the TNFα G308A genotype, four other genes – all previously reported to be associated with vulnerability to performance impairment due to sleep loss – were assayed: ADORA2A (Bodenmann et al., 2012), PER3 (Lo et al., 2012), TLR4 (Wisor et al., 2011), and DQB1*0602 (Goel et al., 2010).

Adenosine A_{2A} Receptor (ADORA2A) Genotyping

Assay procedures for the ADORA2A T1976C polymorphism were based on Rétey et al. (2005). Each sample was separately assayed for the presence or absence of the T and C alleles. For the T allele, samples were amplified with 20 µM forward primer 5' – CGG AGG CCC AAT GGC TAT – 3' and reverse primer 5' – GTG ACT GGT CAA GCC AAC CA – 3'. For the C allele, samples were amplified with 20 µM forward primer 5' – CGG AGG CCC AAT GGC TAC – 3' and the same reverse primer as for the T allele. PCR procedures for both the T and C alleles were carried out in a final reaction volume of 20 µl containing 14 µl PCR master polymerase mix (Custom Genome Services, Pullman, WA), 1 µl of each primer (forward-T or forward-C and reverse), 3 µl nuclease free water, and 1 µl genomic DNA. PCR conditions involved denaturation at 92 °C for 4 min, followed by 35 cycles of: denaturation at 92 °C for 30 s, annealing at 67 °C for 1 min, and extension at 72 °C for 30 s. After another 20 min of extension at 72 °C the reaction ended. The amplified products were electrophoresed on a 2% agarose gel stained with ethidium bromide and visualized under UV light to determine genotype: C/C, C/T, T/T.

PERIOD3 (PER3) Genotyping

Assay procedures for the PER3 tandem repeat polymorphisms were based on Viola et al. (2007). Samples were amplified with 20 µM forward primer 5' – CAA AAT TTT ATG ACA CTA CCA GAA TGG CTG AC – 3' and 20 µM reverse primer 5' – AAC CTT GTA CTT CCA CAT CAG TGC CTGG – 3'. PCR procedures were carried out in a final reaction volume of 24 µl containing 12.5 µl REDTaq ReadyMix PCR Reaction Mix (Sigma-Aldrich, St. Louis, MS), 1 µl of each primer (forward and reverse), 8.5 µl nuclease free water, and 1 µl genomic DNA. PCR conditions involved denaturation at 95 °C for 3 min, followed by 40 cycles of: denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. After another 10 min of extension at 72 °C the reaction ended. The amplified products were electrophoresed on a 2% agarose gel stained with ethidium bromide and visualized under UV light to determine genotype tandem repeats: homozygous for the 4-nucleotide tandem repeat, homozygous for the 5-nucleotide tandem repeat, or heterozygous.

Toll Like Receptor 4 (TLR4) Genotyping

Assay procedures for the TLR4 A896G (Asp299Gly) polymorphism were based on Okayama et al. (2002). Samples were amplified with 20 µM forward primer 5'– GTT GCC ATC CGA AAT TAT AAG AAA AG – 3' and reverse primer 5' – AGC ATA CTT AGA CTA CCA CCT CGA TGG TAT – 3'. PCR procedures were carried out in a final reaction volume of 20 µl containing 10 µl Promega GoTaq Green Master Mix (Fisher Scientific, Hampton, NH), 1 µl of each primer (forward and reverse), 6 µl nuclease free water, and 2 µl genomic DNA. PCR conditions involved denaturation at 95 °C for 2 min, followed by 38 cycles of: denaturation at 95 °C for 30 s, annealing at 51 °C for 30 s, and extension at 72 °C for 30 s. After another 10 min of

extension at 72 °C the reaction ended. Amplified products were digested with *Bst*XI (New England Biolabs, Beverly, MA), a mutation-specific restriction enzyme. The TLR4 896G allele carries the recognition site for this restriction enzyme. The digestion reactions were carried out in a final volume of 10 µl containing 1 µl *Bst*XI, 1 µl 10X NE buffer, and 8 µl PCR product. Products were digested for 3 h at 37 °C, followed by 20 min at 65 °C to inactivate the enzyme. The final digested products were electrophoresed on a 3% agarose gel stained with ethidium bromide and visualized under UV light to determine genotypes: A/A, A/G, or G/G.

*Human Leukocyte Antigen DQB1*0602 (HLA-DQB1*0602) Genotyping*

Assay procedures to determine if an individual was DQB1*0602-positive or -negative were based on Hallmayer et al. (2002). Samples were amplified with 20 µM exon 2 sequence-specific forward primer 5' – CCC GCA GAG GAT TTC GTG TT – 3' and reverse primer 5' – AAC TCC GCC CGG GTC CC – 3', as well as 20 µM DRB1 intron control forward primer 5' – CCC GCA GAG GAT TTC GTG TT – 3' and reverse primer 5' – AAC TCC GCC CGG GTC CC – 3'. PCR procedures were carried out in a final reaction volume of 20 µl containing 10 µl Promega GoTaq Green Master Mix (Fisher Scientific, Hampton, NH), 1 µl of each primer (forward and reverse exon-specific, or forward and reverse intron-specific), 7 µl nuclease free water, and 1 µl genomic DNA. PCR conditions involved denaturation at 95 °C for 5 min, followed by 35 cycles of: denaturation at 95 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 1 min. After another 10 min of extension at 72 °C the reaction ended. The amplified products were electrophoresed on a 3% agarose gel stained with ethidium bromide and visualized under UV light to determine genotype: presence or absence of the DQB1*0602 exon.

Statistical Analyses

Fisher's exact test was used to determine if the TNFα G308A polymorphism was linked with the other polymorphisms assayed here. Furthermore, as was done for the TNFα genotype analyses in the main manuscript, each subject's vulnerability to sleep loss was quantified by averaging the number of PVT lapses over the 24-h period (i.e., one circadian cycle) of total sleep deprivation (TSD) common to all five studies (from 22:00 until 22:00 the next day). These subject-specific averages for vulnerability to sleep loss were first analyzed using non-parametric one-way analysis of rank scores with TNFα G308A genotype as independent variable and each of the additionally assayed genotypes – ADORA2A, PER3, TLR4, or DQB1*0602 – as covariate, and controlling for study. These analyses served to assess whether our TNFα findings could be accounted for by linkage with other polymorphisms previously reported to be associated with vulnerability to sleep loss. Lastly, the subject-specific averages for vulnerability to sleep loss were re-analyzed using non-parametric one-way analysis of rank scores with each of the other genotypes as independent variable, controlling for study. This was followed by one-way analysis of variance (ANOVA) to determine the percentage of variance in psychomotor vigilance performance impairment during TSD that was explained by the genotype and to estimate Cohen's local effect size f^2. The results were compared to the variance explained and effect size of the association between vulnerability to sleep loss and TNFα G308A genotype.

Results and Discussion

Genotype distributions are summarized in the table below. The frequencies we observed are similar to those in published studies.

	Genotype	Genotype Count		Genotype Frequency		
		Expected[a]	Observed	Expected[a]	Observed	Published[b]
ADORA2A	C/C	23.5	29	0.2673	0.3295	0.3169
	C/T	43.9	33	0.4994	0.3750	0.5422
	T/T	20.5	26	0.2333	0.2955	0.1408
PER3	$PER3^{4/4}$	32.5	34	0.3696	0.3864	0.4031
	$PER3^{4/5}$	41.9	39	0.4767	0.4432	0.4884
	$PER3^{5/5}$	13.5	15	0.1537	0.1705	0.1085
TLR4	A/A	82.1	82	0.9329	0.9318	0.9300
	A/G	5.8	6	0.0659	0.0682	0.0730
	G/G	0.1	0	0.0000	0.0000	0.0000
DQB1*0602	Negative		61		0.6932	0.7130
	Positive		27		0.3068	0.2870

[a]Based on Hardy-Weinberg equilibrium.

[b]ADORA2A: Rétey et al. (2007), 142 healthy subjects; PER3: Goel et al. (2009), 129 healthy subjects; TLR4: Santini et al. (2008), 151 healthy subjects; DQB1*0602: Goel et al. (2010), 129 healthy subjects.

Fisher's exact test demonstrated that in our sample, the TNFα G308A polymorphism was not significantly associated with ADORA2A genotype (P=0.41), PER3 genotype (P=0.90), TLR4 genotype (P=0.68), and DQB1*0602 genotype (P=0.52). In non-parametric one-way analysis of rank scores of average PVT lapses during TSD with each of these genotypes as covariate, the effect of TNFα G308A genotype (with A/A and A/G combined) remained statistically significant. This was the case for ADORA2A (TNFα G308A effect: $F_{1,80}$=8.86, P=0.004), PER3 (TNFα G308A effect: $F_{1,80}$=9.55, P=0.003), TLR4 (TNFα G308A effect: $F_{1,81}$=9.68, P=0.003), and DQB1*0602 (TNFα G308A effect: $F_{1,81}$=9.13, P=0.003). Independently, the other genotypes were not significantly associated with vulnerability to sleep loss quantified by rank scores of average PVT lapses, as found for ADORA2A ($F_{2,81}$=1.01 P=0.37), PER3 ($F_{2,81}$=0.09, P=0.92), TLR4 ($F_{1,82}$=0.95, P=0.33), and DQB1*0602 ($F_{1,82}$=0.86, P=0.36). The amount of variance in PVT lapses explained by each genotype in our sample was 1.5% for ADORA2A, 2.1% for

PER3, 0.3% for TLR4, and 1.6% for DQB1*0602. Local effect sizes were negligible for ADORA2A (f^2=0.016), PER3 (f^2=0.022), TLR4 (f^2=0.003), and DQB1*0602 (f^2=0.017). These explained variances and effect sizes are substantially smaller than what we found for the TNFα G308A genotype (i.e., 6.4% variance explained; f^2=0.071). Importantly, these results show that our TNFα findings were not confounded by those other genes reported to be associated with vulnerability to sleep loss.

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