TITLE
Genetics of caffeine and brain-related outcomes – a systematic review of observational studies and randomized trials

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JOURNAL
Nutrition Reviews

DATE DEPOSITED
24 April 2023

Article type: Systematic Review

Title: Genetics of Caffeine and Brain-related Outcomes - A Systematic Review of Observational Studies and Randomised Trials

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Abstract

Context: Although the stimulant and anxiogenic properties of caffeine are widely accepted, research on its specific effects on the brain remains controversial. Growing evidence shows that interindividual differences to caffeine response may be partly due to variations in genes such as *CYP1A2* and *ADORA2A* which have been used to identify individuals as 'fast' or 'slow' caffeine metabolisers and as having a 'high' or 'low' caffeine sensitivity, respectively.

Objectives: To identify, evaluate and discuss current evidence on the associations between common genetic variations, caffeine and brain-related outcomes in humans.

Data sources: PubMed and Embase databases were searched for relevant reports based on a predetermined search strategy.

Data extraction: Included records involved observational and experimental studies on healthy adults who underwent a) genetic analysis for polymorphisms in genes associated with caffeine metabolism and effect and b) measurements of brain-related effects such as anxiety, insomnia and cognition with the consumption of caffeine (habitual intake or supplementation).

Data analysis: Of the 22 records, 15 were randomised controlled trials, six were cross-sectional studies and one was a genome-wide association study. The main outcomes identified were cognition (n = 9), anxiety (n = 7) and sleep disturbance / insomnia (n = 6). Polymorphisms in *CYP1A2* gene were associated with cognitive function, while variations in *ADORA2A* gene were associated with anxiety and sleep disturbance.

Conclusions: The present review has provided evidence that variability in the *CYP1A2* and the *ADORA2A* genes may modulate the association between caffeine and brain-related outcomes. Future studies are warranted to investigate the specific polymorphisms implicated in each brain outcome, which cognitive functions are particularly related to caffeine (simple vs complex), whether there are gender differences in anxiety and how habitual caffeine intake may influence the acute effects of caffeine.

Systematic review registration: PROSPERO registration no. CRD42021257556.

Key words: caffeine, coffee, genetics, brain-related effects, anxiety, cognition, sleep.

1. Introduction

Caffeine is the most widely consumed psychostimulant, being used habitually by more than 80% of the world population. [1] On average, daily caffeine intake in adults worldwide equals to 227 mg, which is approximately two regular 125 ml cups of coffee. [1–3] Caffeine is readily available from a variety of foods and beverages, such as coffee, tea, chocolate and energy drinks, with coffee being the primary dietary caffeine source in Europe and the United States. [1,3,4]

The pharmacokinetics and pharmacodynamics of caffeine have been widely studied; more than 95% of caffeine biotransformation to its main metabolites paraxanthine, theophylline and theobromine, occurs in the liver via the cytochrome P450 enzyme (CYP1A2). [2,5] At the cellular level, caffeine blocks A1 and A2A adenosine receptors in the brain, competitively antagonising their binding with adenosine, a neuromodulator that promotes sleep and suppresses arousal, thereby triggering dopaminergic neurotransmission and promoting wakefulness. [2,6,7]

Caffeine has notable enhancing properties in cognitive function and physical performance, which explain its popularity, especially in shift workers, students, athletes and anyone generally seeking to overcome fatigue or prolong their capacity to complete everyday activities. [6,8–10] Apart from exerting locomotor activity stimulation in the central nervous system, caffeine has also been reported to possess anxiogenic properties for some individuals. [11]

Although the stimulant and anxiogenic properties of caffeine have been known for over a century and are widely accepted, research on its specific effects on the brain remains controversial. [6,12] In fact, a recent review showed mixed results from intervention studies; while some report that caffeine improves simple cognitive functions in doses 32-300 mg, some others have failed to find significant effects. [13] Results from a systematic review of observational studies are also

inconsistent, with only a few studies showing an association between caffeine and cognition and more recent studies detecting associations only among women or for specific exposures. [14]

Inconsistent findings may reflect methodological pitfalls commonly seen with dietary exposures. [15] Observational studies may be biased by misclassification of caffeine exposure due to the use of self-reported data and measures of caffeine-containing drinks. [12,15] In clinical trials, stimulant properties of caffeine may reflect restoration of brain function impaired by caffeine withdrawal. [16] Indeed, participants in randomised studies are often asked to abstain from caffeine overnight or longer, which may hinder cognitive performance in caffeine consumers. [16,17] Additionally, nutrigenetics research has also found a considerable interindividual variability in the magnitude of caffeine effects, or in the lack of an effect when compared to placebo, suggesting that the inconsistencies in previous findings are, at least in part, due to genetic variations. [2]

Growing evidence from genetic studies has associated the interindividual differences to caffeine response with variations in *CYP1A2* and *ADORA2A* genes. [2,18] The rs762551 Single Nucleotide Polymorphism (SNP) in the *CYP1A2* gene has been shown to affect CYP1A2 enzyme activity and has been used to identify individuals as 'fast' or 'slow' caffeine metabolisers. [19,20] Further, it has been hypothesised that variations in the *ADORA2A* gene such as the rs5751876 may impact caffeine-adenosine A2A receptor binding and thus downstream dopaminergic neurotransmission. [11] This may lead to anxiogenic effects following caffeine consumption and individuals can be categorised as having a 'high' or 'low' sensitivity to caffeine. [2,11]

With such widespread consumption of caffeine, the consequences of this stimulant on human health are of particular interest not only to the scientific community but also to the majority of adult population worldwide. To the authors' knowledge, there is currently no systematic review focusing on the associations between brain-related outcomes and SNPs related to physiological

response to caffeine and its metabolism. Better understanding on this topic may provide a basis for further interdisciplinary approaches and personalised recommendations. Therefore, the purpose of the present systematic review was to identify, evaluate and discuss the current evidence on the associations between common genetic variations, caffeine and brain-related outcomes in humans, including indices of cognition, anxiety and insomnia.

2. Materials and Methods

2.1. Search Strategy

This systematic review was guided by the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines and was registered with PROSPERO (CRD42021257556). PubMed and Embase databases were independently searched for relevant reports by two investigators until 21 April 2021. The search strategy (Figure S1) was decided based on consensus and the records identified from both databases were uploaded on Covidence software. [21] To identify possible eligible reports that were not identified by the initial search, a manual search of reference lists of included studies was also conducted.

2.2. Study Selection

Two reviewers selected records for inclusion in the systematic review by independently a) screening records by title and abstract and b) reviewing full texts, according to predetermined inclusion and exclusion criteria. Records that met the population, intervention, comparison, outcome, and study design (PICOS) criteria were eligible for inclusion [22] and are shown in Table 1. The included populations involved healthy adult participants above 18 years. Interventions included a) habitual caffeine intake and caffeine supplementation, reviewed separately and b)

genotyping for polymorphisms in genes associated with caffeine metabolism and effect reported as alleles, haplotypes or genetic scores. Comparators comprised a) different levels of habitual caffeine intake, different doses of caffeine supplementation and placebo and b) the variant allele compared with the ancestral allele, risk haplotypes and different genetic scores. The outcomes included brain-related effects such as mood and anxiety, insomnia and sleep deprivation, as well as indices of cognition such as reaction times, attention and reasoning. All observational and experimental trials were included. Reviewers were blinded to each other's decisions and disagreements between individual judgements were resolved based on consensus.

2.3 Data Extraction

In the present systematic review, outcome data were extracted only from participants for whom both genetic and caffeine intake/supplementation information was available. Data extraction was performed independently by two investigators and conflicts were resolved by consensus. For all included studies, extracted information included the first author’s name, year of publication, study design, participant characteristics (i.e., number, sex, age, and intervention), the genetic variant(s) under study, as well as the main and secondary brain-related effects, including results from statistical analyses. Extracted data were grouped based on the study outcomes.

2.4 Risk of Bias Assessment

The risk of bias of included studies was assessed independently by two reviewers following the Cochrane Review guidelines and conflicts were resolved by consensus. The risk of bias in interventions was assessed using the revised tool for assessing risk of bias in randomised trials (RoB-2). The parallel group and crossover RoB-2 tools were used based on study design and reviewers rated each study on domain level and overall risk of bias as ‘low’, ‘high’, or ‘some

concerns' [23]. For observational studies, the ROBINS-I (Risk Of Bias In Non-randomised Studies of interventions - NRSIs) risk of bias tool was used and reviewers rated each study on domain level and overall, as 'low', 'moderate', 'serious', 'critical', or 'unclear' risk of bias [24].

3. Results

The reporting of the available information is shown in the PRISMA checklist in Figure S2.

3.1. Search Procedure

The search yielded 3,021 records. After removing duplicates (n = 733), 2,228 records were screened by title and abstract. A total of 42 reports were assessed by full text for eligibility, with 22 reports of 19 independent studies meeting the inclusion criteria. A detailed mapping of the records identified, included and excluded, as well as the reasons for exclusions is shown in the PRISMA flow diagram in Figure 1.

3.2. Characteristics of the Included Studies

The characteristics of the included studies are presented in Tables 2-4. [4, 8-11, 26-29, 31-43] Of the included 22 records, nine were crossover randomised controlled trials (RCTs) (41%), six were parallel-group RCTs (27%), six were cross-sectional studies (27%) and one was a genome-wide association study (GWAS) (5%). In these studies, 21 SNPs in 13 genes were identified, while two studies used a genetic score for caffeine metabolism based on two SNPs and one study tested haplotypes including multiple variants instead of individual SNPs.

3.3. Risk of Bias Assessment

The overall and domain risk of bias assessment results, as well as summaries of the results are displayed in Figures 2-5. [4, 8-11, 26-29, 31-43]

3.4. Reporting on the Outcomes

Three major groups of outcomes were identified in the included studies: cognitive performance (n = 9), anxiety (n = 7) and sleep disturbance / insomnia (n = 6). Cognitive performance was assessed either alone (n = 5), during sleep deprivation (n = 3), or during and post-exercise (n = 1). Eight studies reported on deviations of study population from Hardy-Weinberg equilibrium (HWE), while 14 studies did not. HWE is an important tool in genetic studies primarily used to demonstrate whether the study population is representative of the general population. [25] Herein, all records that reported on HWE found that their study samples did not deviate from the HWE principle. Regarding Minor Allele Frequency (MAF), six studies either reported it or reported genotype frequencies that made it feasible to calculate it. For eight studies, although reported on genotype frequencies, it was not possible to estimate the MAF for individuals for whom both genetic and caffeine data were available, while no information on genotype frequencies was available for eight records. In terms of ethnicity, six studies were on unknown population, two studies were on mixed populations and 14 studies were on whites / Caucasian / Europeans. The findings of the included studies by outcome are shown in Tables 2-4.

3.4.1. Cognitive Performance

3.4.1.1 Cognitive performance without co-interventions

Five of the included studies reported genetic variation associated with cognitive performance. [9,26–29] Indices of cognitive performance included abstract reasoning, [26] verbal-numerical reasoning, prospective memory, visual memory and search, processing speed, mental flexibility and executive function, [27,28] alertness, orienting, executive motor control [9] and visual attention. [29]

Casiglia et al. (2017) demonstrated that caffeine in the highest tertile of caffeine intake was associated with significantly higher abstract reasoning in the CC homozygotes ('slow' metabolisers) compared to the lowest ($p < .005$) and middle tertiles ($p < .01$), while habitual caffeine intake was not associated with abstract reasoning in the A carriers ('fast' metabolisers) ($p > .05$ for all tertiles of habitual caffeine intake). On the contrary, Salinero et al. (2017), found no caffeine x *CYP1A2* rs76255 genotype effects on visual attention after caffeine supplementation in a sample of active males and females.

Two analyses from the UK Biobank used two genome-wide significant SNPs (rs2472297 near *CYP1A2* and rs6968554 near *AHR*) of caffeine metabolites [30] to formulate a weighted genetic score ranging from 0 to 4, with the highest score indicating faster caffeine metabolism. Focusing on the sample of Caucasian individuals, one of the studies investigated recent caffeine drinking, [27] defined as caffeine consumption through coffee or tea within the last hour prior to cognitive tests, while the other investigated the effect of habitual coffee, tea and caffeine intake. [28] Although recent caffeine drinking was associated with increasing performance in reaction time (RT) and with decreasing performance in pairs matching with increasing genetic caffeine metabolism score (CMSG), no significant CMSG x recent caffeine drinking interactions were found. [27] Moreover, a weak association between caffeine/tea and Fluid Intelligence (FI) among those with higher CMSG was found ($p < .0003$), while the 'fast' metabolisers (AA genotype of the rs762551) presented with greater decrements in performance in pairs matching with coffee intake than those with CC or AC genotypes ($p < .0001$). Nevertheless, no significant CMSG/genotype x coffee/tea/caffeine interaction was found for these tasks. [28]

Renda et al. (2015) demonstrated that the CC homozygotes ('low' caffeine sensitivity) of the *ADORA2A* rs5751876 showed a significantly higher RT in orienting ($p = .033$) in a sample of

106 males, while the TT homozygotes ('high' caffeine sensitivity) showed a higher RT in motor executive control (p = .005) after caffeine compared to placebo.

3.4.1.2 Cognitive performance & sleep deprivation

Three studies investigated the combined effects of specific genetic variants and caffeine intake on resilience to sleep deprivation by examining indices of cognition. [31–33] Baur et al. (2021) reported that the regular coffee intervention group performed faster and more accurately than the decaffeinated coffee group on sleep restriction days 1 – 4 but not on day 5 in a sample of homozygous C-allele carriers of the *ADORA2A* rs5751876. A second study in 45 males investigated the effects of five *ADORA2A* haplotypes and caffeine on the sleep loss-induced impairment of attention. It was shown that caffeine improved psychomotor vigilance task (PVT) response speed during 40 h of sleep deprivation in non-HT4 (HT1, HT2, HT3, HT5 combined) haplotype carriers only (p < .003). [32] Another study found no differences between *TNFα* G308A genotype groups after caffeine intake compared to placebo (p all > .05) on PVT performance after 48 h of sleep deprivation. [33]

3.4.1.3 Cognitive performance & exercise

Carswell et al. (2020) found that after caffeine supplementation, the 'fast' metabolisers (AA group of the *CYP1A2* rs762551) performed better than the 'slow' metabolisers (AC and CC group) at the PVT during exercise and at rest post-supplementation (p all < .05). However, the study did not detect any differences in caffeine–placebo change scores in RT between 'high' and 'low' sensitivity genotypes of the *ADORA2A* gene during exercise or at rest post-supplementation (p > .05).

3.4.2. Anxiety

Seven of the included studies reported data on genetic variation and the anxiogenic effects of caffeine. [8,11,34–38] Three of the included studies investigated the effects of polymorphisms on self-reported anxiety following caffeine consumption, [8,11,38] while four studies investigated the effects of polymorphisms on startle responses to unpleasant optical or acoustic stimuli following caffeine intake. [34–37]

Alsene et al. (2003) demonstrated that only the TT groups of the rs5751876 and rs2298383 loci reported a significant increase in anxiety after caffeine compared to placebo and this increase was significantly higher compared to the CC and CT genotype groups (p all < .05) in both SNPs. Rogers et al. (2010) demonstrated that *ADORA2A* rs5751876 TT genotype significantly increased self-rated anxiety after 100 mg caffeine compared to placebo ($p < .01$). However, when considering habitual caffeine consumption, 250 mg caffeine increased subjective anxiety only in non-to-low consumers in both TT and CT/CC genotype groups ($p < .05$ and $p < .01$, respectively). On the contrary, Childs et al. (2008) showed that genetic variations in the *ADORA2A* rs2298383 and rs4822492 and *DRD2* rs1110976 but not in the *ADORA2A* rs5751876 gene were associated with anxiety following 150 mg caffeine in no-to-moderate caffeine consumers.

Domschke et al. (2012b) reported that only females with the rs5751876 TT genotype demonstrated significantly higher startle magnitudes for unpleasant pictures in the caffeine condition ($p = .01$). Gajewska et al. (2013) showed that women with the rs5751876 TT genotype exhibited impaired prepulse inhibition compared to TT risk genotype men. Another study demonstrated that the Neuropeptide S receptor gene (*NPSR*) TT risk genotype had a decrease in startle magnitude in response to unpleasant stimuli in caffeine compared to placebo condition ($p \leq .05$). [35] Lastly, a study on the effects of the *COMT* Val158Met variant on startle response

showed no influence of caffeine on startle responses dependent on the *COMT* Val158Met polymorphism. [37]

3.4.3. Sleep Disturbance & Insomnia

Six of the included studies investigated the effects of genetic variability on the effects of caffeine on sleep disturbance and insomnia. [4,39–43]

A GWAS on more than 2 million genetic loci identified eight SNPs that were associated with subjective caffeine-induced insomnia, although no SNPs passed the threshold of genome-wide significance level (7.2×10^{-8}). [39] Erblang et al. (2019) showed that total sleep time (TST) was lower in the T carriers compared to CC genotype of the *ADORA2A* rs5751876 and rs3761422, while it was higher in the TT vs CC group of the rs2298383 and the GG and GC compared to CC genotype of the rs4822492, but only in low caffeine consumers. The risk of sleep complaints was lower in the CT compared to CC genotype for rs5751876 and it was higher in TT compared to CC for rs2298383 and in GG compared to CC genotype for rs4822492 in moderate caffeine consumers.

Holst et al. (2014) revealed that 400 mg of caffeine was associated with increased vigilance to sleep deprivation in 10R/10R homozygotes of the *DAT* 1 gene when compared with 9R allele carriers ($p < .05$), as shown by Electroencephalogram (EEG) activity. Mazzotti et al. (2011) on the other hand, using polysomnography found that, among caffeine consumers, A allele carriers of *ADA* G22A compared to non-carriers showed lower sleep latency ($p = .03$), higher % sleep efficiency ($p = .01$), higher % Rapid-Eye Movement (REM) sleep ($p = .02$), and fewer minutes awake ($p = .04$). No difference was found between genotypes for other sleep parameters, or for any of the sleep parameters among those who did not consume caffeine ($p > .05$).

Nunes et al. (2017) found no difference in sleep variables between the *ADORA2A* rs5751876 genotypes (p all > .05). When stratified by genotype, significant, yet weak correlations were shown between caffeine load and sleep latency, % stage 3 sleep and % REM sleep only in T allele carriers. On the contrary, Retey et al. (2007) demonstrated that the CC genotype of the *ADORA2A* rs5751876 displayed a greater rise in the EEG power in the beta band after caffeine compared to the T carriers (p < .03), suggesting that the CC genotype exhibits acute insomnia following caffeine intake. [44]

4. Discussion

The purpose of the present systematic review was to identify the associations between common genetic variations, caffeine and brain-related outcomes in humans. The findings of this work are discussed below.

4.1. Cognitive Performance

4.1.1 Cognitive performance without co-interventions

Caffeine is normally considered an enhancer of alertness and general cognitive performance. [6,45] Cognitive performance is defined as the performance in functions that require mental effort. [46] Cognitive functions are categorised as either 'simple' or 'complex'; simple functions require very simple perceptual motor skills (e.g., reaction time, short-term memory), whereas complex functions require a greater effort (e.g., executive function, working memory). [47] Genetic studies on the effects of caffeine on specific functions of cognition are limited and are characterised by methodological heterogeneity.

Although caffeine intake has been shown to enhance simple cognitive functions such as reaction times in a dose-dependent manner, the association between caffeine and complex cognitive

functions is often argued. [13] The first study to show that high habitual caffeine intakes are associated with abstract reasoning only in 'slow' metabolisers [26] may partly explain previous controversies in the literature regarding the association between habitual caffeine intake and complex cognitive abilities. Accordingly, the investigations from the UK Biobank found that the 'fast' metabolisers had lower performance in pairs matching with higher habitual coffee intake than those with AC or CC genotypes of the rs762551. [28] After stratifying by a genetic caffeine metabolism score (CMSG), the results suggested that habitual caffeine and tea consumption were associated with decrements in fluid intelligence in 'fast' compared with 'slow' metabolisers, [28] while recent caffeine drinking was associated with improved cognition in simple cognitive functions the faster the genetic caffeine metabolism. [27] Nevertheless, no significant genotype x coffee/tea/caffeine interactions were found for any of these tasks.

On the contrary, a study on light caffeine consumers found no differences in indices of cognition neither between trials nor between rs762551 genotype groups 1 h after supplementation with caffeine or placebo. [29] Finally, in the only study on cognition and the *ADORA2A* gene, the rs5751876 genotypes performed faster in different cognitive indices - the CC genotype performed faster in orienting, while the TT genotype performed faster in motor executive control after caffeine compared to placebo. [9] However, only male subjects were included in the study making the results non-generalisable.

An important factor that needs to be considered is that only the study from Casiglia and colleagues (2017) measured habitual caffeine from all sources, while in the UK Biobank investigations, habitual and recent caffeine drinking estimates were based solely on coffee and tea. In the UK, for example, the major caffeine source in the diet is tea, with coffee and cola drinks in second place and energy drinks in third place. [1] Moreover, the CMSG was derived

using two SNPs (*CYP1A2* rs2472297 and *AHR* rs6968554) that have been presented with the largest effect sizes in a single GWAS of caffeine metabolites and may have not provided a valid measure of genetic caffeine metabolism because of the limited replication of data on these SNPs and because a known SNP associated with caffeine metabolism, *CYP1A2* rs762551 was not included in the scoring.

Moreover, it is important to note that the peak plasma caffeine concentration is shown to be reached in 30-60 min post ingestion and caffeine half-life in plasma is approximately 4-6 h in most adults and it is not yet known to what degree caffeine metabolism is altered between 'fast' and 'slow' metabolisers. [2] Therefore, testing participants within 1 h post-caffeine ingestion would mostly measure caffeine absorption and not metabolism, which is determined by CYP1A2 enzyme. Moreover, it is unknown at what time point there would be a large enough difference in the circulating levels of caffeine between 'fast' and 'slow' metabolisers to have a significant impact on the stimulant effects of caffeine. [48]

In summary, based on genetic studies on caffeine and cognition, important parameters that should be considered are habitual caffeine intake, since it is a known inducer of CYP1A2 enzymatic activity in a dose-dependent manner [49] and how it is measured, the rate of acute caffeine metabolism based on *CYP1A2* rs2472297 and rs762551 and *AHR* rs6968554 polymorphisms, as well as the nature of cognitive functions under study. More research is needed in both males and females stratified by *ADORA2A* rs5751876 genotype to investigate whether this SNP is implicated in the association between caffeine and cognition.

4.1.2 Cognitive performance & sleep deprivation

Sufficient sleep of 6 – 8 h and of good quality is essential for general health and optimal cognitive performance. [50] Whereas the neurobiological mechanisms are not yet fully understood, changes in levels of adenosine in the brain appear to underly the sleep loss-induced reduction in cognitive functions such as working memory and sustained attention. [44] By blocking the binding of adenosine with the A2A receptors, caffeine countermeasures the detrimental effect of prolonged wakefulness by potentiating dopaminergic signalling, which leads to motor activation and subsequent alertness. [51] Hence, caffeine intake, particularly in the morning or early afternoon to enhance wakefulness in response to sleep restriction is very common. [52] The present review identified limited data regarding the effect of genetics and caffeine on cognition in a sleep-deprived state.

The included studies found that caffeine caffeine attenuates the impairment in cognitive functions such as attention, orienting, memory and executive control caused by sleep deprivation in C homozygous of the *ADORA2A* rs5751876 [31] and in non-HT4 haplotype carriers of *ADORA2A* compared with the HT4 haplotype. [32] Although both studies tried to mimic real-life caffeine intakes which are very common in Europe, [1] only the CC genotype group of the *ADORA2A* rs5751876 genotype was included in one study [31] and the second studied only male subjects stratified by *ADORA2A* haplotypes instead of distinct genotypes. [32]

The selective recruitment was based on the notion that these individuals are genetically sensitive to the effects of caffeine on cognition in rested [9] and sleep-deprived states, [44] yet did not allow for comparisons between distinct genotype groups. Additionally, haplotypes are often ambiguous because of unknown linkage within the gene and, although haplotype frequencies are suitable for case-control studies (binary traits), they cannot provide a method of testing the statistical significance with a specific trait. [53]

Further, although the A allele carriers of the *TNFα* rs1800629 polymorphism have been found to be relatively resilient to psychomotor vigilance impairment during sleep deprivation as compared to individuals homozygous for the G allele, [54] Skeiky et al. (2020) found no differences in RT between genotypes after caffeine intake.

Overall, genetic studies on cognition during sleep deprivation are limited. Further studies are needed to elucidate how distinct *ADORA2A* genotypes interact with different indices of cognition and the sleep–wake cycle and whether SNPs of other plausible genes in the dopaminergic system are implicated in these associations. For example, evidence suggests that the T carriers of the *ADORA2A* rs5751876 variant experience caffeine-induced anxiety [11] and that these individuals demonstrate low habitual caffeine intakes, most probably because of this anxiogenic effect. [55,56] These observations may provide a biological basis for habitual caffeine consumption that would drive the acute effects of caffeine in cognition after sleep deprivation and require further exploration.

4.1.3 Cognitive performance & exercise

Caffeine antagonises the effect of adenosine in the central nervous system, thereby decreasing feelings of tiredness and enhancing arousal, vigilance, and willingness to exert effort during exercise [57]. In the only study up to date on the effects of caffeine and genetics on cognition and exercise, caffeine improved cognitive performance in RT in 'fast' compared with 'slow' metabolisers based on *CYP1A2* genotype both during and after exercise, but no differences were observed between *ADORA2A* genotypes. Nonetheless, it needs to be considered that only one heterozygous carrier of the *ADORA2A* C allele was included. Future studies with larger sample sizes are required to determine the influence of the *ADORA2A* gene on the cognitive effects of caffeine during exercise.

4.2. Anxiety

The most extensively researched SNP in association with the anxiogenic effects of caffeine is the *ADORA2A* rs5751876 silent polymorphism, because of its association with panic disorder and anxiety in Caucasians [2]. There are two proposed explanations for the functional relevance of this polymorphism in anxiety: a) the variant can alter mRNA translation or stability and b) it is in linkage disequilibrium (LD) with a functional variant in the *ADORA2A* gene such as the rs35320474 polymorphism. [11,34]

Three reports from mixed samples consisting of predominantly Caucasians support that the TT genotype of the specific variant is associated with increases in self-rated anxiety following caffeine consumption. [8,11,38] However, when data for European-American participants only were considered, this effect was no longer significant in one of the studies. [38]

Interestingly, two studies on measured anxiety showed a possible gender-specific regulation of anxiety in response to caffeine, with female TT homozygous of the *ADORA2A* rs5751876 experiencing higher levels of anxiety. [34,36] One possible explanation for these gender differences would reflect the hormonal differences between males and females and variations of circulating oestrogens. [45] Nevertheless, the studies tested women using oral contraceptives and they were not tested during menstruation to control for such hormonal changes. On the other hand, previous data using functional magnetic resonance imaging (fMRI) show that caffeine effects may be also specific to different lateralisation in the dopaminergic response between genders (negative emotional stimuli activates the left hemisphere in women and the right hemisphere in men) and how males and females perceive and process anxiety. [58]

Two additional *ADORA2A* variants, rs2298383 and rs4822492, were also identified to be associated with self-rated caffeine-induced anxiety, however they lack replication and it remains unclear whether they have a functional role or are in LD with other functional polymorphisms. [11,38,59] Additionally, the present review identified single reports on variants in genes that are biologically plausible modulators of caffeine effects on anxiety: the dopamine D2 receptor (*DRD2*), [38] the neuropeptide S (*NPSR*) [35] and the catechol-O-methyltransferase (*COMT*) [37] genes. The *DRD2* and *COMT* genes are associated with the counteractive signalling between adenosine A2A and dopamine D2 receptors [60] and the inactivation of dopamine and norepinephrine, [61] while the *NPSR* rs324981 polymorphism has been found to be influencing emotion processing of anxiety-relevant stimuli. [62]

Summarising the above, it is suggested that caffeine-sensitive individuals who habitually consume low-moderate caffeine doses are affected by caffeine in doses that can be consumed in one cup of coffee. It is also indicated that caffeine-naïve individuals may experience the anxiogenic consequences of caffeine regardless of genetic variations [8] or perhaps they do not habitually consume caffeine because it has an anxiogenic effect on them. Further investigations using similar measures of anxiety with higher caffeine doses and different variants in SNPs implicated in neurotransmission are needed to reach to safe conclusions on the effect of habituality and gene x gene interactions in anxiety.

4.3. Sleep Disturbance & Insomnia

The majority of evidence on the genetics of caffeine and sleep are focusing on the *ADORA2A* gene. Retey et al. (2007) reported that caffeine can cause an insomnia-like electroencephalogram (EEG) pattern only in CC homozygous individuals. Nunes et al. (2017) reported that caffeine was associated with shorter sleep duration only in T allele carriers. Erblang et al. (2019) reported

that both alleles may be associated with different sleep parameters; T allele associated with shorter sleep duration in low habitual caffeine consumers and CC genotype associated with more sleep complaints in moderate habitual caffeine users. Differences in study design may account for the inconsistencies across studies. For example, Retey et al. (2007) supplemented participants with a measured caffeine dose after caffeine abstinence for two weeks, suggesting more accurate data on caffeine intake. Nunes et al. (2017) used an index (caffeine load) that incorporates the number of caffeine doses the individuals had taken before the polysomnography and the time since the last dose. Finally, Erblang et al. (2019) reported habitual caffeine intake from a self-administered questionnaire and characterised caffeine intake as low, moderate and high. Additionally, both Retey et al. (2007) and Nunes et al. (2017) assessed sleep using polysomnography, while Erblang et al. (2019) used self-reported data.

In the only GWAS up to date on SNPs implicated in caffeine-induced insomnia, no SNPs reached the genome-wide significance level and, although association analyses revealed eight variants to be associated with insomnia, [39] none of these loci has been replicated in genetic association studies. However, risk of insomnia was assessed through a dichotomised scale based on whether participants reported ever or never experiencing caffeine-induced insomnia, which may be a source of information bias. Moreover, the assignment of participants in two groups may have resulted in a loss of power in the study as risk alleles related to more severe or minor caffeine-induced insomnia may have been identified.

Single studies on genes related to neurotransmission were also identified. [40,41] ADA is an enzyme responsible for the clearance of extracellular adenosine and regulates sleep, while the *ADA* rs73598374 variant has been associated with better sleep duration and intensity in healthy adults. [51] Moreover, the 10R/10R genotype dopamine transporter 1 (*DAT1*) VNTR polymorphism has

been associated with reduced DAT protein expression in the striatum when compared with 9R allele carriers. [63] Although both studies found genotype differences in sleep quality parameters with caffeine intake, results require replication.

4.4. Quality of Evidence

The present systematic review used three different tools for risk of bias assessment: the RoB-2 tools for randomised parallel group and crossover trials and the ROBINS-I tool for observational studies. The included randomised trials displayed an overall low risk of bias, while three studies raised some concerns and two studies were of high risk. The domains that raised concerns were selection and detection biases, indicating that the studies provided insufficient information on the sequence generation process and the blinding of allocated interventions by outcome assessors. A high risk of bias appeared in detection and attrition bias domains, suggesting that the outcome is likely to be influenced by lack of blinding and that there is missing outcome data that was not reported, respectively. [64]

On the contrary, the non-randomised trials overall displayed a serious risk of bias, with two studies displaying moderate and one study displaying critical risk. Domains with serious or critical risk included bias due to missing data and detection biases, as well as bias due to confounding and selection of participants. Bias due to confounding in non-randomised trials is very common [24] and results from the use of self-reported measures, for example subjective sleep quality, which may have lower reliability than objective measures such as polysomnography. Moreover, the selective recruitment of participants based on specific characteristics suggests that the study population may not be representative of the target population. [24]

Although the quality of the included observational studies seems to be low, it needs to be considered that the fundamental underlying principle of the ROBINS-I tool is that a non-randomised trial is compared against a target RCT. [23,24] This means that, using this stringent tool, no observational study can be of low overall risk of bias and that a good quality observational study, which is comparable with a RCT, would be of moderate risk of bias. The present systematic review identified two observational studies that are of moderate risk of bias and it needs to be considered that the study that displayed an overall critical risk is a GWAS and it is uncertain whether the ROBINS-I tool is applicable to this study design. This indicates that both the randomised and the non-randomised trials in this systematic review may provide fair quality evidence.

As no meta-analysis was conducted, the GRADE (Grading of Recommendations, Assessment, Development and Evaluations) framework for appraising quality of evidence by brain-related outcome was not feasible. Nonetheless, some issues need to be addressed regarding quality of evidence in this systematic review. The included studies were on three different brain-related outcomes of caffeine: cognition, anxiety and insomnia/sleep disturbance. Among the nine studies on cognition, five studied cognition alone, three studies explored cognitive performance during sleep deprivation and one study during and post exercise. Seven studies were investigating anxiety and six studies were on sleep disturbance and insomnia. Therefore, there is a variety of outcomes and the number of studies for some of them was limited. In addition, studies on the same outcome incorporated different outcome measures based on the study design (intervention vs observational) and the selection of different methods of assessing cognition (i.e., different cognitive tasks assessing executive control or memory), anxiety (i.e., subjective vs objective measures of anxiety) or sleep (subjective measures of sleep quality vs polysomnography).

Regarding the first comparator of this review, the genetic variability, most studies tested individual SNPs (21 SNPs in total), two studies formulated a genetic score based on more than one SNP and one study tested haplotypes. With such diversity in genetic information, only a few SNPs are replicated in the literature. As far as the second comparator, caffeine, both habitual caffeine intake and caffeine interventions were considered. Still, studies compared: a) tertiles of habitual caffeine intake (lowest/middle/highest); b) different doses of caffeine supplementation vs placebo; c) recent acute caffeine intake vs no caffeine intake or d) different caffeine loads based on quantity of recent caffeine drinking and the number of hours since the last caffeine-containing drink. Moreover, some studies estimated habitual caffeine intake of participants solely on coffee and tea and may have omitted important sources of caffeine. [1] Finally, four studies were in males and results may not be generalisable to females. On the other hand, two investigations studied no/low habitual caffeine consumers, who may not be representative of the general adult population worldwide. [12]

Accordingly, based on the studies selected for the aim of the current systematic review, caution is recommended when forming conclusions regarding the impact of individual SNPs on the brain-related effects such as cognition, anxiety and sleep disturbance/insomnia of habitual or acute caffeine intake in humans.

4.5. Strengths and Limitations

A strength of the present review is the inclusion of both experimental and observational study designs on the genetics of caffeine and brain-related effects. Indeed, if a review includes only randomised trials, it may omit other outcomes because of the importance of long-term effects of an exposure to human health or because only a small number of randomised trials is available on the topic. [64]

The separation of randomised and observational studies was primarily a result of recognition that randomisation is the only way to fully protect against confounding and that confounding is always a concern in even the most rigorously conducted observational studies. [65] For this reason, three well-established tools, specific to different study designs were used to assess risk of bias of the included studies. Particularly, the use of a stringent tool, the ROBINS-I tool to compare the quality of observational studies against target RCTs ensured a high quality approach for this review. [24] Finally, no studies were excluded based on language.

A possible limitation of the current systematic review is that non-peer-reviewed studies were excluded. Using grey literature is a method to reduce publication bias through inclusion of research that is yet unpublished or has received less exposure and is highly desirable in systematic reviews. [64] Therefore, the present review might have not fully addressed publication bias and studies that report dramatic effects were more likely to be identified compared with studies that report smaller effect sizes. [66]

5. Conclusions

In conclusion, the present review has provided evidence that variability in the *CYP1A2* and the *ADORA2A* genes are associated with brain-related outcomes of caffeine. Nevertheless, it is not yet clear what specific genotypes are implicated in each brain outcome, which functions of cognition are particularly associated with caffeine (simple vs complex), whether there are gender differences in anxiety and how habitual caffeine intake may influence the acute effects of caffeine. The review also demonstrates that variability in additional genes may be involved in caffeine pharmacokinetics and brain neurotransmission collectively influence individual responses to caffeine; however, these studies lack replication.

Future studies in this area are recommended to utilise interdisciplinary approaches to investigate the complex interactions between genetic and environmental factors on brain function. Careful design to overcome the common methodological challenges of caffeine research is warranted. For example, the selection of caffeine-naïve or low caffeine consumers is not representative of the general population. Individuals who consume caffeine habitually may help investigate issues of caffeine tolerance, caffeine withdrawal and withdrawal reversal. Moreover, there is need for studies that examine brain-related effects of caffeine not based solely on single sessions or a period of days, but also for weeks, months and possibly years.

Acknowledgements

Funding and Sponsorship

This research received no external funding.

Author Contributions

AK: Conceptualisation, Design, Data collection and Analysis, Writing – Original Draft. AK: Data collection, Writing – Review and Editing. CA-MG: Data collection, Writing – Review and Editing. LP: Conceptualisation, Design, Writing – Review and Editing, Supervision. YM: Conceptualisation, Design, Writing – Review and Editing, Supervision. All authors read and approved the final manuscript.

Declaration of Interest

Dr Yiannis Mavrommatis serves as a scientific consultant at MyHealthChecked, a wellness company specialised in personalised healthcare. No other author declares any conflicts of interest.

Supporting Information

The following are available: Table S1: Search Strategy, Table S2: PRISMA Checklist.

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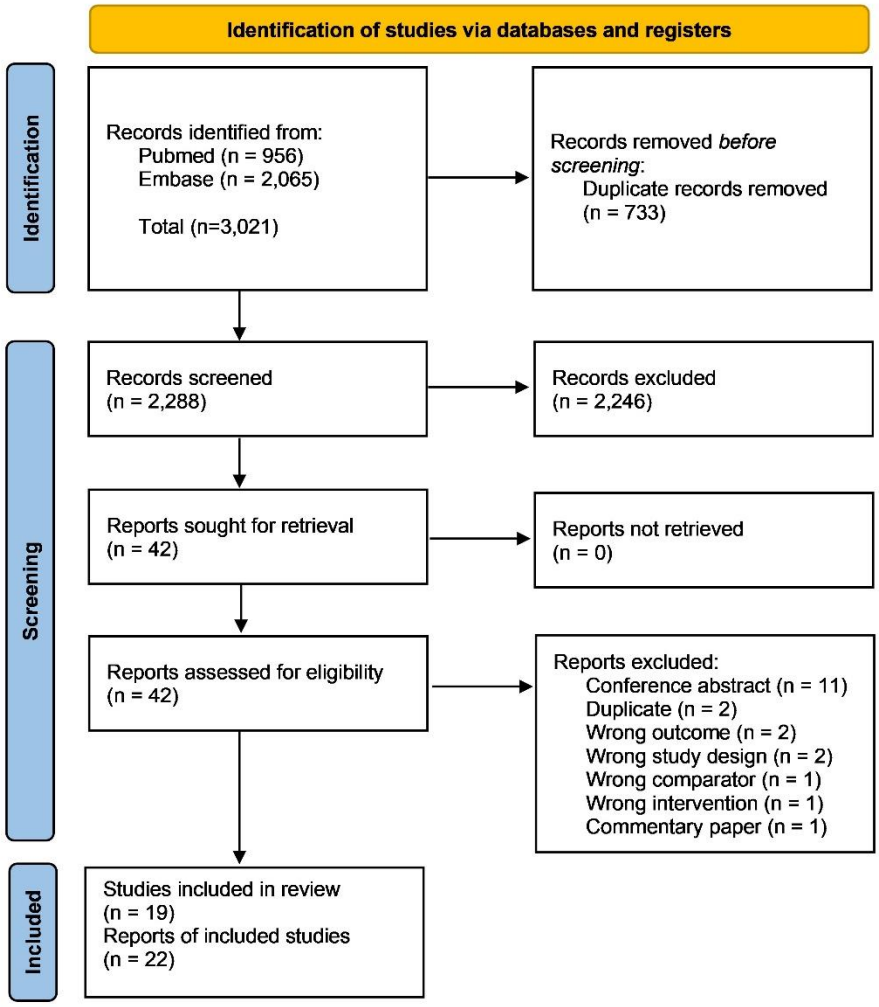

Figure 1 The PRISMA flow diagram. Presentation of the procedure of literature searching and selection with numbers of records at each stage. From: Page et al., The PRISMA 2020 statement: an updated guideline for reporting systematic reviews, 2020.

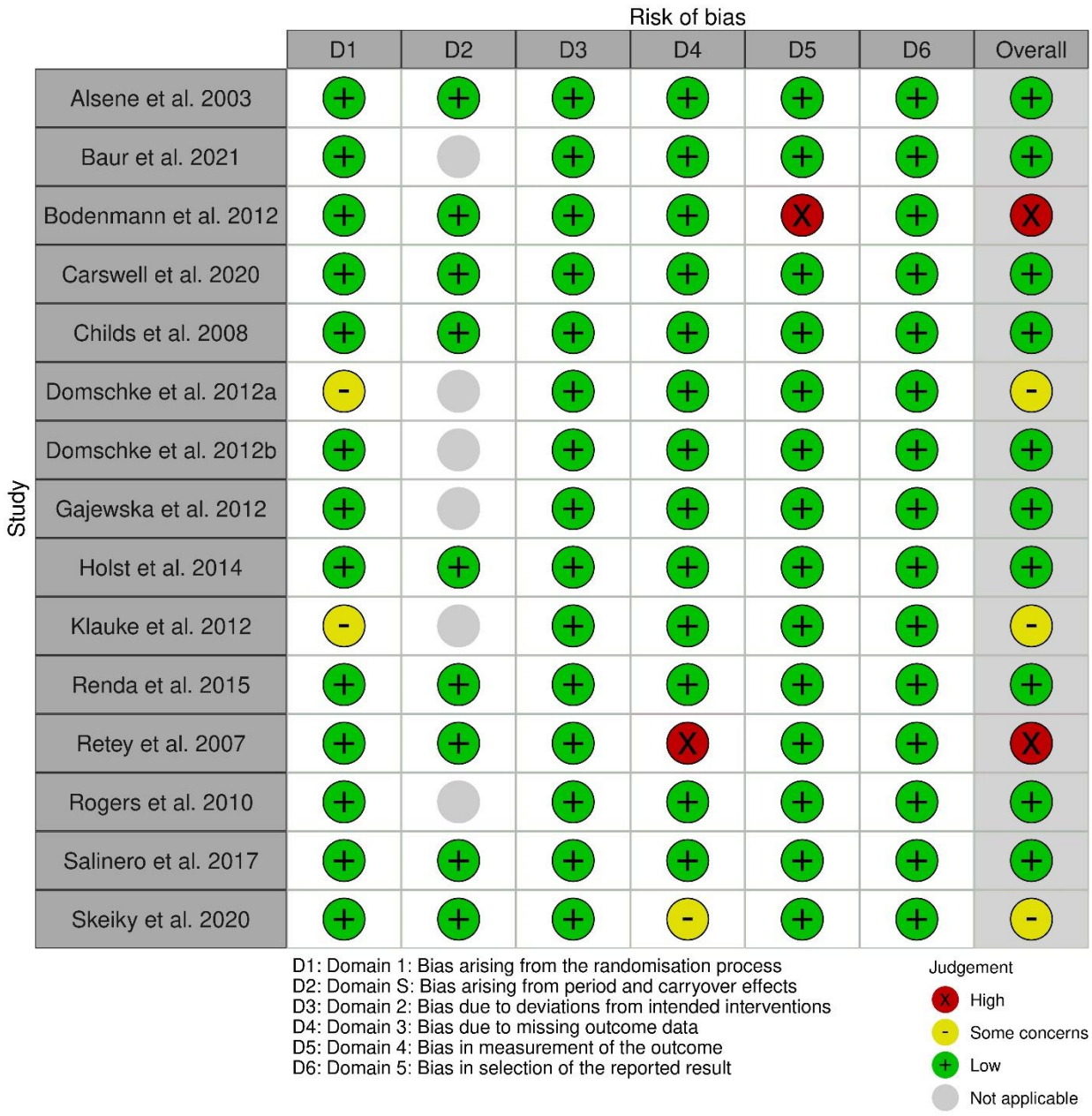

Figure 2 Risk of bias assessment using the RoB-2 tool for crossover and parallel-group RCTs. The majority of RCTs (n =10, 67%) were of low overall risk of bias, while three studies (20%) were of unclear overall risk of bias and two studies (13%) were classified as high overall risk of bias. Some concerns were raised in random sequence generation (n = 2) and in bias due to missing outcome data (n = 1), while high risk of bias was demonstrated for bias due to missing outcome data (n = 1) and for bias in measurement of outcome (n = 1). From: McGuinness & Higgins, Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments, 2020.

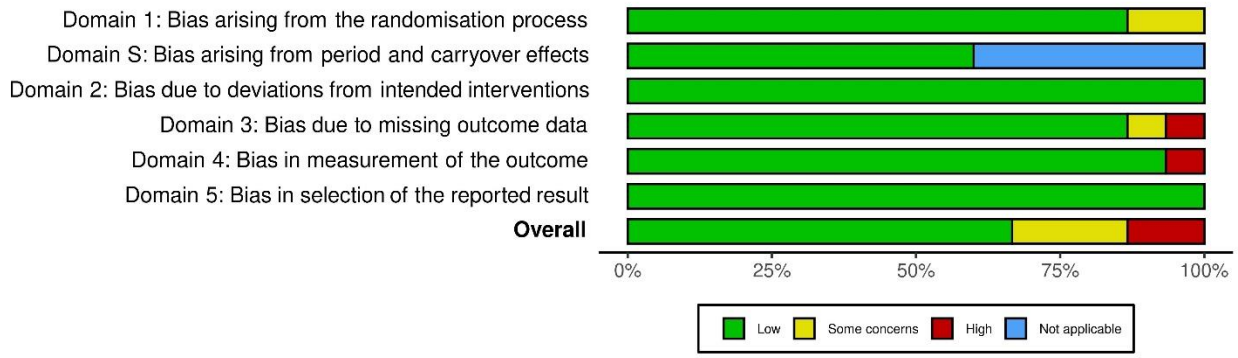

Figure 3 Summary of risk of bias assessment for randomised controlled trials (crossover RCTs, n = 9 and parallel-group RCTs, n = 6, total n = 15). Bias arising from the randomisation process (low risk (n = 13, 86.7%), some concerns (n = 2, 13.3%); Bias arising from period and carryover effects (applicable only for crossover RCTs, low risk (n = 9, 100.0%); Bias due to deviations from intended intervention (low risk (n = 15, 100.0%); Bias due to missing outcome data (low risk (n = 13, 86.7%), some concerns (n = 1, 0.1), high risk (n = 1, 0.1); Bias in measurement of the outcome (low risk (n = 14, 93.3%), high risk (n = 1, 0.1); Bias in selection of the reported result (low risk (n = 15, 100.0%); Overall risk of bias (low risk (n = 10, 66.7%), some concerns (n = 3, 20.0%); high risk (n = 2, 13.3%). From: McGuinness & Higgins, Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments, 2020.

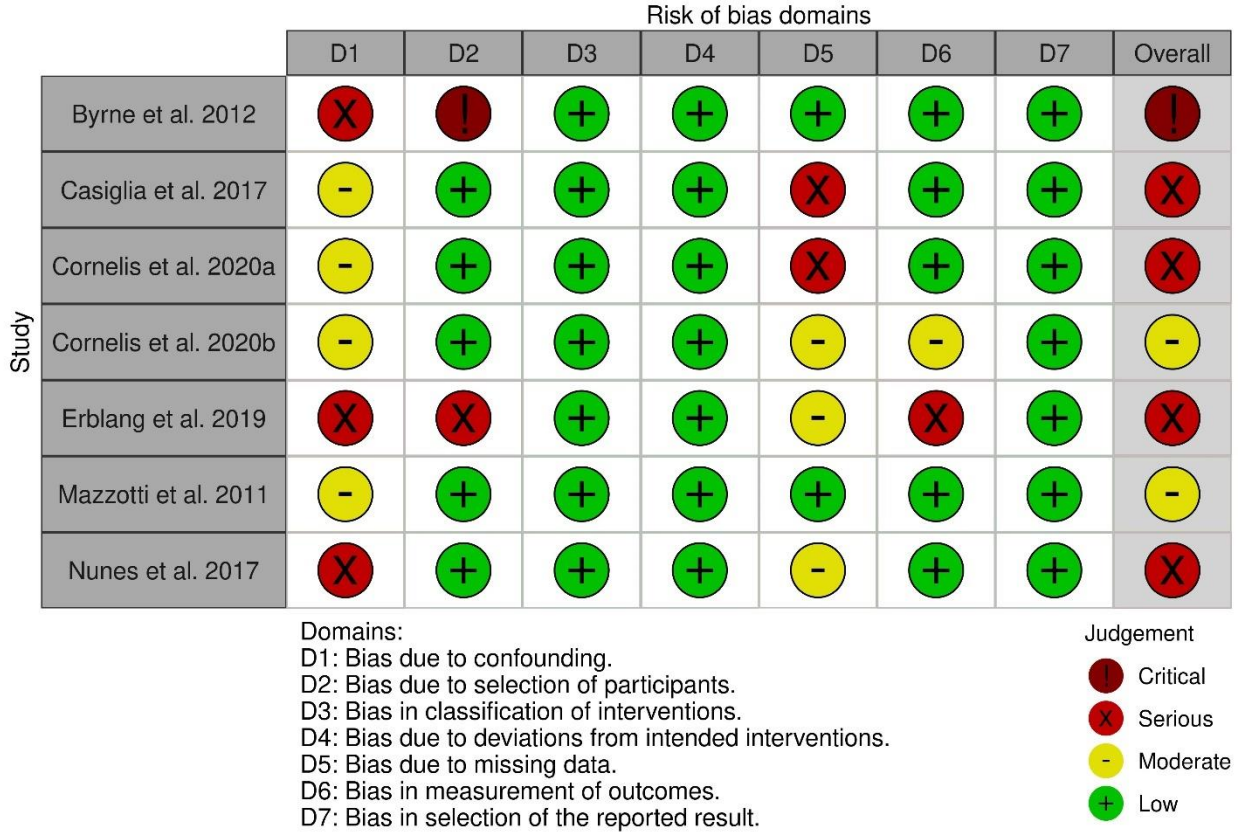

Figure 4 Risk of bias assessment using the ROBINS-I for non-randomised trials of interventions. Overall, two studies (29%) demonstrated a moderate risk of bias, four studies (57%) demonstrated a serious risk of bias, while one study (14%) demonstrated a critical risk of bias. Bias due to confounding was the domain that demonstrated moderate (57%) and high risk of bias (43%) in all studies. The domains that demonstrated low risk of bias in all studies (100%) were bias due to classification of interventions, bias due to deviations from intended interventions and bias in selection of the reported result. A critical risk of bias was demonstrated for bias due to selection of participants in one study (14%). From: McGuinness & Higgins, Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments, 2020.

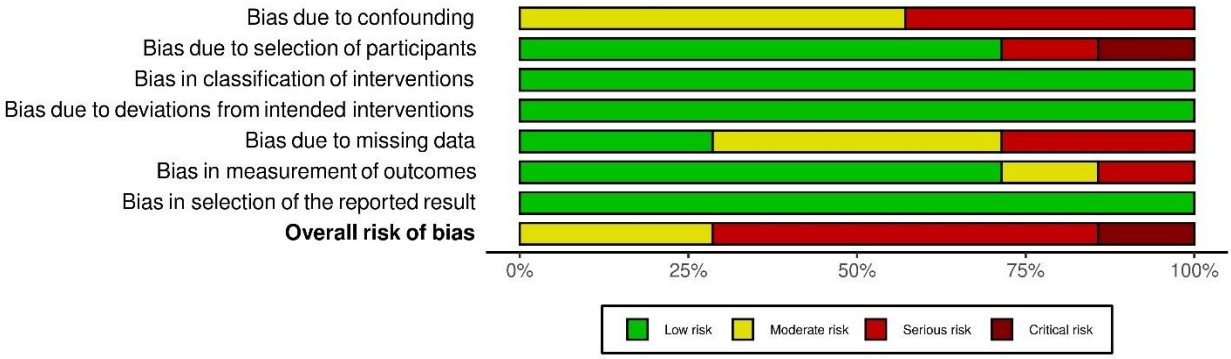

Figure 5 Summary of risk of bias assessment for non-randomised trials of interventions (n = 7). Bias due to confounding (moderate risk (n = 4, 57.1%), serious risk (n = 3, 42.9%); Bias due to selection of participants (low risk (n = 5, 71.4%), serious risk (n = 1, 14.3%), critical risk (n = 1, 14.3%); Bias in classification of interventions (low risk (n = 7, 100.0%); Bias due to deviations from intended interventions (low risk (n = 7, 100.0%); Bias due to missing data (low risk (n = 2, 28.6%), moderate risk (n = 3, 42.9%), serious risk (n = 2, 28.6%); Bias in measurement of outcomes (low risk (n = 5, 71.4%), moderate risk (n = 1, 14.3%), serious risk (n = 1, 14.3%); Bias in selection of the reported result (low risk (n = 7, 100.0%); Overall risk of bias (moderate risk (n = 2, 28.6%), serious risk (n = 4, 57.1%); critical risk (n = 1, 14.3%). From: McGuinness & Higgins, Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments, 2020.

List of tables

Table 1 PICOS criteria for inclusion and exclusion of studies.

Parameter	Inclusion criteria
Population	Healthy adults above 18 years old.
Intervention	a) habitual caffeine intake and acute caffeine supplementation. All caffeine doses reported as grouped variables or continuous variables were considered. b) genotyping for polymorphisms in genes associated with caffeine metabolism and physiological effects in humans. All genetic data reported as alleles, haplotypes or genetic scores were considered.
Comparator	a) different levels of habitual caffeine intake, different doses of caffeine supplementation and placebo supplementation. b) the variant allele, risk haplotype and different genetic scores.
Outcome	a) mood and anxiety (subjective measures and startle response). b) sleep disturbance/insomnia (subjective measures of sleep quality and duration and polysomnography). c) cognitive performance (measures of attention, reaction time, memory, alertness, decision making, reasoning).
Study design	All observational and experimental studies.

Table 2 Summary of records identified from systematic review of genetic studies on caffeine and cognitive performance, cognitive performance during sleep deprivation and cognitive performance during and post-exercise.

Authors	Study design	No of participants	Age (years, range / mean ± SD)	Region, Ethnicity/ Nationality	Gene - SNP(s)	MAF	HWE met	Intervention / exposure	Outcome (measure)	Result
		All* (M / F)								
cognitive performance										
Casiglia et al. (2017)	Cross-sectional	1,374 (601 / 773)	51.4 ± 15.3	Italy, Unknown	*CYP1A2* rs762551	0.33	yes	tertiles of habitual caffeine intake	Abstract reasoning (scores)	There was a significant genotype x caffeine interaction (p = .04). The CC homozygotes had significantly higher mean (SD) abstract reasoning in the 3d tertile of habitual caffeine intake (4.37 ± 0.24) compared to the 1st (3.39 ± 0.24; p < .005) and 2nd tertiles (3.49 ± 0.23; p < .01). Abstract reasoning in the A carriers was independent of caffeine intake (p > .05 for all tertiles of habitual caffeine intake).
Cornelis et al. (2020a)	Cross-sectional	295,492 (137,567 / 157,925)	37 – 73	UK, white British	rs6968554 (near A*HR*) rs2472297 (near *CYP1A2*) *CYP1A2* rs762551	NE NE NE	NA NA NA	caffeine vs no caffeine intake within the last hour prior to tests	Prospective memory** (scores) Pairs matching (n errors) FI** (n correct) Vigilance/RT (ms)	No significant CMSG x recent caffeine drinking interactions on cognitive function were found. Recent caffeine drinking was associated with increasing RT performance (β = -9.02, CI: -14.15, -3.89, p < .0006) and with decreasing Pairs Matching performance (β = 0.05, CI: 0.01, 0.08, p < .004) with increasing CMSG score. Stratified analysis suggested that recent caffeine
Cornelis et al. (2020b)	Cross-sectional	320,333 (147,332 / 173,001)	37 – 73	UK, white British	rs2472297 (near *CYP1A2*) rs6968554 (near *AHR*) *CYP1A2* rs762551	NE NE NE	NA NA NA	0 vs < 1 vs 1 vs 2–3 vs 4–5 vs 6–7 vs ≥ 8 cups of habitual coffee or tea intake / day	Prospective memory** (scores) Pairs matching (n errors) FI** (n correct) Vigilance/RT (ms) SDS (n correct) Trail Making Test A and B RT (ms)	Caffeine and tea intake were associated with decrements in FI performance among those with higher CMSG (p < .0003), but no significant CMSG × caffeine/tea interactions were observed The AA genotype of the rs762551 presented with greater decrements in performance in pairs matching with higher coffee intake than those with CC or AC genotypes (p <.0001); however, no significant rs762551 × coffee interaction was found.
Renda et al. (2015)	Crossover RCT	106 (106 / 0) low / moderate	18 - 40	Italy, Unknown	*ADORA2A* rs5751876 *AMPD1* rs17602729 *ADRA1A* rs1048101 *ADRA2B* rs29000568 *ADRB1* rs1801252 *ADRB1* rs1801253 *ADRB2* rs1042713 *ADRB2* rs1042714	NE NE NE NE NE NE NE NE	yes yes yes yes yes yes yes yes	3 mg/kg body mass of caffeine vs placebo	Alerting (ms) Orienting (ms) Verbal executive control (ms) Motor executive control (ms)	The CC homozygotes of the *ADORA2A* rs5751876 showed a significantly higher RT performance in orienting (ΔRT = 5.8 ms [CI: 0.5;11.0], p = .033), while the TT homozygotes showed higher RT performance in motor executive control (ΔRT = 19.2 ms [CI: 6.1; 29.4], p = .005) after caffeine compared to placebo. No other gene x caffeine interactions were identified.

		caffeine consumers			*ADRB2* rs1800888 *ADRB3* rs4994	NE NE	yes yes			
Salinero et al. (2017)	Crossover RCT	21 (14 / 7) light caffeine consumers	28.9 ± 7.3	Spain, Unknown	*CYP1A2* rs762551	NE	NA	3 mg/kg body mass of caffeine vs placebo	**Vigilance:** Mean RT (ms) Stage 1, 2, 3, 4 RT (ms)	There were no differences between genotype groups (AA homozygotes and C-allele carriers) in any variable measured during the visual attention test.
cognitive performance & sleep deprivation										
Baur et al. (2021)	Parallel group RCT	26 (14 / 12)	20 - 40	Switzerland, Western Europeans	*ADORA2A* rs5751876 CC homozygotes	NA	NA	400 + 200 g regular coffee containing 300 mg caffeine vs decaffeinated coffee	**Vigilance:** Speed (s^{-1}) Lapses (n) Accuracy (%) LSNR (db) **Visual search:** Speed target present (s^{-1}) Speed target absent (s^{-1}) Accuracy (%) **Working memory & executive control:** 1, 2 and 3-back speed (s^{-1}) 1, 2 and 3-back accuracy (%)	**Vigilance** The impairment in speed, lapses, and accuracy on the PVT after sleep deprivation was attenuated in the regular coffee group when compared to the decaffeinated coffee group ('day' x 'group' interactions: speed: $F_{6,672} = 7.72$; lapses: $F_{6,672} = 3.69$; accuracy: $F_{6,672} = 4.52$; p all < .001). The LSNR was higher than in the decaffeinated coffee group on restriction days 1 through 3 ('day' x 'group' interactions: $F_{6,672} = 9.54$, $p < .001$. **Visual search** The regular coffee group performed faster when the target was present ('day' x 'group' interaction: $F_{6,2759} = 3.08$, $p = .005$) on day 5 and when the target was absent ('day' x 'group' interaction: $F_{6,2759} = 4.83$, $p < .001$) on days 4 and 5 and more accurately throughout sleep restriction ('day' x 'group' interaction: $F_{6,672} = 4.35$, $p < .001$) compared to the decaffeinated coffee group. **Visuo-spatial working memory & executive control** The regular coffee group performed faster and more accurately than the decaffeinated coffee group on most days during sleep restriction ('day' x 'group' interaction: speed: $F_{6,2062} = 9.52$; accuracy: $F_{6,2062} = 5.13$; $p_{all} < .001$). **Verbal working memory & executive control** The regular coffee group performed faster and more accurately than the decaffeinated coffee group on all 3 workload levels on sleep restriction days 1 through 4 ('day' x 'group' interaction: speed: $F_{6,2065} = 8.11$; accuracy: $F_{6,2062} = 4.23$; $p_{all} < .001$).
Bodenmann et al. (2012)	Crossover RCT	45 (45 / 0)	20 - 30	Switzerland, Caucasian	HT4 and non-HT4 *ADORA2A* haplotypes including 8 variants: rs5751862, rs5760405, rs2298383, rs3761422,	NE	yes	2 x 200 mg caffeine vs placebo	**Vigilance:** Speed (s^{-1}) z scores	A significant haplotype x caffeine x session effect was found ($F_{26,219} = 2.1$, $p < .003$). Response speed scores were higher after caffeine compared with placebo in non-HT4 haplotype carriers of *ADORA2A* only after 15, 21, 24, 27 and 30h of wakefulness (p all < .05).

					rs2236624, rs5751876, rs35320474, rs4822492					
Skeiky et al., (2020)	Crossover RCT	12 (6 / 6)	27.4 ± 6.9	US, Unknown	*TNFα* rs1800629	NE	yes	200 mg caffeine vs 300 mg caffeine vs placebo	**Vigilance:** LSNR (db)	A non-significant genotype x caffeine effect was observed ($F_{2,20} = 0.21$, $p = .81$). No differences in performance between A allele carriers and GG homozygotes after 200 or 300 mg caffeine intake compared to placebo (p all > .05) during 48 h of TSD.
cognitive performance & exercise										
Carswell et al. (2020)	Crossover RCT	18 (12 / 6) active individuals	24.0 ± 4.0	UK, Unknown	*ADORA2A* rs5751876 *CYP1A2* rs762551	NE NE	NA NA	3 mg/kg body mass of caffeine vs placebo	**Vigilance:** Δspeed (s^{-1}) ΔRT (ms) Δlapses (n) Δslowest 10% response speed (s^{-1}) Δfastest 10% RT (ms)	'Fast' metabolisers showed lower ΔRT scores ($p < .01$, Cohen's $d = 1.6$) and higher Δspeed and Δslowest 10% response speed ($p < .01$, Cohen's $d = 1.5$ and 1.9, respectively) during exercise and lower Δfastest 10% RT and Δlapses at rest ($p < .05$, Cohen's $d = 1.1$ and $p < .01$, Cohen's $d = 1.7$, respectively) after caffeine compared with 'slow' metabolisers. No differences emerged between *ADORA2A* genotypes during exercise or at rest ('high' vs. 'low' sensitivity; p *all* > .05).

M: male; F: female; SD: Standard deviation; SNPs: Single Nucleotide Polymorphisms; MAF: Minor Allele Frequency; HWE: Hardy Weinberg Equilibrium; NE: Not estimable based on data in published work: not distinct genotypes but genetic scores were used for analyses; NA: Not available; *individuals for whom both genetic data & data on caffeine intake were available; RT: Reaction Time; ** added part-way through the baseline assessment period; FI: Fluid Intelligence; SDS: Symbol Digit Substitution; CI: Confidence Interval; CMSG: genetic caffeine metabolism score, derived by summing the number of single-nucleotide polymorphism alleles multiplied by their β-coefficients and recalibrated such that it ranged from 0 to 4, with higher scores predicting faster caffeine metabolism; ΔRT: difference in RT; PVT: Psychomotor Vigilance Task; LSNR: Log of the signal-to noise ratio; Δspeed: difference in speed, Δlapses: difference in number of lapses, Δslowest 10% response speed: difference in the slowest 10% response speed, Δfastest 10% RT: difference in fastest 10% RT; TSD: Total sleep deprivation.

Table 3 Summary of records identified from systematic review of genetic studies on caffeine and anxiety.

Authors	Study design	No of participants All* (M / F)	Age (years, range / mean ± SD)	Region, Ethnicity/ Nationality	Gene - SNP(s)	MAF	HWE met	Intervention / exposure	Outcome (measure)	Result
Alsene et al. (2003)	Crossover RCT	94 (51 / 43) no / low caffeine consumers	early 20s	US, Mixed - White (54), Black (15), Asian (20), Hispanic (5)	*ADORA2A* rs5751876 *ADORA2A* rs2298383	0.14 0.48	yes yes	150 mg caffeine vs placebo	POMS & VAS subjective anxiety (scores)	Only the TT group of the rs5751876 and rs2298383 polymorphism locus reported a significant increase in mean (SEM) anxiety after caffeine compared to placebo ($p < .05$) and this increase was significantly higher compared to the CC and CT genotype groups (POMS: 2.91 ± 0.59 vs -0.06 ± 0.59 vs 0.54 ± 0.41, respectively and VAS: 0.17 ± 0.04 vs -0.02 ± 0.05 vs 0.05 ± 0.05, respectively; *p all* < .05).
Childs et al. (2008)	Crossover RCT	62 (32 / 30) no / low caffeine consumers	20.7 ± 0.3	US, subset of European-Americans	*ADORA2A* rs5751876 *ADORA2A* rs2298383 *ADORA2A* rs4822492 *DRD2* rs1110976	0.41 0.44 0.44 0.14	yes yes yes yes	150 mg caffeine vs placebo	VAS subjective anxiety (scores)	Individuals with the CC genotype at the rs2298383 and rs4822492 reported significantly higher mean (SEM) peak change in anxiety after caffeine compared with baseline than those with the TT genotype at the rs2298383 locus and GG at the rs4822492 locus (11.25 ± 5.09 vs -8.19 ± 3.51 and 11.32 ± 5.16 vs -8.19 ± 3.51, respectively: *p all* < .007). Individuals with the G/− genotype of the *DRD2* rs1110976 reported higher mean (SEM) peak change in anxiety after caffeine compared with baseline than G/G individuals (4.34 ± 3.92 vs -2.78 ± 2.15; $p = .005$). No significant differences were observed among the *ADORA2A* rs5751876 genotypes.
Domschke et al. (2012a)	Parallel group RCT	110 (56 / 54)	18 - 50	Germany, Caucasian	*ADORA2A* rs5751876	NE	NA	150 mg caffeine vs placebo	POMS & VAS subjective anxiety (scores) startle magnitudes	Women but not men showed a significant genotype x caffeine interaction for unpleasant pictures ($F_{1, 46} = 6.83$, $p = .01$) with higher startle magnitudes for TT risk genotype in the caffeine condition and higher startle magnitudes in non-risk CC/CT genotypes in the placebo condition. There was a significant genotype x intervention interaction on POMS 'Depression – Anxiety' ratings: $F_{2, 212} = 5.25$, $p = .02$) but no significant genotype x intervention on VAS ratings: all $F_{2, 212} < 1.28$, $p > .28$.
Domschke et al. (2012b)	Parallel group RCT	116 (57 / 59)	18 - 50	Germany, Caucasian	*NPSR* rs324981	0.45	yes	150 mg caffeine vs placebo	startle magnitudes	A significant interaction between picture valence, NPSR genotype, and challenge condition ($F_{2,216} = 3.61$, $p = .03$) was identified. TT genotype had increased mean (SEM) startle magnitude in response to neutral stimuli (51.49 ± 0.43 vs 49.67 ± 0.53, $p \leq .05$) and a decrease in startle magnitude in response to unpleasant stimuli (49.81 ± 0.52 vs. 51.78 ±

										0.58, $p \leq .05$) in caffeine compared to placebo condition, respectively. No change was observed in AA/AT genotypes.
Gajewska et al. (2013)	Parallel group RCT	114 (57 / 57) low / moderate caffeine consumers	26.6 ± 6.2	Germany, Caucasian	*ADORA2A* rs5751876	NE	NA	150 mg caffeine vs placebo	Prepulse modification (%)	A significant genotype × intervention × gender × SOA on the % startle inhibition was observed ($F_{4,424} = 4.48$, $p = .001$). The TT genotype women reacted with a reduced % prepulse inhibition compared to TT genotype men in response to caffeine at 120 ms SOA: 32.85 % vs 66.41%, respectively; $t_{26} = 2.26$, $p = .03$ and 240 ms SOA: -4.55 % vs 39.41 %, respectively; $t_{26} = 2.63$, $p = .01$). No significant effects were observed between genotype groups.
Klauke et al. (2012)	Parallel group RCT	90 (45 / 45)	26.5 ± 6.2	Germany, European	*COMT* Val158Met	0.4	yes	150 mg caffeine vs placebo	startle magnitudes	No significant genotype x caffeine interaction was found and no differences in affect-modulated startle responses after caffeine based on the *COMT* Val158Met polymorphism.
Rogers et al. (2010)	Parallel group RCT	379 (180 / 199)	18 - 62	UK, Mixed - (95%) white Europeans	*ADORA2A* rs5751876	0.39	yes	100 + 150 mg caffeine vs placebo	MAPSS subjective anxiety	A significant genotype x caffeine interaction was found ($F_{2, 365} = 6.57$, $p = .002$**). The TT genotype significantly increased mean (SEM) anxiety after 100 mg caffeine compared to placebo (TT: 1.65 ± 0.15 vs CT/CC: 0.95 ± 0.17, $p < .01$). When considering habitual caffeine consumption, caffeine increased mean (SEM) anxiety only in non- and low consumers in all genotype groups (TT: 1.76 ± 0.18, $p < .05$ vs CT/CC: 1.1 ± 0.06, $p < .01$) after both caffeine sessions (100 + 150 mg).

M: male; F: female; SD: Standard deviation; SNPs: Single Nucleotide Polymorphisms; MAF: Minor Allele Frequency; HWE: Hardy Weinberg Equilibrium; NE: Not estimable based on data in published work; NA: Not available; *individuals for whom both genetic data & data on caffeine intake were available; SEM: Standard Error of the Mean; POMS: Profile of Mood States; VAS: Visual Analog Scale; Startle magnitudes: the difference between the highest peak 21–200ms after and the average during 50ms before startle probe presentation (anxiety-relevant, neutral, or pleasant picture); Prepulse modification (%): percent difference of the startle magnitude due to the preceding prepulse compared to control startle trials with positive values indicating prepulse inhibition of the startle response (PPI) and negative values indicating prepulse facilitation of the startle response (PPF); SOAs: Stimulus Onset Asynchronies; MAPSS: Mood, Alertness and Physical Sensations Scale; ** After 100 mg caffeine and baseline anxiety included as covariate.

Table 4 Summary of records identified from systematic review of genetic studies on caffeine and sleep disturbance and insomnia.

Authors	Study design	No of participants All* (M / F)	Age (years, range / mean ± SD)	Region, Ethnicity/ Nationality	Gene - SNP(s)	MAF	HWE met	Intervention / exposure	Outcome (measure)	Result
Byrne et al. (2012)	GWAS	2,110 (543 / 1,567)	NA	Australia, Caucasian	2,380,486 SNPs	NA	NA	drinking coffee in the evening	having ever experienced caffeine-induced insomnia vs having never experienced caffeine-induced insomnia	No SNPs reached the genome-wide significance level (7.2×10^{-8}) for caffeine-induced insomnia. Association analysis after adjusting for age, sex and insomnia factor score identified 8 loci related to caffeine-induced insomnia: rs521704 near the *GBP4* gene ($p = 1.9 \times 10^{-6}$, OR [95% CI] = 0.70 [0.62 – 0.78]); rs13172305 near the *RP11-772E11.1* gene ($p = 3.40 \times 10^{-6}$, OR [95% CI] = 1.76 [1.39 - 2.24]); rs11878836 near the *AC008556.1* gene ($p = 3.40 \times 10^{-6}$, OR [95% CI] = 1.37 [1.10 - 1.70]); rs561042 near the *GBP4* gene ($p = 6.20 \times 10^{-6}$, OR [95% CI] = 0.77 [0.66 - 0.91]); rs12725617 near the *LPHN2* gene ($p = 7.30 \times 10^{-6}$, OR [95% CI] = 0.74 [0.61 - 0.90]); rs12407812 near the *GBP1* gene ($p = 8.90 \times 10^{-6}$, OR [95% CI] = 1.41 [1.21 - 1.64]); rs9665295 near the *NEBL* gene ($p = 9.20 \times 10^{-6}$, OR [95% CI] = 2.55 [1.68 - 3.87]) and rs2103117 near the *RP1-21O18.1* gene ($p = 9.80 \times 10^{-6}$, OR [95% CI] = 0.61 [0.49 - 0.76]).
Erblang et al. (2019)	Cross-sectional	1,023 (618 / 405)	32.5 ± 9.6	France, European ancestry	*six ADORA2A* SNPs: rs5751862 rs2298383 rs3761422 rs2236624 rs5751876 rs4822492	 0.46 0.44 0.38 0.22 0.41 0.44	 yes yes yes yes yes yes	low (0-50 mg) vs moderate (51-300 mg) vs high (≥300 mg) habitual caffeine intake / day from all sources	TST (h) Sleep complaints (n, %)	Significant genotype x caffeine interactions were found for rs2298383, rs3761422, rs5751876 and rs4822492 ($p_{all} < .04$) for TST. Mean (95% CI) TST was lower in the CT and TT compared to CC group of the rs5751876 (7.05 ± 0.32 vs 6.92 ± 0.48 vs 7.53 ± 0.30, respectively) and in the CT and CC compared to TT group of the rs3761422 (7.00 ± 0.32 vs 6.85 ± 0.45 vs 7.56 ± 0.28, respectively), while it was higher in the TT vs CC genotype groups of the rs2298383 (7.52 ± 0.32 vs 6.93 ± 0.44) and the GG and GC compared to CC group of the rs4822492 (7.52 ± 0.30 vs 7.26 ± 0.32 vs 6.93 ± 0.44, respectively) only for low caffeine consumers. The risk (OR, 95% CI) of sleep complaints was lower in the CT compared to CC genotype group for rs5751876 (0.6, 0.4–0.9 vs 1) but it was higher in TT compared to CC for rs2298383 (1.5, 1.1–2.8 vs 1) and in GG compared to CC genotype group for rs4822492 (1.8, 1.1–2.9 vs 1) in moderate caffeine consumers. No other differences were shown between genotypes in any of the outcomes.

Holst et al. (2014)	Crossover RCT	16 (16 / 0)	18 - 35	Switzerland, Caucasian	*DAT1* VNTR	NE	NA	2 x 200 mg caffeine vs placebo	Wakefulness EEG power (%) NREM sleep EEG power (%)	A significant genotype x caffeine interaction was found on beta activity (21-24 Hz) ($F_{1,14} \geq 4.25$; $p_{all} < .05$). Caffeine administered during sleep deprivation enhanced beta (21–24 Hz) EEG activity in wakefulness compared to placebo (151.6% ± 9.5 vs placebo: 109.3% ± 9.7; $p < .05$) in 10R/10R homozygotes, yet not in 9R allele carriers. No genotype differences were observed for NREM sleep.
Mazzotti et al. (2011)	Cross-sectional	958 (421 / 537)	42.6 ± 14.4	Brazil, European ancestry	*ADA* rs73598374	0.05	yes	0 vs ≥ 1 cup of caffeine-containing drinks	Lights off time (h:m) Lights on time (h:m) Sleep latency (min) REM sleep latency (min) TST (min) Sleep efficiency (%) Stage 1, 2 and 3-4 sleep (%) REM sleep (%) Minutes awake Arousals / hour	Among caffeine consumers, A allele carriers showed lower mean (SD) sleep latency (12.41 min ± 15.26 vs 17.40 min ± 22.51 for non-carriers; $p = .03$), higher % sleep efficiency (84.93% ± 12.12 vs 81.52% ± 12.45 for non-carriers; $p = .01$), higher % REM sleep (20.77% ± 6.37 vs 18.95% ± 6.41 for non-carriers; $p = .02$), and fewer minutes awake (51.04 min ± 43.85 vs 61.04 min ± 44.62 for non-carriers; $p = .04$). Among those who did not consume caffeine, no differences were found between genotypes in any of the sleep parameters ($p_{all} > .05$).
Nunes et al. (2017)	Cross-sectional	926 (412 / 514)	42.8 ± 14.6	Brazil, European ancestry	*ADORA2A* rs5751876	0.46	yes	0.2 ± 0.3 caffeine load**	Sleep latency (min) REM sleep (%) Stage 1, 2 and 3-4 sleep (%)	Significant correlations between caffeine load and sleep latency ($r = 0.12$; $p = .003$; $\beta = 0.174$), % stage 3-4 sleep ($r = 0.09$; $p = .022$; $\beta = -0.077$), and % REM sleep ($r = 0.08$; $p = .04$; $\beta = -0.00004$) only in T allele carriers. No differences were found among genotype groups in any of the outcomes ($p_{all} > .05$).
Retey et al. (2007)	Crossover RCT	19 (19 / 0)	NA	Switzerland, Unknown	*ADORA2A* rs5751876	0.41	NA	2 x 200 mg caffeine vs placebo	non-REM sleep EEG power density (%)	The CC genotype displayed a greater rise in the EEG power in the beta band (16.625–20.125 Hz) after caffeine compared to the CT and TT genotypes: mean (SEM): 115.45% ± 3.09 vs 106.91% ± 2.98 vs 100% ± 5.00, respectively; $p < .03$

M: male; F: female; SD: Standard deviation; SNPs: Single Nucleotide Polymorphisms; MAF: Minor Allele Frequency; HWE: Hardy Weinberg Equilibrium; NE: Not estimable based on data in published work; NA: Not available; *individuals for whom both genetic data & data on caffeine intake were available; OR: Odds Ratio; TST: Total Sleep Time; EEG: Electroencephalogram; REM: Rapid-Eye Movement; NREM: Non-REM; ** caffeine load: total number of cups taken divided by the number of hours since the last caffeine-containing beverage was consumed on the day of polysomnography.