PharmGKB summary: caffeine pathway

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Background

Caffeine is a naturally occurring stimulant found in coffee, tea, chocolate, and used as an additive in other beverages and adjuvant analgesic in some pain medications [1,2]. It has long been known that genetic variation influences caffeine responses, indeed caffeine has been used as a probe drug for phenotyping CYP1A2 activity [3]. This brief summary highlights the candidate genes involved in the caffeine metabolism pathway and discusses the pharmacogenomic (PGx) variants, both pharmacokinetic and pharmacodynamic, their interaction with caffeine and its impact on disease risk. Caffeine is the most widely used drug in the world [4]. Since its use is so widespread it can be hard to assess the effect of the drug in isolation, and often PGx associations have been studied on the basis of intake of coffee or caffeinated beverages. These are also discussed here. Caffeine acts through multiple mechanisms, the most important of which is the antagonism of adenosine receptors (*ADORA1* and *ADORA2A*) [2]. Recent studies have suggested a role for caffeine in neuroprotection and as a potential treatment for Parkinson disease (PD) [5]. For caffeine to be applied as a therapeutic agent in genomic medicine, it is important to examine the evidence and limitations of the current knowledge of caffeine PGx.

Metabolism

Caffeine is almost completely metabolized with 3% or less being excreted unchanged in urine [3,6]. The main route of metabolism in humans (70–80%) is through *N*-3-demethylation to paraxanthine also known as 1,7-dimethylxanthine or 17X [3,6,7] (see Fig. 1). This reaction is carried out by CYP1A2 in the liver [6]. Experiments with human liver microsomes estimate that 1-*N*-demethylation to theobromine accounts for approximately 7 to 8% of caffeine metabolism with 7-*N*-demethylation to theophylline also around 7 to 8% [8]. The remaining 15% of caffeine undergoes C-8 hydroxylation to form 1,3,7-trimethyluric acid [8].

CYP1A2 is responsible for more than 95% of the primary metabolism of caffeine [9]. Therefore, caffeine is used as a probe drug for CYP1A2 activity with the relative ratios of urinary metabolites used as an indicator of the flux through different parts of the pathway [6]. Other than paraxanthine, the major metabolites of caffeine in urine are 1-methylxanthine (1X), 1-methyluric acid (1U), 5-acetylamino-6-formylamino-3-methyluracil (AFMU), and 1,7-dimethyluric acid (17U) [6]. These are formed by the secondary metabolism of paraxanthine by cytochrome P450 (CYP)1A2, CYP2A6, *N*-acetyltransferase 2, and xanthine dehydrogenase (also known as xanthine oxidase) [6]. *In vitro* studies in cell lines show involvement of CYP2E1 in the formation of theobromine and theophylline, whereas studies of recombinant proteins in microsomes do not support this but instead suggest that it contributes to the formation of 1,3,7-trimethyluric acid [8,10]. Microsome experiments have shown that CYP2C8, CYP2C9, and CYP3A4 also participate in the primary metabolism of caffeine [3,8,10]. The intrinsic clearance of other CYPs compared with CYP1A2 is lower for all routes of metabolism and lower by a factor of 10 when compared with the 3-demethylation reaction of CYP1A2. However, when comparing the intrinsic clearance for the other branches of the pathway, several are of the same order as CYP1A2 (see Table 2 in Kot and Daniel [8]). Although the impact of candidate genes other than *CYP1A2* may have limited relevance in the majority of cases, in situations where CYP1A2 activity is altered, such as coadministration of other CYP1A2-metabolized drugs, the relative flux through other parts of the pathway and other polymorphic enzymes may become more important.

Caffeine has a half-life of 4 to 5 h, which may be prolonged in patients with hepatic diseases, infants and neonates (up to 100 h), or during pregnancy [6]. Smoking increases clearance of caffeine because of its actions on CYP1A2 [11] (see below and PharmGKB *CYP1A2* VIP at *http://www.pharmgkb.org/search/annotatedGene/cyp1a2/index.jsp*).

Pharmacogenomics

There have been several studies that examined the PGx of caffeine (see Table 1 for summary). Most have looked at the role of variants in *CYP1A2* with several also considering those in *ADORA2A* and have found associations with various phenotypes (discussed below).

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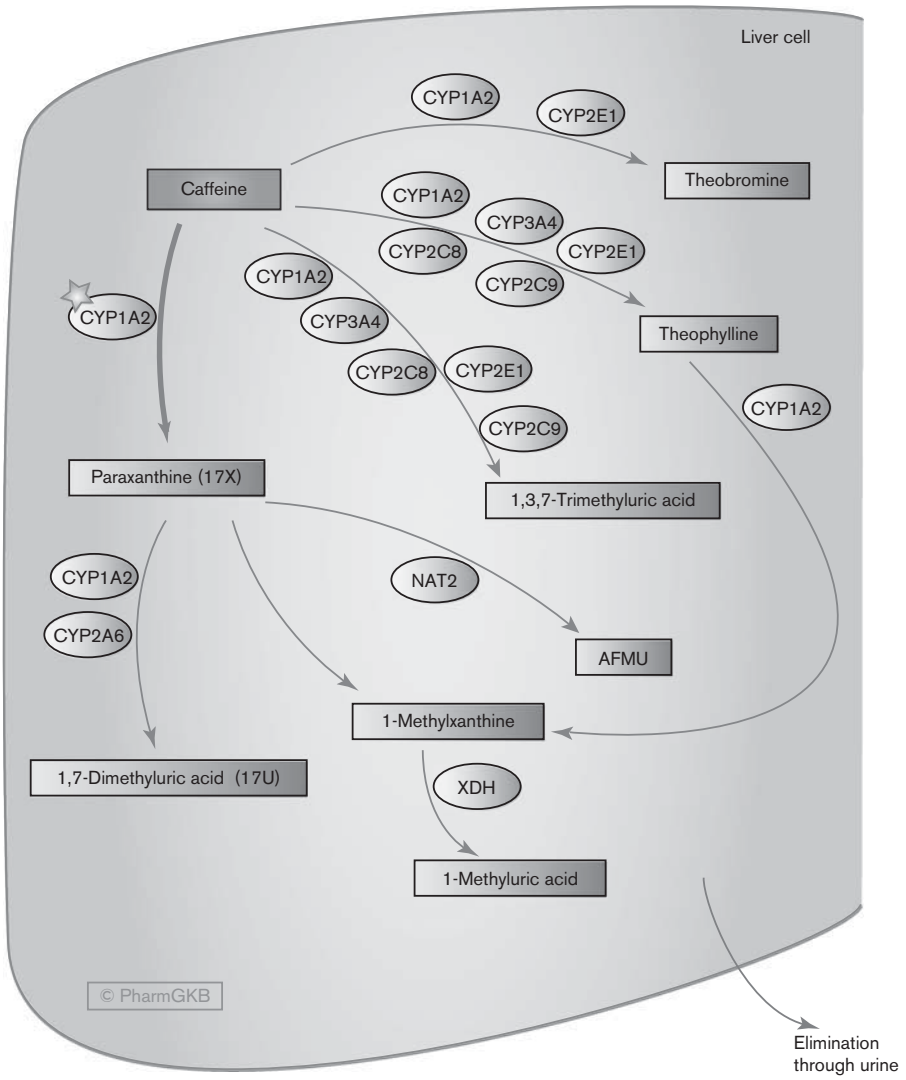
Fig. 1



Stylized liver cell depicting candidate genes involved in the pharmacokinetics of caffeine. The major route of metabolism to paraxanthine is highlighted by a bold arrow, and the key enzyme, CYP1A2, by a star. A fully interactive version is available online at *http://www.pharmgkb.org/pathway/PA165884757*. AFMU, 5-acetylamino-6-formylamino-3-methyluracil; CYP, cytochrome P450; NAT2, *N*-acetyl transferase 2; 17U, 1,7-dimethyluric acid; 17X, 1,7-dimethylxanthine; XDH, xanthine dehydrogenase.

The *CYP1A2*1F* allele is the most commonly studied variant with respect to caffeine. The variant that defines this haplotype is rs762551 (*CYP1A2*: −163C > A) [24]. A study of *CYP1A2*1F*, where other haplotypes containing rs762551 were excluded, showed that rs762551AA was associated with increased metabolism of caffeine in Swedish smokers as well as in Swedish and Serbian heavy coffee consumers [12,13]. Other haplotypes that included rs762551 (*CYP1A2**1L, *1V, and *1W) did not have significantly altered metabolism of caffeine [12]. A previous study reported association of the *CYP1A2*1K* allele with significantly reduced CYP1A2 activity in nonsmokers compared with *1A or *1F, using caffeine as a probe substrate [24]. To date, no studies have shown the mechanism by which the intronic rs762551 variant influences CYP1A2 activity and it may be that other variants in linkage with this locus may be responsible for the phenotypes (for more details on *CYP1A2* see *http://www.pharmgkb.org/vip/PA27093*).

Poor metabolizer variants in *CYP2A6*, which is the major enzyme responsible for the formation of 17U, influence the ratio of 17U to 17X [33]. Nonsmoking individuals with two inactive alleles (*CYP2A6**9 homozygotes and *4/*9 heterozygotes) had lower values of 17U/17X than those with one inactive allele, whose values were lower than those with two active alleles (*CYP2A6**1A, *1B1, or *1B1 × 2) [33]. The effect was less pronounced in smokers, although this may be because of smaller sample numbers [33].

Table 1 Summary of pharmacogenomic studies of caffeine indicating variants or alleles tested, phenotypes associated, and details of type of caffeine and population

Allele/genotype	Associated phenotype	Reference	Study parameters
CYP1A2 rs762551			
*1F/*1F haplotype, rs762551 Genotype AA	Increased caffeine metabolism compared with non-*1F carriers	17370067 [12]	Caffeine intake: 100 mg caffeine dose Study number and race: Swedish smokers, *n*=35 (the effect was not seen in Swedish nonsmokers, or in a cohort of Korean smokers, *n*=28 with low *1F allele frequency)
rs762551 Genotype AA	Increased caffeine metabolism	20390257 [13]	Caffeine intake: heavy coffee consumers (the association was not seen in nonheavy consumers) Study number and race: Swedish, *n*=42, Serbian, *n*=17
rs762551 Allele C	Not associated with habitual consumption of caffeine	17616786 [14]	Caffeine intake: habitual consumption of caffeine Study number and race: Hispanic Americans (*n*=2735)
rs762551 Genotype CC	Decreased risk of Parkinson's disease in coffee drinkers compared with genotype AA	21281405 [15]	Caffeine intake: association was seen in coffee consumers compared with those who never consume coffee Study number and race: mixed population, *n*=948 cases, *n*=1286 controls
rs762551	Not associated with caffeine-related protection from Parkinson's disease	18759349 [16]	Caffeine intake: coffee drinking habits assessed Study number and race: mixed population, *n*=782 matched case–control pairs
rs762551	Not associated with caffeine-related protection from Parkinson's disease	18075470 [17]	Caffeine intake: caffeine consumption Study number and race: Asian, *n*=418 cases, *n*=468 controls
rs762551 Genotype CC and AC	Increased risk of myocardial infarction in coffee consumers	16522833 [18]	Caffeine intake: coffee intake questionnaire Study number and race: Hispanic Americans, *n*=2104 cases, *n*=2104 controls
rs762551 Genotype CC and AC	Decreased risk of breast cancer, in carriers of BRCA1 mutations	17507615 [19]	Caffeine intake: coffee consumption (either caffeinated or decaffeinated), compared with individuals who have never consumed coffee Study number and race: White, *n*=89 cases and *n*=49 controls (coffee consumers) vs. *n*=30 controls and *n*=36 cases (never consumed coffee)
rs762551	Not associated with risk of ovarian cancer and caffeine, coffee, or tea intake	18941913 [20]	Caffeine intake: high vs. low caffeine consumption Study number and race: *n*=1354 cases, *n*=1851 controls (unknown race)
rs762551	Not associated with risk of bladder cancer and coffee consumption	18798002 [21]	Caffeine intake: coffee consumption (cups per day) Study number and race: Spanish, *n*=1034 cases, *n*=911 controls
rs762551 Genotype CC	Increased risk of recurrent pregnancy loss	15849225 [22]	Caffeine intake: maternal coffee consumption Study number and race: Japanese, *n*=58 cases, *n*=148 controls
rs762551 Genotype CC and AC	Increased risk for neural tube defects	20641098 [23]	Caffeine intake: maternal coffee consumption Study number and race: mixed population, *n*=306 cases, *n*=669 controls
CYP1A2 Alleles			
*1K allele (Key SNP: −730C>T rs12720461)	Reduced caffeine metabolism, compared with *1A, *1F, or *1J alleles	12920202 [24]	Caffeine intake: 100 mg caffeine dose Study number and race: Ethiopian, *n*=173 (association only seen in nonsmokers, *n*=153)
*1A, *1F, *1J	No significant effect on caffeine metabolism	12920202 [24]	Caffeine intake: 100 mg caffeine dose Study number and race: Ethiopians, *n*=173 (nonsmokers: *n*=153, smokers, *n*=20)
*1A, *1D, *1L, *1V, *1W alleles	No significant effect on caffeine metabolism	17370067 [12]	Caffeine intake: 100 mg caffeine dose Study number and race: Swedes, *n*=114 (nonsmokers), *n*=35 (smokers), Koreans, *n*=121 (nonsmokers), *n*=28 (smokers)
Additional CYP1A2 variants			
rs2470890 Genotype CC	Decreased risk of Parkinson's disease in coffee drinkers	21281405 [15]	Caffeine details: coffee consumption Study number and race: mixed population, *n*=941 cases, *n*=1264 controls
rs35694136	Not associated with caffeine-related protection from Parkinson's disease	18759349 [16]	Caffeine intake: coffee drinking habits assessed Study number and race: mixed population, *n*=910 matched case–control pairs
rs2470893	Associated with increased coffee consumption	21490707 [25]	Caffeine intake: coffee drinking habits assessed Study number and race: White, *n*=47341
rs2472297 Allele T	Associated with increased coffee consumption	21357676 [26]	Caffeine intake: coffee drinking habits assessed Study number and race: mixed population, *n*=6611
ADORA2A rs5751876			
rs5751876 Genotype TT	Decreased caffeine consumption	17616786 [14]	Caffeine intake: habitual consumption of caffeine Study number and race: Hispanic American, association found in *n*=2735 (full cohort), *n*=1767 (nonsmoker subset), and *n*=968 (current smokers subset)
rs5751876 Genotype TT	Increased anxiety in response to caffeine	12825092 [27]	Caffeine intake: 150 mg caffeine administration Study number and race: mixed population, *n*=100
rs5751876 Genotype TT	Increased anxiety in response to caffeine	18305461 [28]	Caffeine intake: 150 mg caffeine administration Study number and race: mixed population, *n*=102 (not significant in the European-American subset in this cohort, *n*=62)
rs5751876 Genotype TT	Increased anxiety in response to caffeine	20520601 [29]	Caffeine intake: initial 100 mg caffeine administered and then 150 mg 90 min later, or a placebo administered at both time points (capsules) Study number and race: mixed population, *n*=379 [predominantly (95%) White European]
rs5751876	No increase in anxiety in response to caffeine	22012471 [30]	Caffeine intake: 150 mg caffeine administration Study number and race: White, *n*=110
rs5751876 Genotype CC	Increased likelihood of being sensitive to caffeine compared with genotype TT	17329997 [31]	Caffeine intake: questionnaire of caffeine sensitivity and sleeping habits Study number: 58 cases (self-rated caffeine sensitive), 84 controls (self-rated caffeine insensitive) (race unknown)
rs5751876 Genotype CC	Increased likelihood of insomnia when exposed to caffeine	17329997 [31]	Caffeine intake: 2 doses of 200 mg caffeine or placebo, at 11 and 23 h of wakefulness Study number and race: *n*=19 (race unknown)

Table 1 (*continued*)

Allele/genotype	Associated phenotype	Reference	Study parameters
rs5751876 Genotype TT and CC	Not associated with vasodilator response	17558310 [32]	Caffeine intake: administration of adenosine, followed by caffeine (90 μg/min/dl) for 10 min Study number and race: $n=20$ (race unknown)
Additional ADORA2A variants			
rs2298383 Genotype CC	Increased anxiety in response to caffeine	18305461 [28]	Caffeine intake: 150 mg caffeine administration Study number and race: European-Americans, $n=62$
rs4822492 Genotype CC	Increased anxiety in response to caffeine	18305461 [28]	Caffeine intake: 150 mg caffeine administration Study number and race: European-Americans, $n=62$
rs3761422 Genotype TT	Increased anxiety in response to caffeine	20520601 [29]	Caffeine intake: initial 100 mg caffeine administered and then 150 mg 90 min later, or a placebo administered at both time points (capsules) Study number and race: mixed population, $n=379$ [predominantly (95%) White European]
rs35320474 Genotype TT	Increased anxiety in response to caffeine	12825092 [27]	Caffeine intake: 150 mg caffeine administration Study number and race: mixed population, $n=100$
rs3032740	Not associated with caffeine-related protection from Parkinson's disease	18759349 [16]	Caffeine intake: coffee drinking habits assessed Study number and race: mixed population, $n=1100$ matched case–control pairs
rs5751862	Not associated with increased anxiety in response to caffeine	20520601 [29]	Caffeine intake: initial 100 mg caffeine administered and then 150 mg 90 min later, or a placebo administered at both time points (capsules) Study number and race: mixed population, $n=379$ [predominantly (95%) White European]
rs5760405	Not associated with increased anxiety in response to caffeine	20520601 [29]	Caffeine intake: initial 100 mg caffeine administered and then 150 mg 90 min later, or a placebo administered at both time points (capsules) Study number and race: mixed population, $n=379$ [predominantly (95%) White European]
rs11704959	Not associated with increased anxiety in response to caffeine	20520601 [29]	Caffeine intake: initial 100 mg caffeine administered and then 150 mg 90 min later, or a placebo administered at both time points (capsules) Study number and race: mixed population, $n=379$ [predominantly (95%) White European]
rs2298383	Not associated with increased anxiety in response to caffeine (in multiple testing)	20520601 [29]	Caffeine intake: initial 100 mg caffeine administered and then 150 mg 90 min later, or a placebo administered at both time points (capsules) Study number and race: mixed population, $n=379$ [predominantly (95%) White European]
rs2267076	Not associated with increased anxiety in response to caffeine	20520601 [29]	Caffeine intake: initial 100 mg caffeine administered and then 150 mg 90 min later, or a placebo administered at both time points (capsules) Study number and race: mixed population, $n=379$ [predominantly (95%) White European]
CYP1A1 variants			
rs4646421	Not associated with risk of bladder cancer and coffee consumption	18798002 [21]	Caffeine intake: coffee consumption (cups per day) Study number and race: Spanish, $n=1002$ cases, $n=892$ controls
rs2198843	Not associated with risk of bladder cancer and coffee consumption	18798002 [21]	Caffeine intake: coffee consumption (cups per day) Study number and race: Spanish, $n=1018$ cases, $n=919$ controls
rs2472299	Not associated with risk of bladder cancer and coffee consumption	18798002 [21]	Caffeine intake: coffee consumption (cups per day) Study number and race: Spanish, $n=1000$ cases, $n=890$ controls
CYP2A6 alleles			
*4/*9 and *9/*9	Reduced metabolite ratio of 17U/17X	20155256 [33]	Caffeine intake: 100 mg caffeine administration Study number and race: Serbian, $n=100$
CYP2E1 variants			
rs2070676	Not associated with risk of bladder cancer and coffee consumption	18798002 [21]	Caffeine intake: coffee consumption (cups per day) Study number and race: Spanish, $n=1018$ cases, $n=918$ controls
rs8192766	Not associated with risk of bladder cancer and coffee consumption	18798002 [21]	Caffeine intake: coffee consumption (cups per day) Study number and race: Spanish, $n=1017$ cases, $n=919$ controls
AHR variants			
rs4410790	Associated with increased coffee consumption	21490707 [25]	Caffeine intake: coffee drinking habits assessed Study number and race: White, $n=47\,341$
rs6968865 Allele T	Associated with increased coffee consumption	21357676 [26]	Caffeine intake: coffee drinking habits assessed Study number and race: mixed population, $n=6611$
GRIN2A variants			
rs4998386 Allele T	Decreased risk of Parkinson's disease in heavy coffee drinkers	21876681 [34]	Caffeine intake: heavy vs. light caffeine consumption Study number and race: White, $n=1458$ cases, $n=931$ controls

Studies examining the effects of variants in the adenosine receptor ADORA2A and caffeine-related behavior and responses have had mixed results. *ADORA2A* rs5751876 TT is associated with decreased habitual consumption of caffeine as compared with genotypes CC + CT, and this association is more pronounced in smokers [14]. *ADORA2A* rs5751876 TT, rs2298383 CC, and rs4822492 CC were all associated with increased anxiety in response to caffeine in a healthy population that did not routinely consume much caffeine [28]. When the analysis was restricted to European-Americans it lacked sufficient power and no association was seen [28]. The association with *ADORA2A* rs5751876 TT and increased caffeine-induced anxiety were also seen in a mostly White

European nonsmoking or light smoking population [29], and a cohort of American college students with relatively low routine caffeine intake [27]. Although these studies were relatively small, the association did hold up to multiple testing and false discovery correction. A recent study in White healthy volunteers did not replicate this association, although the authors state that this may have been because of differences between the American and German anxiety measurement assessment scales or dose of caffeine [30]. They did observe qualitative differences in startle reflex that were more pronounced in women and involved the interaction of rs5751876 genotype, caffeine, and type of stimuli, although they did not compare genotype groups directly [30]. *ADORA2A* rs5751876 is not associated with vasodilator response when exposed to adenosine and caffeine [32]. Conversely, the CC genotype for *ADORA2A* rs5751876 is associated with increased likelihood of being sensitive to caffeine and increased likelihood of insomnia when exposed to caffeine [31].

The heritability of coffee consumption has been estimated at around 50% [33]. Recent independent genome wide association studies (GWAS) have identified variants in *CYP1A2* and *AHR* that influence caffeine intake [25,26]. In a meta-analysis of four large GWAS studies from Europe and the USA (totaling 6611 subjects), an effect of approximately 0.2 cups a day per allele was observed for rs2472297 T in the regulatory region of *CYP1A2* and rs6968865 T in *AHR* [26]. Another large meta-analysis of GWAS (47 341 White individuals) associated single nucleotide polymorphisms rs2472304 between *CYP1A2* and *CYP1A1* and rs4410790 near *AHR* with habitual caffeine intake [25]. The polymorphic sites identified in the coffee consumption GWAS were not in linkage with any known functional variants. However, the regions where these variants are located are involved in the transcriptional regulation of *AHR* and *CYP1A2*, therefore the variants may have a functional consequence or be linked to functional variants, however, these regions have not been fully characterized.

Gene-drug-disease relationships

Some studies have looked at the relationship between variants, caffeine, and disease risk. Most have looked at PD, a few studies have looked at cancer risk, some looked at risk for pregnancy complications and one study investigated risk for cardiovascular events. It should be noted that the associations seen in these studies have not been replicated. See Table 1 for a list of variants tested and study information.

In the Parkinson's Epidemiology and Genetic Associations Studies in the United States, the *CYP1A2* rs762551 genotype CC and rs2470890 genotype CC were associated with decreased risk of PD in coffee drinkers [15]. Although two variants in *ADORA2A* were associated with reduced risk for PD, there was no caffeine interaction for *ADORA2A* noted in this study [15]. As mentioned above, the CC genotype of rs762551 is not the genotype associated with inducibility of *CYP1A2* in response to smoking or heavy coffee drinking, which may suggest that caffeine is processed more slowly and has a greater effect in these individuals. Subsequent studies, however, have failed to replicate these associations. None of the variants tested in *ADORA2A* (rs3032740) or *CYP1A2* (rs35694136 and rs762551) were associated with caffeine-related protection from PD in a study of people from Midwest USA with mostly European ancestry [16]. A study in an Asian population also failed to find any interaction between *CYP1A2* rs762551, caffeine, and PD, although a significant association was seen between moderate-to-high caffeine intake and lower risk for PD [17]. Since it is likely that the rs762551 variant in *CYP1A2* is not the functional variant but only in linkage with it, the different haplotype structures in the different populations may have affected the ability to reproduce the association. A recent GWAS identified a new candidate gene for PD [34]. The *GRIN2A* rs4998386 T variant carriers had lower risk for PD among heavy coffee drinkers compared with the CC genotype [34]. The association was not seen in those who drink no or less than the median intake of coffee [34]. *GRIN2A* encodes an NMDA-glutamate-receptor subunit and regulates excitatory neurotransmission in the brain [34]. Caffeine has also been proposed as a modulator of Alzheimer disease and other dementias but no studies have yet reported the role of genomic variants in this effect [35].

In a study of patients with breast cancer-predisposing *BRCA1* variants, the C allele of *CYP1A2* rs762551 was associated with decreased risk for breast cancer in coffee drinkers compared with those who never consumed coffee [19]. This protective effect of coffee was not seen in the rs762551 AA homozygotes [19]. This was a very small study and has not been replicated. Studies of *CYP1A2* and coffee consumption and risk for ovarian cancer [20] or bladder cancer [21] found no association. In-vitro studies suggest that the protective effects of caffeine against cancer may be because of growth inhibition through phosphatase and tensin homolog and the phosphatidyl inositol 3-kinase/protein kinase B pathway [36]. Phosphatase and tensin homolog, phosphatidyl inositol 3-kinase, and protein kinase B are part of the Ras signaling pathway involved cellular growth and are the targets of several new anticancer drugs [37].

Studies of the effects of caffeine on pregnancy outcomes have shown increased risk for spontaneous pregnancy loss in women with high caffeine intake particularly among smokers [38,39]. Early studies that used metabolite phenotyping rather than genotyping suggested that women with low activity of caffeine metabolizing enzymes xanthine dehydrogenase or *N*-acetyltransferase 2 might be at increased risk for recurrent pregnancy loss, but results for CYP1A2 were unclear [40]. However, another

study found that high CYP1A2 activity was associated with risk for pregnancy loss and risk was increased with increasing caffeine intake [41]. Studies examining the role of *CYP1A2* variants showed an association between recurrent pregnancy loss, homozygous *CYP1A2*1F*, and caffeine intake above 100 mg per day, with an even greater risk for intake above 300 mg/day [22]. Another recent study showed that infant *CYP1A2*1F* genotype and maternal caffeine intake were associated with risk for neural tube defects [23].

In a study of South Americans, the authors reported that 'slow' caffeine metabolizers, than those with the *CYP1A2* rs762551 C allele, had increased risk of myocardial infarction [18]. However, this has not been validated. This study was criticized by others because the slow caffeine metabolizer phenotype for rs762551 C has only been observed in the context of smoking or heavy coffee consumption and this was not addressed in the study [42].

Conclusion

Since the use of caffeine is so widespread, knowledge of its pharmacokinetics and pharmacogenomics, the genes and variants that impact its metabolism and effects, is of importance for public health. Although there may be some beneficial effects of caffeine or coffee intake for particular individuals in the prevention of diseases, for others caffeine use may be associated with increased risk of disease, drug interactions, adverse events, and harm. Current studies have failed to validate clear relationships between gene variants, caffeine intake, and phenotypes. Work is needed to better define the functional variants that are involved in caffeine response. In addition, the components of coffee in addition to caffeine should be considered as these may have confounding effects in their actions on *AHR* and *CYP1A2*.

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Conflicts of interest

There are no conflicts of interest.

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