© IJHG 2020 Int J Hum Genet, 20(4): 219-228 (2020)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2020/20.04.765

Coffee and Green Tea Decoction Intake in the Tunisian Population According to Genetic Polymorphisms of Genes Involved in Habitual Caffeine Intake

Sabri Denden[1,2], Mohamed Hichem Sellami[3], Ridha Kibech[3], Amel Haj Khelil[1] and Mohamed Hédi Hamdaoui[4]

[1]Laboratory of Human Genome and Multifactorial Diseases (LR12ES07), Faculty of Pharmacy of Monastir, University of Monastir, Monastir, Tunisia
[2]High School for Health Sciences and Technicals, University of Tunis El Manar, Tunis, Tunisia
[3]Blood transfusion department, National Blood Transfusion Centre, University of Tunis El Manar, Tunis, Tunisia
[4]Research Unit on the Antioxidant Compounds, Oxidative Stress, Trace Elements and Metabolic Diseases, High School for Health Sciences and Technicals, University of Tunis El Manar, Tunis, Tunisia

KEYWORDS Coffee. Genetic Polymorphism. Green Tea. Beverage Intake

ABSTRACT The relationship between genetic variation and caffeinated beverages intake has been investigated in various human populations, but not among North Africans who have distinct caffeinated beverage dietary habits. Therefore, this study analysed the association between rs6968865 (*AHR*), rs382140 (*NRCAM*), rs9526558 (*CAB39L*), rs7754744 (*PDSS2*) and rs68157013 (*TAS2R43)* SNPs from previous GWASs on habitual caffeine consumption and caffeinated beverage intake in 568 healthy blood donors from the Tunisian population. The *AHR* caffeine metabolism gene SNP was associated with coffee intake but not with green tea decoction. However, the association of SNPs with caffeine metabolism (*CAB39L* and *PDSS2*), addiction to caffeine (*NRCAM*) and perceived caffeine bitterness (*TAS2R43*) genes was only observed or was stronger with the highest caffeine-containing beverage green tea decoction. The researchers further detected an opposite association of *PDSS2* gene SNP with coffee and tea intake. The study provides additional data on caffeinated beverage intake genetics in a population with specific dietary habits.

INTRODUCTION

Coffee and tea are the most consumed caffeine sources worldwide (Grigg 2002; Reyes and Cornelis 2018). It is known that these beverages have a potential beneficial effect, since they contain various active compounds such as polyphenols (particularly catechins), caffeine and other beneficial components (Cornelis 2019; Musial et al. 2020). Several studies have identified interactions between long-term caffeinated beverage consumption and risk of disease. Tea has been shown to reduce the risk of many diseases, including cardiovascular (Sueoka et al. 2001), neurodegenerative (Weinreb et al. 2004), cancer (Kavanagh et al. 2001) and inflammatory diseases (Dona et al. 2003). However, there is great controversy regarding the effects of coffee intake, as heavy coffee consumption has negative associations with Type 2 diabetes (van Dieren et al. 2009), Parkinson's disease (Hernan et al. 2002), Alzheimer's disease (Liu et al. 2016), some cancers (Je and Giovannucci 2012; Wilson et al. 2013; Loftfield et al. 2015; Petrick et al. 2015) and higher risk of hypertension (Nurminen et al. 1999; Ludwig et al. 2014) and myocardial infarction (Cornelis et al. 2006). Thus, understanding individual differences in caffeinated beverage consumption is of interest in relation to many human traits and diseases.

Caffeinated beverage preferences vary by demographic, social and environmental factors (Brice and Smith 2002). However, studies provide powerful evidence for a genetic determination of beverage consumption. Previous twin stud-

Address for correspondence:
Sabri Denden
High School of Health Sciences and Technicals,
B.P. 176 Bab-Souika 1006 Tunis, Tunisia
Phone: (+216)73461000,
Fax: (+216)73461830,
E-mail: sabri.denden@esstst.utm.tn

ies on caffeine intake reported heritability estimates for tea and coffee consumption ranging from 0.26 to 0.36 for tea and from 0.41 to 0.51 for coffee (Luciano et al. 2005; Teucher et al. 2007). Coffee and tea intake were considered as caffeine-related traits, so lower heritability for tea drinking was attributed to its lower caffeine content (Yang et al. 2010). Genome-wide association studies (GWAS) on habitual caffeine consumption have mainly targeted coffee intake, as coffee frequently contains more caffeine than tea. Indeed, theses studies were conducted only by the monitoring of average daily coffee consumption (Sulem et al. 2011; Amin et al. 2012; Coffee and Caffeine Genetics Consortium et al. 2015; Cornelis et al. 2016; Pirastu et al. 2016; Nakagawa-Senda et al. 2018) or using calculation algorithms assuming the content of caffeine in different foods and beverages that estimated tea cup caffeine amount as one-third (Cornelis et al. 2011) to one-half (Rodenburg et al. 2012; McMahonet al. 2014) of that contained in a coffee cup. Thus, few studies specifically assessed genetic association with habitual tea intake.

On the other hand, the way caffeinated beverages are made and consumed may vary widely, for example in many areas in North Africa, where green tea decoction is a very popular beverage. This tea, prepared by the cooking of the dried tealeaves in boiling water, is characterised by its higher content of polyphenols and caffeine (almost double the content of green tea infusion) (Snoussi et al. 2014).

Objectives

According to specific caffeinated beverage consumption patterns in the Tunisian population, it seems interesting to assess the association between genes discovered by previous genome-wide association studies on habitual coffee intake among populations of European ancestry and caffeinated beverage intake in the Tunisian population. Using a candidate gene approach, the researchers targeted four GWAS-significant loci, namely, *AHR* (Sulem et al. 2011*), NRCAM*, *CAB39L* (Amin et al. 2012) and *PDSS2* (Pirastu et al. 2016). Besides the dietary intake-based estimation, a coffee-liking ascertainment approach showed that *TAS2R43* gene SNPs were associated with coffee liking (Pirastu et al. 2014). The researchers therefore examined the association with *TAS2R43* locus involved in bitter taste perception. In addition, the researchers analysed the interactions effects between genetic variations and variables known to modulate caffeine consumption (age, sex and smoking).

MATERIAL AND METHODS

Study Subjects and Beverage Intake Data Assessment

Data on coffee and tea drinks consumption were obtained from consenting healthy blood donors self-referred to the National Blood Transfusion Centre in Tunisia (N=568, age= 35.17±10.18 years; 440 men and 128 women). Smoking categories were categorised as never smokers (39.61%), former smokers (13.73%) and current smokers (46.65%).The median number of cigarette packs per year was 7.80 ± 10.66 (ranging from 0 to 46). Coffee and tea intake data were obtained by beverage frequency questionnaire. The participants were asked: "how many cups per day of drink?" and "how many days of drink per week?" A beverage intake score (B_{score}) was then calculated according to the following formula: B_{score}= number of beverage cups consumed per day x (number of days of consumption per week/7). The local Ethics Committee approved the study.

Genotyping

Top-scoring rs6968865 (*AHR*), rs382140 (*NRCAM*), rs9526558 (*CAB39L*), rs7754744 (*PDSS2*) and rs68157013 (*TAS2R43*) SNPs from previous GWASs were selected for this study. Genomic DNA was extracted from blood samples of participants using standard salting-out method. Genotyping was performed by RFLP-PCR. PCR Primers pairs were as follow: rs6968865 (*AHR*), 5'AGCACCCTAACTCCATGCG3' and 5'TCTCCACTGAGTAT CCAAGGAAT3', rs382140 (*NRCAM*), 5'GTTTTGCCGTGAGCCAAAGT3' and 5'GCCCT CCTGTTTCCTCAGTG3', rs9526558 (*CAB39L*), 5'CCCATGCCTGTGTGACAGTA3' and 5'AGGATGGATGACCCCCAGAT3', rs7754744 (*PDSS2*), 5'ATTGGTGGCGGCAAGAA AAC3' and 5'GCCAAGCGTGAGATTAGGGA3', rs68157013 (*TAS2R43*), 5'ATGGT-

TGA TCACTGCCCAGAT3' and 5'TTC-CAGTCTGGTAGTGGTTAC3'. PCR products were digested with *EcoRV*, *BsiSI*, *Hpy188III*, *AluI* and *HinfI* restriction enzymes, respectively.

Statistical Analysis

Data analysis was performed with SPSS software version 24 (SPSS Inc., Chicago, IL, USA). A p-value of less than 0.05 was considered as statistically significant. Dominant and recessive minor allele models were assumed for SNPs effects. The p-values were calculated by using linear regression where the B_{score} was the dependent variable and the SNP genotype was the independent variable. Age, sex and smoking status were used as covariates. Missing data was not imputed in the analysis. ORs, 95% CIs and effect sizes (η^2_p) were given for significant effects. Two-way interaction models were developed to assess the joint effect of genotype, smoking status, age and sex on beverage consumption. The researchers also performed a sensitivity analysis using stratifications by smoking status (never plus former smokers (non smokers) versus current smokers (smokers)), median age (≤median age (35 years) versus > median age) and sex (men versus women).

RESULTS

Association of SNP Genotypes with Beverage Intake

Table 1 summarises the association between SNPs and beverages intake. *AHR* rs6968865 SNP T allele (in the dominant model) showed the strongest significant increase in coffee intake (OR=1.68, 95%CI: 1.33-2.13, p^a <0.001). Only tendency to association was detected with *TAS2R43* rs68157013 SNP (coffee intake increase in C allele carriers, p=0.072), *NRCAM* rs382140 (coffee intake decrease in A allele carriers, p=0.016, p^a=0.07) and *PDSS2* rs7754744 SNP (coffee intake decrease in A allele carriers, p=0.001, p^a=0.055).

As for tea intake, the highest positive associations were reported with the minor alleles of *CAB39L* rs9526558 SNP (the G allele at the recessive model, OR=1.58, 95%CI: 1.23-2.04, p^a <0.001) and PDSS2 rs7754744 SNP (the A allele at the recessive model, OR=1.40, 95%CI: 1.16-1.68, p^a <0.001). Moderate effects were observed with *NRCAM* rs382140 SNP A allele (decreased consumption at the recessive model, OR=0.79, 95%CI: 0.64-0.97, p^a=0.025) and *TAS2R43* rs68157013 SNP C allele (increased consumption at the dominant model, OR=1.13, 95%CI: 1.006-1.28, p^a=0.040).

SNP-covariates Interaction Analysis

Table 2 displays the results for SNPs interactions with median age, sex and smoking status caffeine intake covariates. For *AHR* rs6968865 SNP T allele, with the strongest association with coffee intake, only a borderline interaction was recorded with the smoking status (F=3.70, p=0.055, partial eta squared=0.008). In sensitivity analysis (Table 3), the genetic effect was more pronounced in non-smokers (OR=3.10, 95%CI: 1.77-5.43, p^a=0.000). Moderate significant interaction effects on coffee intake were recorded for *PDSS2* rs7754744 SNP (A-allele dominant model) X sex (F=4.45, p=0.035, partial eta squared=0.009) and *TAS2R43* rs68157013 SNP (C-allele dominant model) X Median age (F=5.71, p=0.017, partial eta squared = 0.011). When examining within subgroups, the association was found only in men (for *PDSS2* rs7754744 SNP, OR=0.73, 95%CI: (0.59-0.90), p^a=0.004) and age ≤35 years (for *TAS2R43* rs68157013 SNP, OR=1.68, 95%CI: (1.30-2.18), p^a=0.000).

With regards to tea intake, the examination of the strongest locus associated with habitual consumption, *CAB39L* rs9526558 SNP, showed that it interacts with smoking status (F=9.03, p=0.003, partial eta squared=0.018), median age (F=3.73, p=0.054, partial eta squared=0.008) and sex (F=6.56, p=0.011, partial eta squared=0.013) in the G-allele recessive model (Table 2). A further analysis by subgroups showed that the genetic effect was only in men (OR=1.58, 95%CI: 1.23-2.03, p^a=0.000), age ≤35 years (OR=2.10, 95%CI: 1.44-3.07, p^a=0.000) and non-smokers (OR=3.10, 95%CI: 1.77-5.43, p^a=0.000) (Table 3). As for the interaction with *PDSS2* rs7754744 SNP, it was driven by a larger genotype (AA versus GG+GA) effect in men (F=13.78, p=0.000, partial eta squared=0.029) and smokers (F=10.87, p=0.001, partial eta squared=0.023).

Table 1: The effect of SNP genotypes on beverage intake

SNP genotype	B_{score} *for coffee intake Mean (SD)*	B_{score} *for tea intake Mean (SD)*	*SNP genotype*	B_{score} *for coffee intake Mean (SD)*	B_{score} *for tea intake Mean (SD)*
AHR rs6968865 A>T			*CAB39L rs9526558 A>G*		
AA (N=152)	1.186 (1.02)	0.370 (0.765)	AA+AG (N=492)	1.458 (1.13)	0.345 (0.69)
*AT+TT (N=368)	1.599 (1.25)	0.344 (0.69)	* GG (N=44)	1.584 (0.96)	0.753 (0.88)
p	0	0.315	p	0.477	0
OR (IC à 95%)	1.51 (1.20-1..89)	-	OR (IC à 95%)	-	1.50 (1.20-1.87)
p[a]	0	-	p[a]	-	0
OR (IC à 95%)	1.68 (1.33-2.13)	-	OR (IC à 95%)	-	1.58 (1.23-2.04)
AHR rs6968865 A>T			*PDSS2 rs7754744 A>G*		
AA+AT (N=384)	1.521 (1.22)	0.377 0 (0.77)	GG (N=296)	1.63 (1.18)	0.32 (0.63)
*TT (N=136)	1.337 (1.11)	0.276 (0.50)	*GA+AA (N=244)	1.29 (1.15)	0.46 (0.85)
p	0.132	0.167	p	0.001	0.035
OR (IC à 95%)	-	-	OR (IC à 95%)	0.71 (0.58-0.87)	1.14 (1.01-1.30)
p[a]	-	-	p[a]	0.055	0.063
OR (IC à 95%)	-	-	OR (IC à 95%)	-	-
NRCAM rs382140 A>G			*PDSS2 rs7754744 A>G*		
GG (N=320)	1.64 (1.27)	0.34 (0.69)	GG+GA (N=456)	1.44 (1.15)	0.33 (0.64)
*GA+AA (N=200)	1.28 (0.95)	0.30 (0.58)	*AA (N=84)	1.63 (1.29)	0.66 (1.07)
p	0.016	0.598	p	0.176	0
OR (IC à 95%)	0.77 (0.62-0.95)	-	OR (IC à 95%)	-	1.38 (1.16-1.64)
p[a]	0.07	-	p[a]	-	0
OR (IC à 95%)	-	-	OR (IC à 95%)	-	1.40 (1.16-1.68)
NRCAM rs382140 A>G			*TAS2R43rs68157013 G>C*		
GG+GA (N=472)	1.54 (1.16)	0.35 (0.67)	GG (N=284)	1.392 (1.18)	0.29 (0.59)
*AA (N=48)	1.47 (1.18)	0.05 (0.16)	*GC+CC (N=284)	1.570 (1.13)	0.41 (0.79)
p	0.683	0.002	p	0.072	0.042
OR (IC à 95%)	-	0.74 (0.61-0.90)	OR (IC à 95%)	-	1.12 (1.005-1.27)
p[a]	-	0.025	p[a]	-	0.04
OR (IC à 95%)	-	0.79 (0.64-0.97)	OR (IC à 95%)	-	1.13 (1.006-1.28)
CAB39L rs9526558 A>G			*TAS2R43 rs68157013 G>C*		
AA (N=260)	1.541 (1.28)	0.346 (0.72)	GG+GC (N=452)	1.497 (1.13)	0.36 (0.69)
*AG+GG (N=276)	1.40 (0.93)	0.409 (0.72)	*CC (N=116)	1.418 (1.27)	0.30 (0.75)
p	0.145	0.308	p	0.521	0.387
OR (IC à 95%)	-	-	OR (IC à 95%)	-	-
p[a]	-	-	p[a]	-	-
OR (IC à 95%)	-	-	OR (IC à 95%)	-	-

* Risk genotype
[a] adjusted for age, sex and smoking status

Table 2: SNP genotypes main effects and interactions with caffeine intake covariates

	B_{score} *for coffee intake*				B_{score} *for tea intake*			
	df	*F*	*p*	η^2_p	*df*	*F*	*p*	η^2_p
AHR rs6968865 A>T	dominant model (AT+TT vs AA)							
Genotype[a]	1	16.62	0	0.035				
Genotype X Median Age[b]	1	1.02	0.312	0.002				
Genotype X Sex[c]	1	0.05	0.817	0				
Genotype X Smoking status[d]	1	3.7	0.055	0.008				
NRCAM rs382140 A>G	dominant model (GA+AA vs GG)				recessive model (AA vs GG+GA)			
Genotype[a]	1	3.46	0.063	0.007	1	6.15	0.013	0.013
Genotype X Median Age[b]	1	0.18	0.672	0	1	0.39	0.532	0.001
Genotype X Sex[c]	1	2	0.158	0.004	1	0.14	0.701	0
Genotype X Smoking status[d]	1	0.63	0.427	0.001	1	0	0.956	0
CAB39L rs9526558 A>G					recessive model (GG vs AA+AG)			
Genotype[a]					1	12.56	0	0.025
Genotype X Median Age[b]					1	3.73	0.054	0.008
Genotype X Sex[c]					1	6.56	0.011	0.013
Genotype X Smoking status[d]					1	9.05	0.003	0.018
PDSS2 rs7754744 A>G	dominant model (GA+AA vs GG)				recessive model (AA vs GG+GA)			
Genotype[a]	1	3.5	0.062	0.007	1	15.93	0	0.033
Genotype X Median Age[b]	1	1.82	0.178	0.004	1	0.46	0.497	0.001
Genotype X Sex[c]	1	4.45	0.035	0.009	1	13.78	0	0.029
Genotype X Smoking status[d]	1	1.15	0.283	0.002	1	10.87	0.001	0.023
TAS2R43 rs68157013 G>C	dominant model (GC+CC vs GG)				dominant model (GC+CC vs GG)			
Genotype[a]	1	7.37	0.007	0.014	1	4.8	0.029	0.009
Genotype X Median Age[b]	1	5.71	0.017	0.011	1	0	0.986	0
Genotype X Sex[c]	1	0.01	0.909	0	1	0.02	0.885	0
Genotype X Smoking status[d]	1	0.44	0.507	0.001	1	0.17	0.679	0

[a] adjusted for Median age, sex and smoking status
[b] adjusted for sex and smoking status
[c] adjusted for Median age and smoking status
[d] adjusted for Median age and sex

DISCUSSION

The differences between populations in term of caffeinated beverages consumption may be influenced by various factors including the genetic background and lifestyle factors, mainly differences in dietary habits. In the literature, genome-wide association studies on caffeine habitual intake, investigated in populations of European ancestry, have focused on coffee intake as having the highest caffeine content (Sulem et al. 2011; Amin et al. 2012; Coffee and Caffeine Genetics Consortium et al. 2015; Cornelis et al. 2016; Pirastu et al. 2016). Thus, few studies have analysed tea consumption genetics. Furthermore, only tea infusion intake (with lower caffeine content) was evaluated (Cornelis et al. 2011; Rodenburg et al. 2012; McMahon et al. 2014; Cornelis et al., 2016; Taylor et al. 2018) but not tea decoction. Tea decoction is a very popular beverage through extensive areas of North Africa, especially Tunisia. It is prepared by boiling the dried tea leaves in water for 15-30 minutes and is known for its high level of caffeine (almost twice the amount as in tea infusion) (Snoussi et al. 2014).

To gain better insights into the relationship between genetic variation and caffeinated beverages dietary patterns differences between populations, the researchers conducted this first study on caffeinated beverages intake genetic in a North African population, Tunisia. Four SNPs identified in previous coffee intake GWASs in populations of European ancestry were selected, namely, *AHR* (rs6968865), *NRCAM* (rs382140), *CAB39L* (rs9526558), and *PDSS2* (rs7754744) loci. One of the strongest associations from GWASs on caffeine intake was observed with rs6968865, rs382140 and rs9526558 SNPs (Sulem et al. 2011; Amin et al. 2012). As for rs7754744 SNP, it was one of five *PDSS2* gene SNPs in strong linkage disequilibrium that

showed association replication in cohorts from the Italian and Dutch populations (Pirastu et al. 2016). Besides the GWAS-based SNP choice, the researchers further investigated the association with *TAS2R43* gene implicated in bitter taste perception. Top hit rs68157013 SNP, identified by Illumina high density SNP arrays genotyping focusing on *TAS2R* genes SNP (Pirastu et al. 2014), was selected for the study.

The researchers showed that the highest coffee consumption was observed with the T allele of rs6968865 (*AHR*) SNP. Unlike coffee intake, rs6968865 SNP was not associated with tea intake. The finding of the strongest association with coffee intake according to rs6968865 SNP is consistent with previous findings (Cornelis et al. 2011; Sulem et al. 2011). AHR plays a key role in CYP1A2 induction (Nukaya et al. 2009), which is the main enzyme that metabolises caffeine (Butler et al. 1989). Thus, it is not surprising that a gene directly involved in caffeine metabolism is responsible for the highest variation in coffee habitual consumption (3.2%). The rs68157013 (*TAS2R43*) SNP showed only borderline ($p = 0.072$) to moderate ($p^a = 0.040$) associations with coffee and tea intake, respectively. Beyond the GWAS studies based on food intake, the non-functional rs68157013 and rs71443637 alleles in *TAS2R43* gene showed lower liking for coffee when studying coffee hedonic (Pirastu et al. 2014). The reported small percentage of coffee liking variance explained by the genetic variation (0.32%) is in line with beverage intake variation by rs68157013 SNP in the study. Additionally, it has been suggested that beverage preference is mediated by the bitterness perceived by caffeine (Yeomans et al. 2007), and therefore this may differ depending on beverage caffeine content. Surprisingly, the researchers found that the genetic effect of rs68157013 SNP was more pronounced in tea consumption than in coffee consumption. One explanation for this finding is that tea decoction may contain more caffeine per serving than coffee.

Among SNPs that showed strong evidence of association with coffee intake in GWASs, there were *NRCAM* rs382140 SNP ($p=3.3x10^{-07}$) and top hit rs9526558 SNP within *CAB39L* ($p=6.79x10^{-07}$) (Amin et al. 2012). NRCAM encodes neuronal cell adhesion molecule, a protein that is involved in neuron-neuron adhesion and nervous system development (Ishiguro et al. 2006). Several lines of evidence support *NRCAM* genetic variation to be associated with addiction susceptibility and polysubstance abuse (Uhl2006). Therefore, that may influence caffeine consumption. *CAB39L* is found to encode a member of calcium binding protein family, CAB39L, implicated in cytoskeletal rearrangement and cell mobility (Khanna et al. 2004). As a molecule involved in kinases activation, CAB39L is implicated in several cellular pathways including tumour suppressing (Li et al. 2018). The relationship between CAB39L and caffeine is not yet understood. However, CAB39L was found to be upregulated after caffeine treatment in the expression analysis performed in three types of cell lines (Amin et al. 2012), suggesting CAB39L to be involved in caffeine metabolism and/or response. In the present study, the researchers did not find any significant association between coffee intake and *CAB39L* rs9526558 or *NRCAM* rs382140 SNPs. However, these SNPs were associated with tea consumption. Accordingly, the variation in tea beverage intake, explained by *CAB39L* and *NRCAM* genes variants, might be mediated by its higher caffeine amounts.

Regarding the *PDSS2* gene, which encodes decaprenyl-diphosphate synthase subunit 2 synthesising the prenyl side-chain of coenzyme Q, a trend towards a significant decrease in coffee intake was detected with *PDSS2* rs7754744 SNP A allele ($p=0.001$, $p^a=0.055$), while the same A allele, but at the recessive model, showed a strong increase in tea intake($p^a <0.001$). The researchers detected a similar situation of opposite association with coffee and tea intake when analysing the relationship with SNPs at the *CYP1A1-CYP1A2* genes junction (data not published). In the GWAS showing the association of *PDSS2* gene with coffee intake (Pirastu et al. 2016), the authors reported that PDSS2 negatively regulates the expression of caffeine metabolism genes. Based on these findings, it seems that the observed opposite associations with coffee and tea intake in *PDSS2* rs7754744 and *CYP1A1-CYP1A2*SNPs variants may result from the same regulatory pathway. Further studies are still needed to understand precisely the molecular mechanism leading to this beverage intake preference according to specific variants in caffeine metabolism genes.

Table 3: SNP genotypes effects by subgroups of caffeine intake covariates

TAS2R43 rs68157013 G>C	B_{score} *for coffee intake Mean (SD)*	B_{score} *for tea intake Mean (SD)*	*PDSS2 rs7754744 A>G*	B_{score} *for coffee intake Mean (SD)*		B_{score} *for tea intake Mean (SD)*	*CAB39L rs9526558 A>G*	B_{score} *for tea intake Mean (SD)*	*AHR rs6968865 A>T*	B_{score} *for coffee intake Mean (SD)*
Non smokers										
GG(N=144)	0.94 (1.02)	0.33 (0.71)	GG (N=144)	1.10 (1.03)	GG+GA (N=232)	0.45 (0.79)	AA+AG (N=264)	0.41 (0.79)	AA (N=80)	0.62 (0.78)
*GC+CC (N=144)	1.10 (0.93)	0.48 (0.88)	*GA+AA (N=136)	0.92 (0.89)	*AA (N=48)	0.51 (1.10)	* GG (N=12)	1 (1.47)	*AT+TT (N=188)	1.17 (1.03)
p	0.16	0.11	p	0.115	p	0.658	p	0.017	p	0
OR (95% CI)	-	-	OR (IC à 95%)	-	OR (IC à 95%)	-	OR (95% CI)	1.79 (1.10-2.90)	OR (95% CI)	1.73 (1.34-2.23)
p[a]	-	-	p[a]	-	p[a]	-	p[a]	0	p[a]	0
OR (95% CI)	-	-	OR (IC à 95%)	-	OR (95% CI)	-	OR (95% CI)	3.10 (1.77-5.43)	OR (95% CI)	3.10 (1.77-5.43)
Smokers										
GG (N=124)	1.90 (1.19)	0.19 (0.39)	GG (N=136)	2.18 (1.10)	GG+GA (N=200)	0.18 (0.37)	AA+AG (N=216)	0.23 (0.56)	AA (N=68)	1.79 (0.91)
*GC+CC (N=128)	2.07 (1.14)	0.33 (0.64)	*GA+AA (N=92)	1.83 (1.30)	*AA (N=28)	0.92 (1.14)	* GG (N=28)	0.61 (0.54)	*AT+TT (N=160)	2.08 (1.31)
p	0.23	0.047	p	0.03	p	0	p	0.001	p	0.1
OR (95% CI)	-	1.15 (1.002-1.32)	OR (IC à 95%)	0.70 (0.51-0.96)	OR (IC à 95%)	2.09 (1.70-2.58)	OR (95% CI)	1.45 (1.16-1.81)	OR (95% CI)	-
p[a]	-	0.09	p[a]	0.036	p[a]	0	p[a]	0.054	p[a]	-
OR (95% CI)	-	-	OR (IC à 95%)	0.71 (0.51-0.97)	OR (95% CI)	2.17 (1.75-2.70)	OR (95% CI)	-	OR (95% CI)	-
Age d 35 Years										
GG (N=148)	1.29 (1.03)	0.22 (0.47)	GG (N=140)	1.56 (1.06)	GG+GA (N=224)	0.22 (0.56)	AA+AG (N=260)	0.26 (0.59)	AA (N=196)	1.34 (1.06)
*GC+CC (N=136)	1.72 (1.25)	0.33 (0.80)	*GA+AA (N=120)	1.39 (1.32)	*AA (N=36)	0.70 (1.07)	* GG (N=16)	1 (1.26)	*AT+TT (N=168)	1.67 (1.24)
p	0.002	0.144	p	0.259	p	0	p	0	p	0.034
OR (95% CI)	1.52 (1.16-2.00)	-	OR (IC à 95%)	-	OR (IC à 95%)	1.62 (1.28-2.04)	OR (95% CI)	2.09 (1.50-2.90)	OR (95% CI)	1.38 (1.02-1.86)
p[b]	0	-	p[b]	-	p[b]	0	p[b]	0	p[b]	0.003
OR (95% CI)	1.68 (1.30-2.18)	-	OR (IC à 95%)	-	OR (95% CI)	1.76 (1.36-2.27)	OR (95% CI)	2.10 (1.44-3.07)	OR (95% CI)	1.52 (1.15-2.01)
Age >35 Years										
GG (N=112)	1.39 (1.22)	0.38 (0.73)	GG (N=124)	1.61 (1.24)	GG+GA (N=176)	0.40 (0.67)	AA+AG (N=204)	0.39 (0.77)	AA (N=44)	0.92 (0.89)
*GC+CC (N=116)	1.48 (1.01)	0.50 (0.82)	*GA+AA (N=92)	1.28 (0.93)	*AA (N=40)	0.72 (1.17)	* GG (N=20)	0.60 (0.50)	*AT+TT (N=160)	1.52 (1.18)
p	0.524	0.22	p	0.037	p	0.021	p	0.26	p	0.002
OR (95% CI)	-	-	OR (IC à 95%)	0.72 (0.53-0.98)	OR (IC à 95%)	1.37 (1.04-1.80)	OR (95% CI)	-	OR (95% CI)	1.82 (1.24-2.65)
p[b]	-	-	p[b]	0.056	p[b]	0.004	p[b]	-	p[b]	0.003
OR (95% CI)	-	-	OR (IC à 95%)	-	OR (95% CI)	1.50 (1.13-1.97)	OR (95% CI)	-	OR (95% CI)	1.78 (1.21-2.61)
Women										
GG (N=52)	1.14 (1.48)	0.30 (0.93)	GG (N=60)	1.33 (1.36)	GG+GA (N=92)	0.51 (0.93)	AA+AG (N=104)	0.41 (0.84)	AA (N=12)	0.71 (0.95)
*GC+CC (N=68)	1.26 (1.39)	0.37 (0.59)	*GA+AA (N=56)	1.12 (1.52)	*AA (N=24)	0.16 (0.38)	* GG (N=4)	0.00 (0.00)	*AT+TT (N=104)	1.23 (1.50)
p	0.672	0.61	p	0.424	p	0.08	p	0.26	p	0.243
OR (95% CI)	-	-	OR (IC à 95%)	-	OR (95% CI)	-	OR (95% CI)	-	OR (95% CI)	-
p[c]	-	-	p[c]	-	p[c]	-	p[c]	-	p[c]	0.055
OR (95% CI)	-	-	OR (IC à 95%)	-	OR (95% CI)	-	OR (95% CI)	-	OR (95% CI)	-
Men										
GG (N=220)	1.43 (1.10)	0.27 (0.48)	GG (N=220)	1.70 (1.13)	GG+GA (N=325)	0.27 (0.53)	AA+AG (N=380)	0.32 (0.65)	AA (N=136)	1.20 (1.02)
*GC+CC (N=204)	1.66 (1.02)	0.43 (0.85)	*GA+AA (N=176)	1.33 (1.01)	*AA (N=80)	0.90 (1.23)	* GG (N=40)	0.82 (0.89)	*AT+TT (N=248)	1.75 (1.09)
p	0.03	0.023	p	0.001	p	0	p	0	p	0
OR (95% CI)	1.25 (1.02-1.53)	1.16 (1.02-1.32)	OR (IC à 95%)	0.68 (0.55-0.85)	OR (95% CI)	1.86 (1.53-2.25)	OR (95% CI)	1.66 (1.32-2.07)	OR (95% CI)	1.72 (1.38-2.16)
p[c]	0.015	0.052	p[c]	0.004	p[c]	0	p[c]	0	p[c]	0
OR (95% CI)	1.28 (1.05-1.57)	-	OR (IC à 95%)	0.73 (0.59-0.90)	OR (95% CI)	1.78 (1.44-2.19)	OR (95% CI)	1.58 (1.23-2.03)	OR (95% CI)	1.57 (1.25-1.96)

[a] adjusted for age and sex
[b] adjusted for sex and smoking status
[c] adjusted for age and smoking status

Another point examined in this study considers the genetic effect modification by classical caffeine intake covariates. The rs382140 SNP variation in the *NRCAM* gene, likely involved in caffeine addiction, did not show any interaction with caffeine intake modifiers. However, SNPs in *AHR*, *CAB39L* and *PDSS2* genes, implicated in caffeine metabolism, have shown different patterns of interaction with age, sex and smoking covariates on caffeinated beverage intake. Interestingly, gene-covariate interaction effects were more pronounced on tea decoction (the highest caffeine-containing beverage) intake. Indeed, genetic effect modifications on tea intake were observed with *CAB39L* rs9526558 SNP (genetic effect in men, age d"35 years and non-smokers) and *PDSS2* rs7754744 SNP (genetic effect in men and smokers). However, a borderline interaction effect on coffee intake was detected between *AHR* rs6968865 SNP and smoking. According to the data, it seems likely that the interactions of age, sex and smoking factors with *AHR*, *CAB39L* and *PDSS2* genes SNPs are exerted through their known influence in caffeine metabolism, mainly on CYP1A2 activity (Backman et al. 2008; Gunes and Dahl 2008; Gunes et al. 2009). This hypothesis is supported by a previous report on rs762551 functional *CYP1A2* gene SNP, suggesting a genetic effect in men and non-smokers (Rodenburg et al. 2012). In the interaction analysis between bitter taste perception gene *TAS2R43* rs68157013 SNP and caffeine intake covariables, the researchers determined that age modifies the genetic association with coffee intake, but not tea intake. The observation of the genetic effect in only the younger age group may be explained by bitter taste transduction sequence resorption by aging. Indeed, various studies suggest that sensory-specific satiety decreases with age (Rolls and McDermott 1991), and mainly the ability to perceive bitter taste (Cowart et al. 1994; Krishnaa and Jayaraj 2017). The finding is supported by a previous study on the perception of bitter taste in different age groups, showing a high sensitivity to bitterness with specific haplotypes in the *TAS2R38* gene in children, being more sensitive than adults with the same *TAS2R38* haplotype (Rossella et al. 2015).

CONCLUSION

The study provides additional data on the genetics of caffeinated beverage intake around the world. The specificity of the study population (Tunisia), with a higher caffeine content in tea decoction drinks, enabled differentiation of the traits linked to caffeine, and those likely unrelated to caffeine. Indeed, clear relationships between SNPs in caffeine metabolism (*CAB39L* and *PDSS2*), addiction to caffeine (*NRCAM*), caffeine-perceived bitterness (*TAS2R43*) genes and the highest caffeine-containing beverage (tea decoction) were established, while *AHR* rs6968865 SNP was only associated with coffee intake.

RECOMMENDATIONS

In light of the results, the association between coffee intake and *AHR* gene SNPs should be reconsidered as likely unrelated to caffeine. Furthermore, the opposing association of *PDSS2* rs7754744 SNP with coffee and tea intake likely unveil a mechanism of beverage preference that needs to be elucidated by future studies.

ACKNOWLEDGMENTS

The authors would like to thank all blood donors and the staff of the blood transfusion service at the National Blood Transfusion Centre, University of Tunis El Manar, Tunis in Tunisia, for making this publication possible.

ABBREVIATIONS

AHR: aryl hydrocarbon receptor
CAB39L: Calcium Binding Protein 39 Like
CYP1A1: cytochrome P450, family 1, subfamily A, member 1
CYP1A2: cytochrome P450, family 1, subfamily A, member 2
DNA: deoxyribonucleic acid
GWAS: genome-wide association study
NRCAM: neuronal cell adhesion molecule
PCR: polymerase chain reaction
PDSS2: decaprenyl-diphosphate synthase subunit 2
RFLP: restriction fragment length polymorphism

TAS2R43: taste receptor type 2 member 43
TAS2R38: taste receptor type 2 member 38
SNP: single nucleotide polymorphism

REFERENCES

Amin N, Byrne E, Johnson J, Chenevix-Trench G, Walter S, Nolte IM, kConFab Investigators, Vink JM, Rawal R, Mangino M et al. 2012. Genome-wide association analysis of coffee drinking suggests association with *CYP1A1*/*CYP1A2* and *NRCAM*. *Mol Psychiatry*, 17: 1116–1129.

Backman JT, Schröder MT, Neuvonen PJ 2008. Effects of gender and moderate smoking on the pharmacokinetics and effects of the CYP1A2 substrate tizanidine. *Eur J Clin Pharmacol*, 64: 17–24.

Brice CF, Smith AP 2002. Factors associated with caffeine consumption. *Int J Food Sci Nutr*, 53: 55–64.

Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF 1989. Human cytochrome P-450PA (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc Natl Acad Sci USA*, 86: 7696–7700.

Coffee and Caffeine Genetics Consortium, Cornelis MC, Byrne EM, Esko T, Nalls MA, Ganna A, Paynter N, Monda KL, Amin N, Fischer K et al. 2015. Genome-wide meta-analysis identifies six novel loci associated with habitual coffee consumption. *Mol Psychiatry*, 20(5): 647–656.

Cornelis MC, El-Sohemy A, Kabagambe EK, Campos H 2006. Coffee, *CYP1A2* genotype, and risk of myocardial infarction. *JAMA*, 295: 1135–1141.

Cornelis MC, Kacprowski T, Menni C , Gustafsson S, Pivin E, Adamski J, Artati A, Eap CB, Ehret G, Friedrich N et al. 2016. Genome-wide association study of caffeine metabolitesprovides new insights to caffeine metabolism and dietary caffeine-consumption behavior. *Hum Mol Genet*, 25(24): 5472–5482.

Cornelis MC, Monda KL, Yu K, Paynter N, Azzato EM, Bennett SN, Berndt SI, Boerwinkle E, Chanock S, Chatterjee N et al. 2011. Genome-wide meta-analysis identifies regions on 7p21 (AHR) and 15q24 (CYP1A2) as determinants of habitual caffeine consumption. *PLoS Genet*, 7(4): e1002033.

Cornelis MC 2019. The impact of caffeine and coffee on human health. *Nutrients*, 11(2): 416.

Cowart BJ, Yokomukai Y, Beauchamp GK 1994. Bitter taste in aging: Compound-specific decline in sensitivity. *Physiol Behav*, 56(6): 1237-1241.

Dona M, Dell' Aica I, Calabrese F, Benelli R, Morini M, Albini A, Garbisa S 2003. Neutrophil restraint by green tea: Inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. *J Immunol*, 170: 4335–4341.

Grigg D 2002. The worlds of tea and coffee: Patterns of consumption. *Geo Journal*, 57: 283–294.

Gunes A, Dahl ML 2008. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. *Pharmacogenomics*, 9: 625–637.

Gunes A, Ozbey G, Vural EH, Uluoglu C, Scordo MG, Zengil H, Dahl ML 2009. Influence of genetic polymorphisms, smoking, gender and age on CYP1A2 activity in a Turkish population. *Pharmacogenomics*, 10: 769–778.

Hernan MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ 2002. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann Neurol*, 52(3): 276–284.

Ishiguro H, Liu QR, Gong JP, Hall FS, Ujike H, Morales M, Sakurai T, Grumet M, Uhl GR 2006. NrCAM in addiction vulnerability: Positional cloning, drug-regulation, haplotype-specific expression, and altered drug reward in knock-out mice. *Neuropsychopharmacol*, 31: 572–584.

Je Y, Giovannucci E 2012. Coffee consumption and risk of endometrial cancer: Findings from a large up-to-date meta-analysis. *Int J Cancer*, 131: 1700–1710.

Kavanagh KT, Hafer LJ, Kim DW, Mann KK, Sherr DH, Rogers AE, Sonenshein GE 2001. Green tea extracts decrease carcinogen-induced mammary tumor burden in rats and rate of breast cancer cell proliferation in culture. *J Cell Biochem*, 82: 387–398.

Khanna C, Wan XL, Bose S, Cassaday R, Olomu O, Mendoza A, Yeung C, Gorlick R, Hewitt SM, Helman LJ 2004. The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. *Nat Med*, 10: 182–186.

Krishnaa PK, Jayaraj G 2017. Effect of age on taste perception: A survey. *Int J Orofac Biol*, 1(1): 35-38.

Li W, Wong CC, Zhang X, Kang W, Nakatsu G, Zhao Q, Chen H, Go MYY, Chiu PWY, Wang X, et al. 2018. CAB39L elicited an anti-Warburg effect via a LKB1-AMPK-PGC1á axis to inhibit gastric tumorigenesis. *Oncogene*, 37: 6383–6398.

Liu QP, Wu YF, Cheng HY, Xia T, Ding H, Wang H, Wang ZM, Xu Y 2016. Habitual coffee consumption and risk of cognitive decline/dementia: A systematic review and meta-analysis of prospective cohort studies. *Nutrition*, 32(6): 628–636.

Loftfield E, Freedman ND, Graubard BI, Hollenbeck AR, Shebl FM, Mayne ST, Sinha R 2015. Coffee drinking and cutaneous melanoma risk in the NIH-AARP diet and health study. *J Natl Cancer Inst*, 107 (2): dju421.

Luciano M, Kirk KM, Heath AC, Martin NG 2005. The genetics of tea and coffee drinking and preference for source of caffeine in a large community sample of Australian twins. *Addiction*, 100: 1510–1517.

Ludwig IA, Clifford MN, Lean ME, Ashihara H, Crozier A 2014. Coffee: Biochemistry and potential impact on health. *Food Funct*, 5(8): 1695–1717.

McMahon G, Taylor AE, Davey Smith G, Munafò MR 2014. Phenotype refinement strengthens the association of *AHR* and *CYP1A1* genotype with caffeine consumption. *PLoS ONE*, 9(7): e103448.

Musial C, Kuban-Jankowska A, Gorska-Ponikowska M 2020. Beneficial properties of green tea catechins. *Int J Mol Sci*, 21: 1744.

Nakagawa-Senda H, Hachiya T, Shimizu A, Hosono S, Oze I, Watanabe M, Matsuo K, Ito H, Hara M, Nishida Y, et al. 2018. A genome-wide association study in the Japanese population identifies the 12q24 locus for habitual coffee consumption: The J-MICC Study. *Sci Rep*, 8(1): 1493.

Nukaya M, Moran S, Bradfield CA 2009. The role of the dioxin-responsive element cluster between the *CYP1A1* and *CYP1A2* loci in aryl hydrocarbon receptor biology. *Proc Natl Acad Sci USA*, 106: 4923–4928.

Nurminen ML, Niittynen L, Korpela R, Vapaatalo H 1999. Coffee, caffeine and blood pressure: A critical review. *Eur J Clin Nutr,* 53: 831–839.

Petrick JL, Freedman ND, Graubard BI, Sahasrabuddhe VV, Lai GY, Alavanja MC, Beane-Freeman LE, Boggs DA, Buring JE, Chan AT et al. 2015. Coffee consumption and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma by sex: The liver cancer pooling project. *Cancer Epidemiol Biomarkers Prev*, 24(9): 1398–1406.

Pirastu N, Kooyman M, Robino A, van der Spek A, Navarini L, Amin N, Karssen LC, Van Duijn CM, Gasparini P 2016. Non-additive genome-wide association scan reveals a new gene associated with habitual coffee consumption. *Sci Rep,* 6: 31590.

Pirastu N, Kooyman M, Traglia M, Robino A, Willems SM, Pistis G, d'Adamo P, Amin N, d'Eustacchio A, Navarini L et al. 2014. Association analysis of bitter receptor genes in five isolated populations identifies a significant correlation between TAS2R43 variants and coffee liking. *PLoS ONE*, 9(3): e92065.

Reyes CM, Cornelis MC 2018. Caffeine in the diet: Country-level consumption and guidelines. *Nutrients*, 10(11): 1772.

Rodenburg EM, Eijgelsheim M, Geleijnse JM, Amin N, Duijn CM, Hofman A 2012. *CYP1A2* and coffee intake and the modifying effect of sex, age, and smoking. *Am J Clin Nutr,* 96: 182–187.

Rolls BJ, McDermott TM 1991. Effects of age on sensory-specific satiety. *Am J Clin Nutr,* 54(6): 988-996.

Rossella N, Andrea S, Martina G 2015. Age variation in bitter taste perception in relation to the TAS2R38 taste receptor phenotype. *Int J Nutr*, 1(2): 87-98.

Snoussi C, Ducroc R, Hamdaoui MH, Dhaouadi K, Abaidi H, Cluzeaud F, Nazaret C, Le Gall M, Bado A 2014. Green tea decoction improves glucose tolerance and reduces weight gain of rats fed normal and high-fat diet. *J Nutr Biochem,* 25: 557–564.

Sueoka N, Suganuma M, Sueoka E, Okabe S, Matsuyama S, Imai K, Nakachi K, Fujiki H 2001. A new function of green tea: Prevention of life style related diseases. *Ann N Y Acad Sci*, 928: 274–280.

Sulem P, Gudbjartsson DF, Geller F, Prokopenko I, Feenstra B, Aben KKH, Franke B, den Heijer M, Kovacs P, Stumvoll M et al. 2011. Sequence variants at CYP1A1-CYP1A2 and AHR associate with coffee consumption. *Hum Mol Genet*, 20: 2071–2077.

Taylor AE, Davey Smith G, Munafò MR 2018. Associations of coffee genetic risk scores with consumption of coffee, tea and other beverages in the UK Biobank. *Addiction*, 113(1): 148–157.

Teucher B, Skinner J, Skidmore PM, Cassidy A, Fairweather-Tait SJ, Hooper L, Roe MA, Foxall R, Oyston SL, Cherkas LF et al. 2007. Dietary patterns and heritability of food choice in a UK female twin cohort. *Twin Res Hum Genet*, 10: 734–748.

Uhl GR 2006. Molecular genetics of addiction vulnerability. *Neuro Rx*, 3: 295–301.

van Dieren S, Uiterwaal CS, van der Schouw YT, van der AD, Boer JM, Spijkerman A, Grobbee DE, Beulens JWJ 2009. Coffee and tea consumption and risk of type 2 diabetes. *Diabetologia*, 52: 2561–2569.

Weinreb O, Mandel S, Amit T, Youdim MBH 2004. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J Nutr Biochem*, 15: 506–516.

Wilson KM, Balter K, Moller E, Adami HO, Andren O, Andersson SO, Grönberg H, Mucci LA 2013. Coffee and risk of prostate cancer incidence and mortality in the Cancer of the Prostate in Sweden Study. *Cancer Causes Control*, 24: 1575–1581.

Yang A, Palmer AA, de Wit H 2010. Genetics of caffeine consumption and responses to caffeine. *Psychopharmacol*, 211: 245–257.

Yeomans MR, Mobini S, Chambers L 2007. Additive effects of flavour–caffeine and flavour–flavour pairings on liking for the smell and flavour of a novel drink. *Physiol Behav*, 92: 831–839.

Paper received for publication in August, 2020
Paper accepted for publication in August, 2020