Investigating the Impact of Genetic Variability of *CYP1A2*, *ADORA2A*, and *AHR* on Caffeine Consumption and Responses in New Zealanders

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Rebecca Tennent
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Abstract

Background: Single nucleotide polymorphisms (SNPs), in three genes encoding for cytochrome P450 1A2 (*CYP1A2*; rs762551), adenosine A2A receptor (*ADORA2A*; rs5751876), and aryl-hydrocarbon receptor (*AHR*; rs4410790) are known to have links to caffeine consumption and metabolism, and its resultant effects. Exploration of the links between genetic profile and caffeine response will advance understanding of the impact of caffeine consumption on individuals with differing genetic profiles.

Aim: To examine caffeine consumption patterns and post-consumption responses among New Zealand (NZ) individuals and how these differ with genetic variation of *CYP1A2, ADORA2A,* and *AHR.*

Methods: Caffeine intake and post-consumption responses were assessed using a caffeine consumption habits questionnaire (CaffCo). Genetic data for the SNPs (*CYP1A2*, *ADORA2A, AHR*) was analysed using MassARRAY analysis on DNA extracted from saliva samples. CaffCo data was examined according to genotype for the three SNPs, which were then paired to consider links between genes.

Results: 255 participants aged 15 years and over were included in the study. Half (49.4%) of all participants were "fast" metabolisers (*CYP1A2* AA) and 10.2% "ultra-slow" metabolisers (*CYP1A2* CC) of caffeine. Almost half of the participants carried *ADORA2A* CT (46.3%), followed by CC (29.0%), and TT (24.7%). Half (51.8%) of the participants carried *AHR* CT, followed by CC (30.6%), and TT (17.6%) genotypes. Overall, 14.1% of participants reported a caffeine intake >400 mg/day and 52.9% an intake of 80-400 mg/day. Carriers of the genotype *ADORA2A* TT consumed 65 mg/day less caffeine than carriers of the heterozygote genotype (*ADORA2A* CT; p= 0.034). No association was found with the other analysed SNPs.

Conclusions: This novel research is the first in New Zealand to examine all three genes, identifying the genetic variation and caffeine consumption habits in this population. The data show an association between *ADORA2A* TT and decreased caffeine consumption in this population. Future studies are needed to assess caffeine response in relation to these three genes, to understand and develop appropriate strategies for informing genotype based advice on caffeine use.

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ADORA	Adenosine receptor
ADORA2A	Adenosine A2A receptor
AHR	Aryl-hydrocarbon receptor
AHRE	Aryl-hydrocarbon receptor response elements
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
CaffCo	Caffeine consumption habits questionnaire
COMT	Catechol-O-methyl transferase
CYP	Cytochrome P450
CYP1A2	Cytochrome P450 1A2
DRD2	Dopamine Receptor D2
EEG	Electroencephalogram
GWAS	Genome-wide association studies
MI	Myocardial infarction
NZ	New Zealand
PAH	Polycyclic aromatic hydrocarbons
REM	Rapid eye movement
RTD	Ready to drink alcoholic beverage
SBP	Systolic blood pressure
SNP	Single Nucleotide Polymorphism

1 Introduction

1.1 Background

Caffeine consumption is ingrained in daily culture for many New Zealand adults, with at least 73% of New Zealanders consuming caffeine daily (Thomson & Schiess, 2011). The extensive range of caffeine sources in our diet is often seen as unexpected, with the word 'caffeine' strongly associated with the consumption of coffee alone. What makes caffeine so popular is its ability to give consumers a boost of wakefulness, sharper cognition and enhanced mood (Haskell, Kennedy, Wesnes, & Scholey, 2005; Lieberman, Tharion, Shukitt-Hale, Speckman, & Tulley, 2002). An increasing number of people are making a conscious decision to take caffeine in various forms to enhance mental and/or physical performance. This can include sporting, working or study performance, with the stimulating effects of caffeine noted to be a major motivation for consumption (McLellan, Caldwell, & Lieberman, 2016). However, in some individuals this stimulating effect can be detrimental, resulting in sleeplessness, anxiety, nervousness, or tremors (Daly & Fredholm, 1998; Rutherfurd-Markwick & Ali, 2016). Finding the ideal caffeine amount to balance these positive and negative effects is very individual and can be impacted by demographic and environmental factors (including age, drug use, sleep quality), as well as genetic predisposition (Yang, Palmer, & De Wit, 2010).

Genetic make-up can impact caffeine metabolism and subsequent physiological responses, which will collectively influence how an individual responds to caffeine consumption. This, together with factors such as habituation and tolerance will contribute to variation between individuals.

The rate an individual is able to metabolise caffeine is primarily determined by the enzyme Cytochrome P450 1A2, which is coded by the gene *CYP1A2* (Lelo, Birkett, Robson, & Miners, 1986). Genotypic variation influences the inducibility of the enzyme, thus metabolism rate (Sachse, Brockmoller, Bauer, & Roots, 1999). Transcription of this gene is regulated by the aryl-hydrocarbon receptor, a ligand-dependent transcription factor (Jorge-Nebert et al., 2010; Nebert, Dalton, Okey, & Gonzalez, 2004). When activated by a ligand, the AHR complex binds to its response element in the nucleus. In the case of *CYP1A2,* AHR response elements (AHRE)

bind to the *CYP1A2* promotor region and activate its transcription. Therefore, variations in the *AHR* gene indirectly influence caffeine metabolism rate through this transcription activation (Jorge-Nebert et al., 2010).

Given that metabolism rate will determine how long caffeine stays in the body, an individual's ability to metabolise caffeine fast or slow will influence the potential length of time that it can impact on the body's systems (Cornelis et al., 2016; Gu, Gonzalez, Kalow, & Tang, 1992)**.** In the body, caffeine binds as an antagonist to adenosine receptors, which are involved in the sleep cycle and our anxiety responses (Daly, Butts-Lamb, & Padgett, 1983; Ribeiro & Sebastião, 2010). Variations in the *ADORA2A* gene impact on an individual's sensitivity to these effects, which can result in sleep disturbances (Rétey et al., 2007), or increases in anxiety in response to caffeine consumption (Childs et al., 2008).

The current literature centres on the impact of these three genes (*CYP1A2*, *ADORA2A*, and *AHR*) individually, with little emphasis on the genes in relation to each other. However, given the relationship between metabolism and post-consumption responses, it would be valuable to investigate the patterns and correlations between the genetic profile of the three genes and caffeine consumption. In addition, the majority of studies (Cornelis et al., 2014; Denden, Bouden, Haj Khelil, Ben Chibani, & Hamdaoui, 2016; Guessous et al., 2012; Nordestgaard, Thomsen, & Nordestgaard, 2015; Rodenburg et al., 2012) investigating genetic links to caffeine consumption habits have investigated links with coffee consumption alone, and fewer studies (Cornelis et al., 2011; Josse, Da Costa, Campos, & El-Sohemy, 2012) have looked into correlations between caffeine consumption from different sources and genes of interest.

Importantly, variations in our genes is only one factor that influences caffeine consumption. The choice to consume caffeinated products is also influenced by sociocultural, environmental, and lifestyle factors, as well as intrinsic factors (Cornelis, El-sohemy, & Campos, 2007; Gray, 1998). All of these multifactorial influences are individual and not static, changing situationally. Based on this, the majority of people are able to self-regulate and choose the optimal caffeine consumption level for them based on their own experiences (Smith, 2002).

Interest and concern around caffeine's potential health effects has fostered a large body of research into both the benefits and risks of caffeine consumption, and the influence our genetics has on how much we consume and our response post consumption (Cornelis et al., 2016; Heckman, Weil, & de Mejia, 2010). Adaptations to caffeine consumption such as a build-up of tolerance (habituation) is also influenced by genetic polymorphisms (Harland, 2000). Long-term disease risk is another area of emerging research, with caffeine consumption associated with both increased and decreased risks of diseases such as Parkinson's disease and cardiovascular disease (Yang et al., 2010; Zhou, Wang, Yang, & Liu, 2010). Given the positive and negative associations to many areas of overall wellness, both health professionals and members of the public would benefit from additional knowledge on the health impacts of caffeine consumption, and the many factors that are involved.

Uniquely, the current study provides an analysis of a snap-shot recording of consumption patterns and caffeine-related responses, using a previously designed caffeine consumption habits questionnaire (CaffCo). This questionnaire was pilot-tested in a NZ population in 2015 (Rowe, 2015; Appendix A), and allows for a comprehensive habitual analysis in combination with genetic data. The current study adds to the body of evidence assessing the links between responses to caffeine consumption with three genes, two coding for proteins involved in caffeine metabolism: cytochrome P450 1A2 (*CYP1A2*; rs762551); and aryl-hydrocarbon receptor (*AHR*; rs4410790), and the third for the adenosine A2A receptor (*ADORA2A*; rs5751876), the target receptor for caffeine in the central nervous system (Cornelis et al., 2007).

1.1.1 Study Purpose

Information collected on New Zealand caffeine consumption patterns and responses can be utilised to better understand the benefits and risks of caffeine consumption from a public health perspective, to ensure people are better able to make informed decisions about their caffeine intake. This information would be valuable to guide decisions for individuals who tend to avoid caffeine in response to their experience of adverse effects, and conversely, for persons interested in whether they are likely to experience performance-enhancing effects from consuming caffeine.

There is ongoing debate with regards to the polymorphisms of most relevance to caffeine intake and response (Fulton et al., 2018; Yang et al., 2010). Additionally, the proposed genotypes within the selected SNPs that are linked to the responses or consumption patterns are not always in agreement. The three genes in this study were chosen to allow for the potential to investigate the complex interaction between caffeine metabolism (via *CYP1A2* and *AHR* genes) and response (via the *ADORA2A* gene). Each gene of interest is assessed individually, and *ADORA2A* and *AHR* are further analysed in correlation with *CYP1A2* to establish the impact of the rate of caffeine metabolism, and elucidate further connections within the genome. At the individual gene level, the current evidence is strong for the relationships of these genes to caffeine, and it is of interest to investigate the replicability of this in a NZ population. Additionally, we sought to add to the body of evidence on these genes by further investigating them in combination, to provide a deeper understanding of their role, and relevance to health in New Zealanders.

Further exploration of the links between our genetic profile and caffeine response will advance our understanding of the impact of caffeine on individuals with differing genetic profiles. Not only will these associations improve our understanding of the role of dietary and pharmacological caffeine in health, they will help to build towards informing personalised nutrition advice on caffeine use, based on genotype. This emerging field of nutrigenetics aims to optimise health through the knowledge of how genetic variation affects response to nutrients and compounds in foods and beverages.

1.2 Aim, Objectives, and Hypothesis

1.2.1 Study Aim

This study aims to investigate the impact of genetic variations in the *CYP1A2*, *ADORA2A* and *AHR* genes on caffeine consumption and responses to caffeine in individuals 15 years and older living in New Zealand.

1.2.2 Objectives

Using data obtained from a CaffCo questionnaire and associated genetic data:

- Examine the caffeine consumption patterns of individuals living in New Zealand and how these differ depending on genetic variation in the *CYP1A2*, *ADORA2A*, and *AHR* genes.
- Explore the caffeine-related responses (symptoms post-consumption) of individuals living in New Zealand and how these correlate to genetic variation in the *CYP1A2*, *ADORA2A*, and *AHR* genes.

1.2.3 Hypothesis

The primary hypothesis is that individuals carrying genotypes known to be more sensitive to adverse effects post caffeine consumption will report higher incidence of these adverse responses, and consume less caffeine in adaptation to this. A secondary hypothesis is that "ultra-slow" and "slow" caffeine metabolisers are likely to experience more notable adverse responses post caffeine consumption, and consequently consume less caffeine in adaption to this.

1.3 Structure of the Thesis

This thesis is comprised of four chapters, with Chapter 1 providing an introduction to this study and the significance of building knowledge on the links between genetics and caffeine. Chapter 2 is presented in the form of a narrative review and critiques the current literature, focusing on the impact of genetics and how this influences caffeine responses. Chapter 3 consists of a full primary research manuscript, prepared for the journal Psychopharmacology. Finally, Chapter 4 summarises key study findings and limitations, along with recommendations for future research. A reference list and appendices can be found at the end of the thesis.

1.4 Research Support and Contribution

Table 1.1 Researchers' contribution to the study

Contributors	Research Contribution
Rebecca Tennent	Principal Researcher – Thesis Author Recruitment of participants, data collection, data analysis, results and discussion formulation, preparation of thesis manuscript.
Sophie Turner	Associate Researcher Provided assistance with data collection alongside general administration of the caffeine study.
Associate Professor Kay Rutherfurd-Markwick	Academic Supervisor Provided supervision for the caffeine study, including the design and conduct of the study, ethics application, and the writing of this thesis.
Associate Professor Ajmol Ali	Academic Supervisor Provided supervision for the caffeine study, including the design and conduct of the study, ethics application, and the writing of this thesis.
Associate Professor Carol Wham	Academic Supervisor Provided supervision for the caffeine study, including the design and conduct of the study, ethics application, and the writing of this thesis.
Saskia Stachyshyn	Provided hand-over of data collection protocol and data collection sample data for use in this research.
Kyle Southward	Provided knowledge on the wider caffeine study, laboratory work for genetic analysis, and equipment organisation for data collection.
Wendy O'Brien	Assisted with equipment organisation for data collection.

2 Literature Review

Prepared according to author guidelines for submission to the journal titled Psychopharmacology

(Author guidelines detailed in Appendix B)

Narrative Review Outlining the Impact of Genetic Variability of *CYP1A2*, *ADORA2A*, and *AHR* on Caffeine Consumption and Response

2.1 Introduction

Caffeine (1,3,7-trimethylxanthine) is readily available in many forms, commonly found in coffee, tea, and energy drinks, and also found in less expected sources such as kola drinks, chocolate, and other products (Frary, Johnson, & Wang, 2005; Fulton et al., 2018; Heckman et al., 2010; Rutherfurd-Markwick & Ali, 2016). The number of available caffeine-containing products on the market is growing (Fulton et al., 2018; Thomson, Campbell, Cressey, Egan, & Horn, 2014). Recently, caffeine has been added to products such as jelly beans, marshmallows, and chewing gum. An issue of concern is that many individuals may not realise they are consuming caffeine with these products, especially if they are presented and consumed outside of their packaging (Rutherfurd-Markwick & Ali, 2016).

Caffeine is known to elicit a variety of responses and effects which have been widely researched (Lieberman et al., 2002; Wikoff et al., 2017; Yang et al., 2010). Many consumers make a deliberate choice to consume caffeine in seeking positive responses, including the feeling of alertness, improvements in mood and cognition, and a reduction in fatigue (Harland, 2000; Mahoney et al., 2018). Others choose to use caffeine for the beneficial effect on sport and exercise performance (Platritis, Andreou, & Papandreou, 2013). Whilst caffeine is generally well tolerated, in some consumers the effects can be unpleasant. Difficulty with sleeping, anxiety related symptoms, nervousness, heart palpitations and tremors are some of the symptoms reported (Harland, 2000; Kaplan et al., 1997). In some individuals, even small amounts of caffeine can elicit notable physiological responses whilst others can consume large amounts of caffeine with minimal impact (Smith, 2002).

Variation in our genes is one of the factors that has been demonstrated to impact on caffeine response and consumption (Fulton et al., 2018; Yang et al., 2010). Eight loci have been associated with habitual coffee intake patterns by genome-wide association studies (GWAS), the two with the strongest links to caffeine metabolism being *CYP1A2* (rs2472297) and *AHR* (rs6968865; Cornelis et al., 2014). Adenosine A1 and A2A receptors (coded for by *ADORA2A* rs5751876) are the primary targets for caffeine in the central nervous system, where caffeine mediates most of its physiologic effects (Josse et al., 2012; Zhou et al., 2018).

This narrative review aims to investigate the literature related to the roles of genes *CYP1A2, ADORA2A* and *AHR* in caffeine consumption and responses to intake. Topics covered include caffeine's many dietary sources, its physiological effects, and the established genetic links of each of the three genes to caffeine consumption and response. Finally, the fundamental element of consumer choice is explored, impacting every individual regardless of genotype or response to caffeine consumption. Key words used in the literature searches include caffeine, *CYP1A2*, *ADORA2A*, *AHR*, cytochrome P450, adenosine, aryl-hydrocarbon receptor, genetics, genes, coffee, anxiety, sleeplessness, and variations of these words. Databases searched included Discover, Web of Science and Google Scholar, conducted May 2017 through January 2019.

2.2 The Varying Caffeine Content of Dietary Sources

Caffeine is widely consumed, especially in beverages, with coffee noted as the second most popular beverage choice (after water) worldwide at a staggering consumption of 500 billion cups per year (Butt & Sultan, 2011). Caffeine is found naturally in coffee beans, tea leaves, cacao, guarana, and kola nuts, which are added to beverages, foods, and medications, largely for their stimulating effects (Frary et al., 2005). In recent years, energy drinks have become a popular caffeinated beverage choice, with the energy drink market growing exponentially since Red Bull was first released in 1997 (Malinauskas, Aeby, Overton, Carpenter-Aeby, & Barber-Heidal, 2007). In 2010 there were 47 energy drink products on the market in New Zealand (Thomson et al., 2014), this has no doubt continued to expand since.

A large challenge faced by consumers is the difficulty in accurately calculating or being aware of how much caffeine they are consuming. For those who are more sensitive to its effects,

this can be challenging to navigate (Rutherfurd-Markwick & Ali, 2016). A key example of this is coffee, where bean type, environmental conditions, processing and preparation techniques (such as filtering, instant) determine how much caffeine the end product contains; this can range from ~83 mg/1 tsp of instant coffee powder to ~210 mg for a double shot espresso (**Table 2.1**). It also needs to be considered that each individual beverage may further vary based on the volume of the cup and the person who makes it (Gray, 1998).

Furthermore, consumers are unable to accurately rely on caffeine content of coffee beverages based on an assessment of beverage volume. Crozier et al. (2012) investigated 20 commercial espressos from different outlets in Glasgow and found that coffee from three different sources all had 140 mg of caffeine, but beverage volumes of 26, 45, and 70 ml, respectively. The study also found a six-fold variation in caffeine content across the 20 outlets, ranging from 51 – 322 mg/serving (Crozier et al., 2012). Ultimately, many New Zealanders are likely to be consuming more caffeine than they realise or intend. **Table 2.1** demonstrates the vast differences in caffeine content between major sources consumed.

Table 2.1 Caffeine content of products containing caffeine in New Zealand

Product	Caffeine content (mg)*
Coffee, instant[1]	~83 mg/1tsp powder
Coffee, plunger/drip[1]	~120 mg/single shot
Coffee, double shot espresso[1]	~210 mg/double shot
Tea, black[1]	~57 mg/1 teabag
Tea, green[1]	~31 mg/1 teabag
Milk chocolate[1]	~20 mg/100g
Dark chocolate[1]	~60 mg/100g
Kola drinks, regular[†1]	~11 mg/100ml
Kola drinks, diet[†1]	~14 mg/100ml
Energy drinks[2]	~30 mg/100ml
Energy shots[2]	~163 mg/60ml
Alcoholic RTD[2,3]	~15 mg/100ml
Pre-workout powder[4]	~210 mg/10g serve

Sports gels[2]	~78 mg/100g
Caffeine tablets[3]	~50-200 mg/tablet
Medication, Panadol Extra[5]	~65 mg/tablet

[1] Sivakumaran (2017)
[2] Thomson and Jones (2013)
[3] Beer Wine and Spirits Producers (2015), RTD, Ready to drink
[4] Supplements.co.nz
[5] Medsafe (2017)
*Caffeine content is approximate and varies with specific products and preparation techniques
[†]'Kola drinks' is used instead of 'cola' and comprises all kola-type beverages
Table adapted from Stachyshyn (2017)

Caffeine is added to products such as energy drinks and shots, alcoholic beverages, and sports supplements. This can have an additive effect with the natural caffeine in these products (i.e. guarana), giving these beverages a strong stimulating effect (Thomson & Jones, 2013). Sports supplements can vary widely in caffeine content depending on the target purpose and formula of the supplement. This includes pre-workout powder which ranges from ~75 mg - ~390 mg/10g per serve (Supplements.co.nz, 2018) as well as sports gels which are popular with athletes during endurance events (Thomson & Jones, 2013).

It is challenging to measure how much caffeine an individual consumes due to the variations in caffeine amounts within sources, and the incidental caffeine that is consumed outside the more commonly recognised items, such as in medications or muffins and biscuits that contain chocolate. There are no New Zealand specific guidelines which recommend limits for caffeine consumption (Food Standards Australia New Zealand, 2018), however up to 400 mg of caffeine a day (approximately 4-5 cups of instant coffee) is generally considered safe for the general adult population (Heckman et al., 2010; Nawrot et al., 2003; US Food and Drug Administration, 2018). This guideline is based on a volume of studies and reviews examining the potential risks and effects of consumption, including acute toxicity and cardiovascular response, and chronic long-term risk of disease.

2.3 Caffeine, Adenosine and Dopamine

Adenosine is an inhibitory neurotransmitter which promotes sleep and supresses arousal (Ribeiro, Sebastião, & de Mendonça, 2002). Both caffeine and adenosine are able to bind to adenosine receptors due to their similar chemical structure (Ribeiro & Sebastião, 2010).

Throughout the day a build-up of adenosine occurs, as energy in the form of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) is broken down (Elmenhorst et al., 2007). When adenosine binds to its receptors, the activity of other neurotransmitters (e.g. dopamine) in the brain is suppressed and neural activity slowed, resulting in sleepiness (Dunwiddie & Masino, 2001). Caffeine acts as an adenosine receptor antagonist, inhibiting the effects of adenosine when bound in its place (Daly et al., 1983; Ribeiro & Sebastião, 2010). The sought after feeling of alertness often associated with caffeine consumption is a result of this binding, stimulating neurotransmitters and increasing neural activity that would otherwise be slowed when adenosine is bound (Ribeiro & Sebastião, 2010; Yang et al., 2010).

2.4 Genetic Links to Caffeine

The impact of genetics on caffeine consumption habits and experiences is increasingly documented, with new evidence emerging on the genes and SNPs found to be involved. Three SNPs that have been extensively researched and are known to have links to caffeine consumption and metabolism, and it effects and responses to it, will be the focus of this review: cytochrome P450 1A2 (*CYP1A2*; rs762551), adenosine A2A receptor (*ADORA2A*; rs5751876), and aryl-hydrocarbon receptor (*AHR*; rs4410790).

2.4.1 *CYP1A2* – Caffeine Metabolism

The majority of caffeine is metabolised in the liver by the Cytochrome P450 enzyme system. These enzymes are coded for by the *CYP* genes, with *CYP1A2* coding for Cytochrome P450 1A2; the enzyme responsible for 95% of the metabolism of caffeine (Berthou et al., 1991; Miners & Birkett, 1996). The rate of this metabolism determines how long caffeine stays in the body and therefore its potential to impact on the body's systems (Cornelis et al., 2016; Gu et al., 1992). The rate of caffeine metabolism can vary 40-fold between, and even within individuals, largely due to variations in the enzymes' activity (Yang et al., 2010), as the P-450 enzymes represent the rate-limiting step for plasma clearance of caffeine (Lelo et al., 1986; Miners & Birkett, 1996). The SNP rs762551 is the focus in this review, however a number of other SNPs have been found to impact on caffeine metabolism in genes that code for P-450 1A2, including: rs2069514, rs2069526, rs12720461, and rs2472297 (Amin et al., 2012; Denden et al., 2016; Obase et al., 2003; Rodenburg et al., 2012; Sachse et al., 1999; Sulem et al., 2011; Thorn, Aklilu, Klein, & Altman, 2016).

There are three genotypes of the *CYP1A2* SNP (AA, AC, and CC) resulting in differing CYP1A2 enzyme activity. The SNP is in the non-coding region of the *CYP1A2* gene and likely impacts on the levels of expression of the CYP1A2 enzyme by affecting the binding of regulatory proteins to the surrounding gene sequences. This results in the AA genotype metabolising caffeine fast, due to the high inducibility of the genotype (Sachse et al., 1999). Therefore, homozygotes for the A allele (AA) are categorised as "fast" metabolisers, whilst homozygotes for the C allele (CC) are "ultra-slow" metabolisers. Heterozygotes (AC) are referred to as "slow" metabolisers. As homozygous "ultra-slow" metabolisers take the longest to break down caffeine in the body, they are potentially at the highest risk of adverse effects following caffeine consumption. This risk may be heightened based on the genotypes of other genes e.g. *ADORA2A* and *AHR* (Cano-Marquina, Tarín, & Cano, 2013; Rutherfurd-Markwick & Ali, 2016).

CYP1A2 enzyme activity is induced by the binding of aromatic hydrocarbons to the aryl hydrocarbon receptor (Sachse et al., 1999). Variations in both these genes therefore impact on the metabolism of caffeine (Fulton et al., 2018). The impact of *CYP1A2* on consumption is further explored in linkage with *AHR* in **Section 2.4.4**.

2.4.2 *CYP1A2* and Impact on Consumption

Early research (Cornelis et al., 2007) did not identify a link to any *CYP1A2* genotypes and differing caffeine consumption, however further work by Cornelis et al. (2011) demonstrated the potential for CYP1A2 activity to influence caffeine intake, by showing this association with a different *CYP1A2* SNP (rs2472304). The impact of enzyme activity and thus rate of metabolism has been linked to caffeine consumption, demonstrated in studies looking specifically at coffee intake. Non-smoking, "fast" metabolisers (carrying *CYP1A2* AA) have been found to have the highest caffeine intake (Guessous et al., 2012). Rodenburg at al. (2012) agreed, finding coffee intake was lowest in non-smoking women carrying the *CYP1A2* CC genotype ("ultra-slow" metabolisers). Irrespective of smoking status, Rodenburg et al. (2012) found coffee intake was tiered dependent on genotype. Compared to "fast" metabolisers, "ultra-slow" metabolisers reported consuming a third of a cup/day (-0.34 cups/day) less coffee, and "slow" metabolisers -0.19 cups/day less, suggesting that the

presence of one A allele (in heterozygotes) still impacts on metabolism rate (Rodenburg et al., 2012).

In agreement with these findings, a recent meta-analysis by Denden et al. (2016), concluded that the *CYP1A2* AA genotype ("fast" metabolisers) may be linked to increased coffee consumption. This link was significantly increased in men, those of younger age, and of Caucasian ethnicity. Interestingly, this link was not seen in those of Asian ethnicity, suggesting that the genetic background relating to caffeine consumption may differ between ethnicities. Further, work by Cornelis et al. (2007) was carried out in a population of Costa Rican individuals and found no association between *CYP1A2* genotypes and caffeine intake. Other SNPs would need to be analysed to explore this further (Denden et al., 2016). The equivocal results due to ethnicity highlights one of the challenges in pinpointing causation, and demonstrates the large range of factors that could influence caffeine intake.

2.4.3 Other Factors Influencing Caffeine Metabolism Through *CYP1A2*

The activity of the CYP1A2 enzyme is influenced by sex, age, and smoking, as well as genotype (Backman, Schröder, & Neuvonen, 2008; Denden et al., 2016; Gunes et al., 2009; Gunes & Dahl, 2008; Kalow & Tang, 1991; Rodenburg et al., 2012; Simon et al., 2001). These same factors have been linked to coffee intake (Rodenburg et al., 2012). Smoking and sex explain the majority of differences in coffee consumption (24% and 10% respectively), with age and genotype together explaining <1% (Gunes et al., 2009; Rodenburg et al., 2012).

Smoking tobacco increases caffeine metabolism by increasing liver CYP1A2 enzyme activity. Polycyclic aromatic hydrocarbons (PAH) in cigarette smoke are responsible for an increase in CYP1A2 activity, which can almost double caffeine metabolism rate (Backman et al., 2008; Grosso & Bracken, 2005; Tantcheva-Poór, Zaigler, Rietbrock, & Fuhr, 1999). PAHs bind to the aryl hydrocarbon receptor (AHR), activating their transcription factor role and inducing the CYP1A2 enzyme (Josse et al., 2012). Thus, irrespective of genotype, smoking has been correlated to increased caffeine intake (444 mg/day compared to 316 mg/day in non-smokers; Josse et al., 2012). Rodenburg et al. (2012) found smokers drank almost 1 cup more of coffee each day than non-smokers. This relationship may be due to the co-adoption of these behaviours, alongside the increased rate of caffeine clearance in smokers compared to

non-smokers (Josse et al., 2012). In linking this to genotypic variations, Sachse et al. (1999) demonstrated that smokers with *CYP1A2* AA genotype metabolise caffeine 1.6 times faster than other genotypes.

Women appear to have a lower CYP1A2 enzyme activity compared to men (Gunes & Dahl, 2008), which has been demonstrated in consumption patterns with women drinking -0.38 cups of coffee per day less than men (Rodenburg et al., 2012). Increasing age has been associated with decreased CYP1A2 enzyme activity in some (Gunes et al., 2009) but not all (Simon et al., 2001; Tantcheva-Poór et al., 1999) studies. Rodenburg et al. (2012) found this was linked to a decline in coffee consumption, at a rate of 0.07 cups/day per year of age. Interestingly, animal studies have shown that AHR activity also decreases with age, therefore, the effects of genetic variations may become more pronounced as we get older (Josse et al., 2012).

Caffeine metabolism is slowed during pregnancy, likely due to a reduction of CYP1A2 activity alongside other enzymes throughout pregnancy (Grosso & Bracken, 2005). By the third trimester it can take up to 18 hours to metabolise caffeine rather than the normal ~4 hours. These different influences on enzyme activity (sex, age, smoking, and pregnancy) are important to consider when interpreting research results.

2.4.4 *AHR* – Caffeine Consumption

The aryl hydrocarbon receptor (AHR) is the transcription factor protein product of the *AHR* gene, which impacts on caffeine metabolism through regulating *CYP1A1* and *CYP1A2* by binding to a promotor region between them, activating their transcription (Cornelis et al., 2011; Jorge-Nebert et al., 2010; McMahon, Taylor, Davey Smith, & Munafò, 2014; Nukaya, Moran, & Bradfield, 2009). The receptor indirectly alters the tendency to consume coffee by altering the physiological response via its effect on caffeine metabolism (Cornelis et al., 2014).

Differential expression of AHR has a role in the tendency to consume caffeine, with higher coffee intakes being associated with carriers of the C allele of the *AHR* gene (Josse et al., 2012; Sulem et al., 2011). AHR was found to have the strongest association with habitual caffeine consumption in a meta-analysis of 47,341 individuals of European descent, where caffeine

intake was 44 mg/day higher in carriers of the *AHR* CC genotype compared to carriers of the TT genotype (Cornelis et al., 2011). Comparably, a 23 mg/day difference was found between carriers of the TT genotype compared to the combined CC or CT carriers in a Costa Rican population (Josse et al., 2012). These studies demonstrate strong evidence that individuals with the T allele of *AHR*, or TT genotype are likely to consume less caffeine than the CC or CT genotype. Moreover, the C allele of *AHR* is correlated with the methylation of cerebellum *AHR*, which may imply a role in motor pathways in the brain that trigger a desire for coffee consumption (Cornelis et al., 2014).

Established links have been noted with the combination of *AHR* and *CYP1A2* genes (rs2472297) and increased caffeine consumption (Amin et al., 2012; Josse et al., 2012; Sulem et al., 2011), demonstrating the potential that an increased consumption is linked to increased caffeine metabolism due to the amplified CYP1A2 enzyme activity. Similarly, a recent study found that having the combination of *AHR* and *CYP1A1* rs2470893 was associated with a 42% higher coffee intake (Nordestgaard & Nordestgaard, 2016). An even clearer link is seen between *AHR* and *CYP1A2* in smokers, due to the induction of CYP1A2 activity in response to PAHs in cigarette smoke acting on the AHR.

Slower caffeine metabolisers may need less caffeine to achieve comparable stimulating effects than “fast” metabolisers. Additionally, they are likely to have a lower threshold for adverse effects (Cornelis et al., 2016). It has been demonstrated in several studies (Cornelis et al., 2016; 2014; 2011) that these alleles are associated with lower caffeine consumption, supporting the hypothesis that individuals self-regulate their caffeine consumption for optimum responses and/or experiences.

In strong linkage disequilibrium (alleles at different loci are associated) to the *AHR* SNP of interest in this review is rs6968865, located near the AHR (Josse et al., 2012; Nordestgaard, Thomsen, & Nordestgaard, 2015; Sulem et al., 2011). This SNP has also been strongly linked to caffeine consumption, with the T allele found to be associated with increased coffee consumption (Josse et al., 2012; Sulem et al., 2011). Current research demonstrates a clear link between the *AHR* gene (multiple SNPs) and caffeine intake, particularly when genotypes of *AHR* and *CYP1A2* are combined for increased enzyme activity.

2.4.5 *ADORA2A* – Caffeine Response

The adenosine receptors (ADORA) are the main target of caffeine action in the nervous system (Cornelis et al., 2007). Polymorphisms in the *ADORA2A* receptor gene influence various effects on the body, such as anxiety, cardiovascular response, sleep response, and habit formation (Rutherfurd-Markwick & Ali, 2016). Initial indications that A2A receptors are involved in the sleep cycle and anxiety response was found in animal studies, which demonstrated that mice without functioning A2A receptors show no arousal/wakefulness response to caffeine (Huang et al., 2005), and showed increased anxiety behaviours (Ledent et al., 1997). Since these initial findings, human studies have been able to replicate these links. The T allele at *ADORA2A* predisposes individuals to be more sensitive to caffeine-induced anxiety (Alsene, Deckert, Sand, & de Wit, 2003; Childs et al., 2008; Domschke et al., 2012; Rogers et al., 2010) while the C allele predisposes individuals to be more sensitive to caffeine-induced sleep disturbance (Bodenmann et al., 2012; Byrne et al., 2012; Rétey et al., 2007). Therefore, sleep and anxiety responses are favoured by the opposing genotypes (Bodenmann et al., 2012).

When adenosine binds to its A2A receptors (located largely post-synaptically on medium spiny neurons in the striatum), a heteromeric complex is formed between Dopamine D2 and A2A receptors, activating G-proteins (which prompt antagonist effects on each other; Fuxe et al., 2005) When caffeine binds to the A2A receptors instead, the heteromeric complex cannot be formed, and D2 receptor responses are enhanced (increasing dopamine). In this way stimulatory effects of caffeine result from the opposition of the inhibitory actions of adenosine on D2 neurotransmission (Dunwiddie & Masino, 2001). Childs et al. (2008) investigated the links with *ADORA2A* and found an association with the dopamine receptor gene *DRD2* G/- (rs1110976) and anxiety after 150 mg caffeine consumption, and a further enhanced response (higher anxiety) in participants who carried both polymorphisms (*ADORA2A* TT and *DRD2* G/-).

Acute increases in blood pressure, specifically systolic blood pressure (SBP), has been found to be more strongly associated with TT genotype in *ADORA2A*, suggesting genetic variation may affect blood pressure response (Renda et al., 2012). Individual results still had high variability however with some showing no response and others even showing a decrease in

SBP (Renda et al., 2012). In theory, this may expose these TT individuals to higher caffeine-related cardiovascular disease risk, however this claim is still being debated (Renda et al., 2012).

2.4.6 Anxiogenic Response

Individuals homozygous for the TT genotype of *ADORA2A* have been identified as being at higher risk of anxiety compared to either the CC or CT genotypes (Domschke et al., 2012). This is especially enhanced when combined with caffeine consumption, with three studies demonstrating an association of the TT genotype with anxiety after a moderate dose of 150 mg caffeine (Alsene et al., 2003; Childs et al., 2008; Domschke et al., 2012). This demonstrates the interaction of genetic factors and biochemical processes altered by caffeine, impacting on emotional processing and thus anxiety response (Domschke et al., 2012). A 150 mg dose appears to be an optimal dose for detecting genotypic effects alongside anxiogenic effects, as an increase in anxiety was shared by all genotypes at a high dose of 450 mg, while a low dose of 50 mg did not elicit an anxiety response for any genotype (Alsene et al., 2003; Childs et al., 2008; Rogers et al., 2010). It may be that at higher doses, the strong effects of caffeine overtake the subtler genotypic effects (Childs et al., 2008). It is worth noting that these studies involved participants who were only occasional caffeine users.

Rogers et al. (2010), using more regular caffeine-consumers, demonstrated that those who consumed more than 40 mg caffeine daily (similar to the amount in a cup of tea) did not experience an anxiety response despite having the *ADORA2A* TT genotype. This differed from occasional caffeine users (Rogers et al., 2010), and may be due to a build-up of tolerance to the anxiogenic effects of caffeine with frequent consumption (regardless of genotype). When caffeine is consumed habitually over time this may stimulate the synthesis of additional adenosine receptors, leading to a tolerance to the effects of caffeine in some individuals (Harland, 2000). These findings demonstrate that tolerance develops even at modest levels of habitual intake, and even in those who carry *ADORA2A* TT genotype and are most vulnerable to the anxiogenic effects (Rogers et al., 2010). Rogers et al. (2010) also points out that susceptibility to the effect did not necessarily discourage caffeine consumption, given that low consumers (<40 mg/day) tended to report less anxiety regardless of genotype.

The results from the Rogers et al. (2010) study were in contrast to that of Cornelis et al. (2007), where participants carrying *ADORA2A* TT had higher habitual caffeine intake, particularly coffee, despite a higher anxiety response. Cornelis et al. (2007) found that carriers of the *ADORA2A* TT genotype are less likely to be heavy caffeine consumers (>200 mg/day). Reasons for the difference between these findings is unknown, and further research into anxiety responses in individuals with differing habitual caffeine intake levels would be valuable to understand the influences on post caffeine consumption experiences.

The T allele of *ADORA2A* has been associated with panic disorder (Deckert et al., 1998; Hamilton et al., 2004; Hohoff et al., 2010) and also been found to influence anxiety levels in healthy individuals as well as other disorders (Alsene et al., 2003; Deckert et al., 1998; Domschke et al., 2012; Hohoff et al., 2010; Rogers et al., 2010). This is unrelated to caffeine consumption, but helps to explain the finding that those with panic disorder are more likely to experience caffeine-induced anxiety (Domschke et al., 2012).

Importantly, studies investigating the association of the T allele of *ADORA2A* and panic disorder have found conflicting results, with some studies (Lam, Hong, & Tsai, 2005; Yamada et al., 2001) not finding an association between TT genotype and panic disorder. These studies both involved participants from Asian populations, compared to other studies (Deckert et al., 1998; Hamilton et al., 2004; Hohoff et al., 2010) in Western populations. In further contrast, Hamilton et al. (2004) found the C allele (rather than the T allele) of the SNP to be associated with panic disorder. These differences may point to the notion that the SNP may be in linkage disequilibrium with the disease allele, rather than being the disease allele itself. This is possible due to the linkage analysis focusing on loci rather than alleles (Hamilton et al., 2004). Building on this, given the contrasting results between ethnic populations, there may be ethnical variation with this association. The *ADORA2A* gene may be in linkage disequilibrium with another gene that affects panic disorder, with different ethnic populations differing in the extent of this link (Hamilton et al., 2004; Lam et al., 2005). Interestingly, caffeine consumption is lower in individuals with panic disorder, likely due to the predisposition to an anxiety response, resulting in the implicit choice to consume less caffeine (Yang et al., 2010).

Carrying the *ADORA2A* TT genotype appears to increase the likelihood for individuals to experience anxiety, which is enhanced with caffeine intake. This response appears to be muted in habitual consumers, and future work in these consumers would be valuable to better understand the tolerance aspect to caffeine and afford the best education for consumers with this genotype to avoid this negative response.

2.4.7 Sleep Disturbances

Caffeine influences the sleep cycle via adenosine A2A receptors. Human studies have shown that A2A receptor polymorphisms influence sleep changes after caffeine consumption. Those with the *ADORA2A* CC genotype experienced increased sleep impairment and electro-encephalogram (EEG) beta band activity during non-REM sleep after caffeine consumption, compared to the TT genotype (Byrne et al., 2012; Rétey et al., 2007). The EEG pattern seen in those with the CC genotype aligns with patterns seen in insomnia patients. Intermediate genotype CT showed half the increase compared to CC, and TT genotype showed no change in beta activity. These findings demonstrate the link of the C allele of *ADORA2A* to an enhanced sensitivity to caffeine-induced insomnia (Byrne et al., 2012; Rétey et al., 2007).

Exploring sleep difficulty in relation to a range of time intervals post consumption would be valuable to ascertain optimal caffeine intake timing for carriers of a C allele of *ADORA2A* to achieve the ideal balance between increased alertness or stimulation, with minimal negative impact on sleep.

2.4.8 *ADORA2A* and Impact on Consumption

Evidence for the impact of *ADORA2A* genotypes on caffeine consumption is conflicting, with both alleles T (Cornelis et al., 2007) and C (Rétey et al., 2007; Rogers et al., 2010) of *ADORA2A* found to be associated with lower consumption to their alternate allele. These contrasting findings may ultimately support the hypothesis that individuals alter their consumption of caffeine according to their response. With respect to the *ADORA2A* gene, individuals may adjust their consumption in accordance with their experience of either anxiety or sleep disturbances, with some studies finding more emphasis on each experience, depending on the participants involved. Given the lack of consensus in the current evidence, further work

is needed to uncover the impact of the different *ADORA2A* genotypes on consumption, and how strongly these links are based on the experience of adverse symptoms post consumption.

2.4.9 Long-Term Impact (Disease Risk) of Caffeine Consumption Related to Genes of Interest

Genetic polymorphisms affect adaptations to caffeine as well as the acute response, with disease risk being one factor that has been investigated. Long-term caffeine use is associated with risks (both increased and decreased) of diseases (Yang et al., 2010). Associations have been made with cardiovascular diseases (Cornelis, El-sohemy, & Kabagambe, 2006; Guessous et al., 2012) and neurodegenerative disorders (Ascherio et al., 2001; Ross et al., 2000), however the evidence is not yet established enough to be irrefutable (Yang et al., 2010). It is important to bear in mind the multifactorial nature of these diseases when considering genetic associations.

Parkinson's Disease

Caffeine may be protective against Parkinson's disease via its blockade of A2A receptors, protecting dopaminergic neurons (Ascherio et al., 2001; Ross et al., 2000), however not all research agrees (Chuang et al., 2017). Ascherio et al. (2001) found the benefit to be a U-shaped effect, with moderate doses of caffeine providing the highest protection. The variant allele in *ADORA2A* (rs5996696), akin to the T allele in the SNP of interest, was associated with a 30% reduced risk of Parkinson's disease (Ascherio et al., 2001). In consideration of caffeine metabolism, *CYP1A2* SNPs were not found to be associated with Parkinson's disease risk on their own, however links with protection were strongest in individuals who were both slow metabolisers as well as carrying the variant genotype in *ADORA2A* (Ascherio et al., 2001).

Myocardial Infarction

An increased risk of nonfatal myocardial infarction (MI) has been found in those with a C variant of the *CYP1A2* gene ("slow and "ultra-slow" caffeine metabolisers). This may be due to the increased length of time it takes to clear caffeine from the system (Cornelis, El-Sohemy, Kabagambe, & Campos, 2006). Additionally, as caffeine blocks adenosine receptors, vasodilation is reduced, contributing to the reactive inflammatory response during

myocardial ischemia (Cornelis et al., 2006). Another contribution to this increased risk may be a decreased ability to metabolise catecholamines in response to caffeine ingestion. Links have been made with heavy caffeine use and lower catechol-O-methyl transferase (COMT) activity, the main enzyme responsible for metabolising catecholamines. This lower activity may mean that catecholamines have more opportunity to damage myocardial cells, leading to an increased risk for myocardial infarction (Yang et al., 2010).

Hypertension and Stroke

Similarly, with MI, an increased risk of hypertension has been found in slow caffeine metabolisers, with *CYP1A2* CC demonstrating a 34% higher chance of having hypertension compared to AA and AC genotypes in non-smokers (Guessous et al., 2012). Interestingly, no association was found between the genotypes in smokers, likely due to the increased caffeine metabolism smokers may experience, dulling this genotypic association (Guessous et al., 2012). In linking to consumption, non-smoking "fast" metabolisers were found to have a higher caffeine intake, and associated lower odds of hypertension, demonstrating a protective role of caffeine intake on hypertension (Guessous et al., 2012). Lopez-Garcia et al. (2009) and Larsson & Orsini (2011) also found that high coffee consumption may reduce the risk of stroke. This may be explained by the protective effect of caffeine intake on hypertension, the largest modifiable risk factor for ischemic stroke (Sacco, 1997).

2.5 Additional Factors Influencing Caffeine Consumption

The decision to consume any food or beverage is multifactorial (Baranowski, Cullen, & Baranowski, 1999), and may differ between various caffeine sources, and in different population groups. Additional to the impact of genetics on our positive or negative caffeine responses, consumption is influenced by sociocultural factors (such as the Western 'café culture', and societal expectations and norms), environmental and lifestyle factors (such as stress and smoking; Gray, 1998), and other intrinsic factors (such as perceived adverse health effects; Cornelis et al., 2007). Alongside all of these aspects, there is always the element of *choice.* This is important to be mindful of when considering consumption data, as regardless of an individual's reaction to, or ability to clear caffeine, there is always the *choice* to consume or not to consume, and the extent to do so. Most individuals, especially if they are sensitive

to caffeine's effects, can establish a gauge of approximately how much caffeine they can comfortably consume, and do so accordingly (Smith, 2002). However, there will also always be those that seek to intentionally over-consume caffeine for the functional outcomes (e.g. to stay awake for longer periods; Lieberman et al., 2015).

2.6 Summary

This review describes the complex relationship between caffeine consumption and response in relation to genetic profile. Despite the volume of research, an individual's response to caffeine and its consumption involves a magnitude of factors. This challenge is amplified by an individual's capacity of choice that always has the potential to override other predictors. What is clear is that caffeine consumption and response is linked to all three genes of interest *CYP1A2*, *AHR*, and *ADORA2A* in varying ways.

3 Research Manuscript

Prepared according to author guidelines for submission to the journal titled Psychopharmacology (Author guidelines detailed in Appendix C)

Investigating the Impact of Genetic Variability of *CYP1A2*, *ADORA2A*, and *AHR* on Caffeine Consumption and Response

Rebecca Tennent[1], BSc; Ajmol Ali[1], PhD; Carol Wham[1], PhD; Kay Rutherfurd-Markwick[2], PhD.

[1]School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand

[2]School of Health Sciences, Massey University, Auckland, New Zealand

Abstract

Rationale: Exploration of the links between genetic profile and caffeine response will advance the understanding of the impact of caffeine consumption on individuals with differing genetic profiles. Three single nucleotide polymorphisms (SNPs) have known links to caffeine consumption, metabolism, and post-consumption effects and responses: cytochrome P450 1A2 (*CYP1A2*; rs762551), adenosine A2A receptor (*ADORA2A*; rs5751876), and aryl-hydrocarbon receptor (*AHR*; rs4410790).

Objectives: To examine caffeine consumption patterns and post-consumption responses among New Zealand individuals and how these differ with genetic variation of *CYP1A2*, *ADORA2A*, and *AHR*.

Methods: Caffeine intake and post-consumption responses were assessed using a caffeine consumption habits questionnaire (CaffCo). Genetic data for the SNPs was analysed using MassARRAY analysis on DNA extracted from saliva samples.

Results: 255 participants aged 15 years and over were included in the study. Half (49.4%) of all participants were "fast" metabolisers (*CYP1A2* AA) and 10.2% "ultra-slow" metabolisers (*CYP1A2* CC) of caffeine. Almost half of the participants carried *ADORA2A* CT (46.3%), followed

by CC (29.0%), and TT (24.7%). Half (51.8%) of the participants carried *AHR* CT, followed by CC (30.6%), and TT (17.6%) genotypes. Overall, 14.1% of participants reported a caffeine intake >400 mg/day and 52.9% an intake of 80-400 mg/day. Carriers of the genotype *ADORA2A* TT consumed 65 mg/day less caffeine than carriers of the heterozygote genotype (*ADORA2A* CT; *p*= 0.034). No association was found with the other analysed SNPs.

Conclusions: These results identify the genetic variation and caffeine consumption habits in New Zealanders over 15 years. The data show an association between *ADORA2A* TT and decreased caffeine consumption. Future studies are needed to assess caffeine response in relation to these genes, to understand and develop appropriate strategies for informing genotype based advice on caffeine use.

Key words: *caffeine metabolism, caffeine response, cytochrome P450, aryl-hydrocarbon receptor, genetics, SNP, adenosine, anxiety, sleeplessness*

3.1 Introduction

At least 73% of New Zealanders consume caffeine daily (Thomson & Schiess, 2011), providing consumers with a boost of wakefulness, enhanced cognition, and improved mood (Haskell et al., 2005; Lieberman et al., 2002). Many individuals make a conscious decision to consume caffeine in search of these positive responses (McLellan et al., 2016). In contrast, other consumers find the stimulating effects unfavourable, resulting in sleeplessness, anxiety, nervousness, or tremors (Daly & Fredholm, 1998; Rutherfurd-Markwick & Ali, 2016) and may intentionally avoid caffeine ingestion. Increasing interest in the impact of our genes in response to caffeine intake has prompted research in this area in the past two decades, with polymorphisms in selected genes influencing the rate of caffeine metabolism (Sachse et al., 1999), and the physiological responses post-consumption (Harland, 2000; Kaplan et al., 1997; Rutherfurd-Markwick & Ali, 2016) which collectively affect the individuals' overall response to caffeine. This, together with factors such as habituation and tolerance (Harland, 2000), contribute to variation in caffeine experiences between individuals.

Single nucleotide polymorphisms (SNPs), in three genes encoding for cytochrome P450 1A2 (*CYP1A2*; rs762551), adenosine A2A receptor (*ADORA2A*; rs5751876), and aryl-hydrocarbon receptor (*AHR*; rs4410790) are known to have links to caffeine consumption and metabolism, and its resultant effects. Caffeine is primarily metabolised in the liver by the cytochrome P450 1A2 enzyme (Berthou et al., 1991; Miners & Birkett, 1996). The rate that this enzyme works at determines the length of time caffeine stays in the body (Gu et al., 1992), and can vary 40-fold between, and even within, individuals (Yang et al., 2010). The three *CYP1A2* genotypes (AA, AC, and CC) result in differing enzyme activity, with the AA genotype metabolising caffeine "fast" and the CC genotype metabolising caffeine slowly ("ultra-slow"; Sachse et al., 1999). The CYP1A2 enzyme is induced by the binding of aromatic hydrocarbons to the AHR, a transcription factor protein (Sachse et al., 1999). Variation in both of these genes (*CYP1A2* and *AHR*) therefore impacts on the metabolism of caffeine (Fulton et al., 2018).

In the body, adenosine receptors (ADORA) are the main target of caffeine action (Cornelis et al., 2007). Caffeine binds to adenosine receptors as an antagonist, stimulating the release of neurotransmitters that would otherwise be slowed when adenosine is bound, resulting in an energising effect in the consumer (Daly et al., 1983; Ribeiro & Sebastião, 2010). Genetic variation in the *ADORA2A* gene has been associated with increased susceptibility to anxiety post-caffeine consumption (*ADORA2A* TT; Alsene et al., 2003; Childs et al., 2008; Domschke et al., 2012), or sleep disturbances (*ADORA2A* CC; Byrne et al., 2012; Rétey et al., 2007), depending on the genotype. Anxiety effects in particular have been noted in occasional caffeine users (Alsene et al., 2003; Childs et al., 2008; Rogers et al., 2010), inferring an increased vulnerability to this adverse effect in infrequent consumers.

The tendency to consume caffeine has been linked to genotype variation in the *AHR* gene, with higher coffee intake associated with carriers of the C allele (Josse et al., 2012; Sulem et al., 2011). Increased caffeine consumption has also been associated with fast caffeine metabolism, involving *CYP1A2* (rs2472297) and *AHR* (Amin et al., 2012; Josse et al., 2012; Sulem et al., 2011). Additionally, "ultra-slow" and "slow" caffeine metabolisers are more likely to consume less caffeine than "fast" metabolisers (Cornelis et al., 2016; 2014; 2011). The data is less clear for *ADORA2A*, with contrasting findings of both the T (Cornelis et al., 2007) and C (Rétey et al., 2007; Rogers et al., 2010) alleles linked to decreased consumption. These

contrasting results support the notion that most individuals are able to self-regulate their caffeine consumption for optimal responses. In addition, tolerance and habituation may have an added influence on the prevalence of adverse responses (Rogers et al., 2010).

Given the relationship between metabolism and post-consumption responses, the present study aims to investigate the patterns and relationships between the genetic profile of the three genes: *CYP1A2*, *AHR*, and *ADORA2A*, and caffeine consumption. This is the first study examining the impact of genetics on caffeine experiences in a New Zealand (NZ) population. Current literature suggests that those carrying genotypes known to be more sensitive to adverse effects post caffeine consumption, and those who metabolise caffeine at a slower rate, are likely to consume less caffeine. NZ has a unique ethnic diversity, with over 100 ethnicities living in Auckland City alone (Education Review Office, 2016). This ethnic disparity is an important consideration, given the conflicting results linked to differing ethnicities (Denden et al., 2016). Gaining a deeper understanding of the genetic influences related to caffeine will improve understanding of the role of dietary and pharmacological caffeine in health, and elucidate areas for further research.

3.2 Methods

3.2.1 Study Design and Participants

A cross-sectional study was undertaken among New Zealanders 15 years and over to measure caffeine consumption habits and experiences, and relate these to genetic variations in the three genes of interest (*ADORA2A* (rs5751876), *CYP1A2* (rs762551), and *AHR* (rs4410790)). Data on caffeine consumption habits and experiences, participant characteristics and demographics, and self-reported clinical characteristics (e.g. weight, height) was collected using a previously developed and pilot-tested questionnaire CaffCo (Rowe, 2015; Appendix A), conducted through Qualtrics online survey software (Qualtrics, 2015). A saliva sample was collected for genomic DNA analysis of the three genes (*ADORA2A* (rs5751876), *CYP1A2* (rs762551), and *AHR* (rs4410790)). Participants had the option to complete the questionnaire at the time of saliva collection or via an online link at their discretion.

Participants were asked to refrain from consuming food and drink for 30 min prior to saliva collection. A saliva sample of approximately 1 ml was collected by drooling into a labelled sterile tube. Preservation buffer (Appendix D) was then added in a ratio of approximately 1:1, to ensure the DNA remained stable at room temperature during collection days (Nunes et al., 2012). Samples were stored at -20°C prior to DNA extraction. Genomic DNA from saliva samples was prepared using a standard DNA extraction protocol (Appendix D). Analysis of the 3 SNPs (*ADORA2A* (rs5751876), *CYP1A2* (rs762551), and *AHR* (rs4410790)) from the extracted DNA samples was carried out using MassARRAY analysis (GeneWorks, Australia). Full standard operating procedures for the 'The Caffeine Study' are provided in Appendix E.

Participant recruitment took place at university campuses in Auckland and Palmerston North between June and August 2016 as part of "The Caffeine Study" (Stachyshyn, 2017). Participants were provided with an information sheet (Appendix F), and gave written consent (Appendix G) before providing a saliva sample for genetic analysis. A total of 374 participants provided a saliva sample that was successfully analysed for the three SNPs of interest. After exclusion of participants with incomplete questionnaire data, a total of 255 participants were included in the study (**Figure 3.1**). Ethical approval for this study was granted by the Massey University Human Ethics Committee (Southern A, Application 17/01; Appendix H).

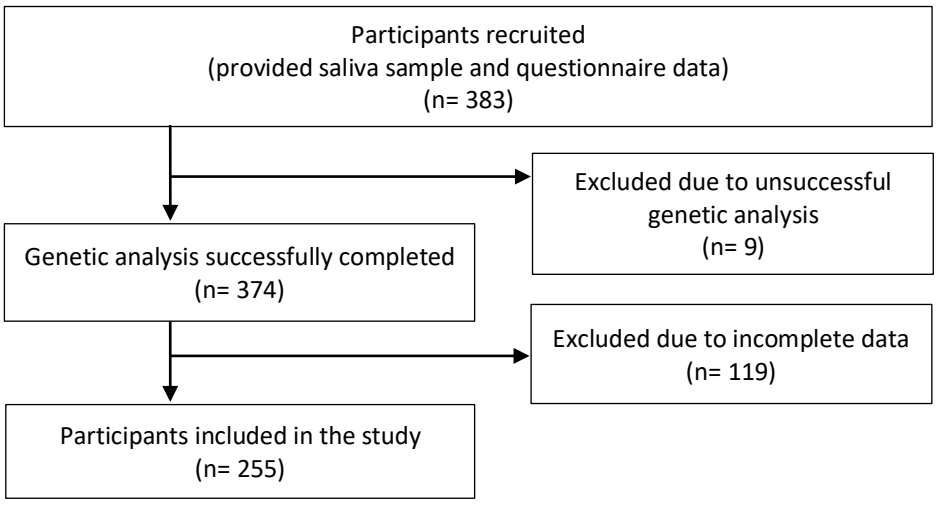

Fig. 3.1 Flow diagram of participant recruitment and final inclusion in the study

3.2.2 Data Analysis

Ethnicity data from 17 chosen ethnicities was categorised into five groups (European, Māori, Pacific Peoples, Asian, Middle Eastern/ Latin American/ African) according to a prioritisation system to consolidate results (Statistics New Zealand, 2017) and ensure minority groups were represented.

An estimation of total daily caffeine consumption (mg/day) was calculated for each participant based on source and frequency, using consumption data from the CaffCo questionnaire. To calculate daily consumption, frequencies were assigned a factor (e.g. once a week, factor 1/7= 0.143; Table 2, Appendix E); this factor was then multiplied by the mg of caffeine contained in the source (Table 1, Appendix E).

Participants were categorised into three intake groups (<80 ("low"), 80-400 ("moderate"), >400 ("high") mg/day caffeine) based on Food Standards NZ classifications for intake (Australia New Zealand Food Authority Expert Working Group on Caffeine, 2000) and categorisations from other studies (Heckman et al., 2010; Josse et al., 2012; Nawrot et al., 2003; Rogers et al., 2010). Additionally, for participants who provided body weight data (n=228), mg/kg/day was calculated to enable a relative comparison between participants.

Caffeine intake data was analysed by genotype to explore any relationships between the genotypes of the three SNPs. SNPs were then paired to consider links between genes. *CYP1A2* was paired with *ADORA2A* and *AHR* to determine the combined impact of the rate of metabolism with post-caffeine responses and consumption patterns.

Of the available options in the questionnaire, five "adverse symptoms" post-caffeine consumption were chosen for analysis based on their relationship to the effects linked to the three SNPs in the literature: "nervous", "jitters", "twitches", "fast or uneven heartbeat", and "unable to sleep". Three of these symptoms ("nervous" (n=39), "jitters" (n=74), and "twitches" (n=39)) were combined into one group (n=95), and re-named "anxiety indicators". "Adverse symptom" data was analysed by *ADORA2A* genotype alone, as well as in combination with *CYP1A2* to examine the impact of the rate of metabolism on "adverse symptom" prevalence.

3.2.3 Statistical Analysis

CaffCo questionnaire data was exported from Qualtrics into Microsoft Excel (2013) and matched to genetic data for each participant. All statistical analyses were performed using the IBM SPSS statistics software, version 24.0 (IBM Corporation, New York, USA). Scale variables were tested for normality and, as the data was not normally distributed, findings are reported as median (interquartile range). Categorical data was reported as frequency and percentage. Statistical significance was set at $p<0.05$ for all tests.

As all scale data was not normally distributed, Kruskal-Wallis tests were used. For variables that showed statistically significant differences between groups, post-hoc tests using Mann-Whitney U-tests and pairwise comparisons were conducted. If significance was shown for any Mann-Whitney U-test, effect size (*r*) was calculated to show practical significance using $r=z/\sqrt{N}$ (Fritz, Morris, & Richler, 2012). An effect size value of 0.1 indicated a small effect, a value of 0.3 indicated a medium effect and a value of ≥ 0.5 indicated a large effect (Field, 2013).

3.3 Results

3.3.1 Participant Characteristics

Participant characteristics, including gender, age, ethnicity, and genotype for each of the three SNPs tested are presented in **Table 3.1.** Participants were predominantly European and Asian, with most aged between 19 to 30 years. Approximately half (49.4%) of the respondents were classified as "fast" metabolisers (genotype *CYP1A2* AA), 40.4% were classified as "slow" metabolisers (CA), and 10.2% were classified as "ultra-slow" metabolisers (CC) of caffeine. Within *ADORA2A*, heterozygous CT was most prominent (46.3%), followed by CC (29.0%), and TT (24.7%). Within *AHR*, half (51.8%) of the participants carry heterozygous CT, followed by CC (30.6%), and TT (17.6%) genotypes.

No association was found between gender and genotype distribution (**Supplementary Table 1**), therefore gender was not stratified in the analysis. However, choice of caffeine source did differ by gender. Tea ($p= 0.010$), coffee ($p= 0.038$) and chocolate ($p <0.001$) were all consumed

by a higher proportion of females than males, and more males consumed caffeine-containing sports supplements (*p*= 0.045; **Supplementary Table 2**).

Table 3.1 Participant characteristics

		N= 255 n (%)
Gender		
Male		123 (48.0)
Female		132 (52.0)
Age Group		
16-18 years		40 (15.7)
19-30 years		189 (74.4)
31-50 years		20 (7.9)
51-70 years		5 (1.6)
71+ years		1 (0.4)
Ethnicity		
European*		123 (48.2)
Māori		14 (5.5)
Pacific Peoples†		13 (5.1)
Asian‡		93 (36.5)
Middle Eastern / Latin American / African §		12 (4.7)
Genotypes		
CYP1A2	CC	26 (10.2)
	CA	103 (40.4)
	AA	126 (49.4)
ADORA2A	CC	74 (29.0)
	CT	118 (46.3)
	TT	63 (24.7)
AHR	CC	78 (30.6)
	CT	132 (51.8)
	TT	45 (17.6)

*NZ European, European, Russian, Irish, Croatian; †Samoan, Cook Island Māori, Tongan, Fijian, Niuean, Tokelauan, Tuvaluan; ‡ Chinese, Southeast Asian, Korean, Japanese, Filipino, Malaysian, Taiwanese, Indonesian, Vietnamese, Indian, Sri Lankan, Mongolian; § Middle Eastern, Latin American, African, South African.

3.3.2 Caffeine Consumption

An estimation of total daily caffeine consumption (mg/day) was calculated for each participant based on source and frequency, using consumption data from the CaffCo questionnaire. Overall, 32.9% (n=84) of participants were considered "low" caffeine consumers (<80 mg/day), 14.1% (n= 36) "high" consumers (>400 mg/day), with the remaining 52.9% (n=135) of participants reporting "moderate" intake (80-400 mg/day). Participants with genotype *ADORA2A* CT reported significantly higher caffeine consumption compared to carriers of TT, (p = 0.034, effect size r = 0.16), consuming 168 mg/day and 103 mg/day, respectively (**Table 3.2**). The data did not reveal any differences between genotypes when intake was grouped into "low", "moderate", and "high" caffeine intake (**Supplementary Table 3**). No association was found between daily caffeine intake when expressed on a per kg body weight basis by genotype (**Supplementary Table 4**). **Figure 3.2 (A-I)** and **3.3 (A-I)** combine participants into their nine genotype carrier groups for *CYP1A2* and *ADORA2A*, and *CYP1A2* and *AHR*, respectively. No association was found between median caffeine intake and either the combined genotype groups of *CYP1A2* and *ADORA2A*, nor *CYP1A2* and *AHR* (**Figure 3.2; Figure 3.3; Supplementary Table 5**).

Table 3.2 Estimated median daily caffeine intake by genotype

Genotypes	Median intake (Interquartile range) mg/day	Kruskal-Wallis test statistic (H)	*P* value
CYP1A2			
CC	182 (64, 332)	0.643	0.725
CA	132 (65, 273)		
AA	143 (58, 298)		
ADORA2A			
CC	130 (61, 275)	6.779	0.034* TT-CT
CT	168 (79, 311)		r= 0.159[†]
TT	103 (49, 223)		
AHR			
CC	131 (58, 291)	0.874	0.646
CT	160 (66, 295)		
TT	134 (73, 265)		

*Significant at the 0.05 level.

[†]Effect size (r) was calculated to show practical significance using $r = z/\sqrt{N}$ (Fritz, Morris, & Richler, 2012). An effect size value of 0.1 indicated a small effect, a value of 0.3 indicated a medium effect and a value of ≥0.5 indicated a large effect (Field, 2013).

Figure 3.2 shows the impact of the rate of caffeine metabolism (*CYP1A2*) and "adverse symptom" vulnerability (*ADORA2A*) on caffeine intake. Participants most vulnerable to anxiety (*ADORA2A* TT) across groups A (*ADORA2A* TT / *CYP1A2* CC), B (*ADORA2A* TT / *CYP1A2* AC), and C (*ADORA2A* TT / *CYP1A2* AA) generally reported a lower caffeine intake (120, 102, and 108 mg/day, respectively), with fewer consumers in the "high" intake category (10.0%, 14.3%, and 6.3%, respectively), and the highest frequency of consumers in the "low" category (40.0%, 42.9%, and 43.8%, respectively) of all nine carrier groups. Those most vulnerable to sleep impairment (*ADORA2A* CC) across groups G (*ADORA2A* CC / *CYP1A2* CC) and I (*ADORA2A* CC / *CYP1A2* AA) reported a higher frequency of participants in the "high" intake category (28.6% and 21.2% respectively). Group H (*ADORA2A* CC / *CYP1A2* AC) was an exception to this, reporting the lowest frequency of "high" intakes of all nine carrier groups (2.9%). The highest median caffeine intake was reported from participants in group G (*ADORA2A* CC / *CYP1A2* CC) with 217 mg/day, followed by group E (heterozygous for both genes) consuming 186 mg/day (**Supplementary Table 5**).

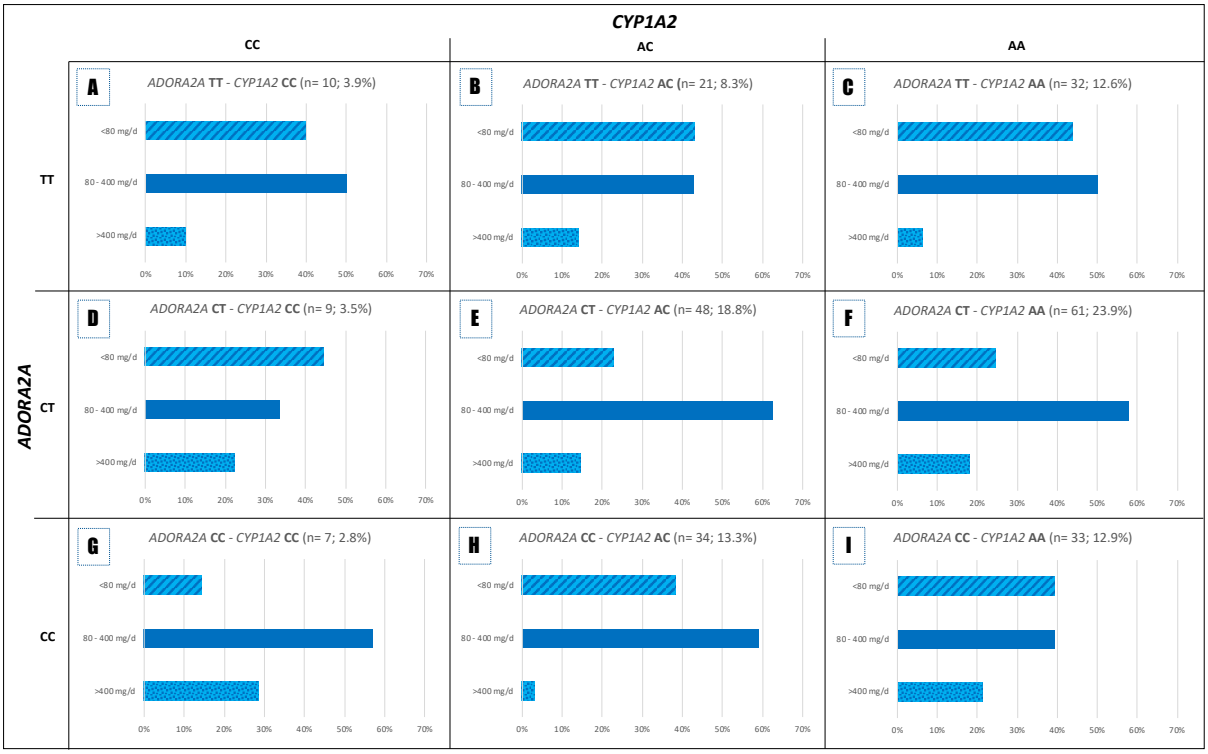

Fig. 3.2 Daily caffeine intake of participants by genotype combination *ADORA2A* and *CYP1A2*. A demonstration of the impact of caffeine-metabolism rate and 'adverse symptom' vulnerability on daily caffeine intake

Each bar in each graph A - I represents the percentage of participants within the named genotype combination. Caffeine intake <80 mg/d - low intake; 80 - 400 mg/d - moderate intake; >400 mg/d - high intake.

Prediction of caffeine-related behaviour based on literature:

A - Slow metaboliser; most vulnerable to caffeine-related anxiety (Predicted overall lowest intake group alongside group 7). B - Intermediate metaboliser; most vulnerable to caffeine-related anxiety. C - Fast metaboliser; most vulnerable to caffeine-related anxiety. D - Slow metaboliser; some vulnerability to caffeine-related sleep impairment. E - Intermediate metaboliser; some vulnerability to caffeine-related sleep impairment. F - Fast metaboliser; some vulnerability to caffeine-related sleep impairment (Predicted overall highest intake group). G - Slow metaboliser; most vulnerable to caffeine-related sleep impairment (Predicted overall lowest intake group alongside group 1). H - Intermediate metaboliser; most vulnerable to caffeine-related sleep impairment. I - Fast metaboliser; most vulnerable to caffeine-related sleep impairment.

Figure 3.3 (A-I) shows the impact of the rate of caffeine metabolism and the role of *AHR* in the upregulation of *CYP1A2* expression on caffeine intake. Of all nine gene carrier groups, group G (*AHR* CC / *CYP1A2* CC) reported the highest frequency of consumers in the "high" caffeine intake category (33.3%). The highest median caffeine intake was reported from participants in group A (*AHR* TT / *CYP1A2* CC) with 297 mg/day, followed by groups F (*AHR* CT / *CYP1A2* AA) and G (*AHR* CC / *CYP1A2* CC) consuming 161 mg/day (**Supplementary Table 5**).

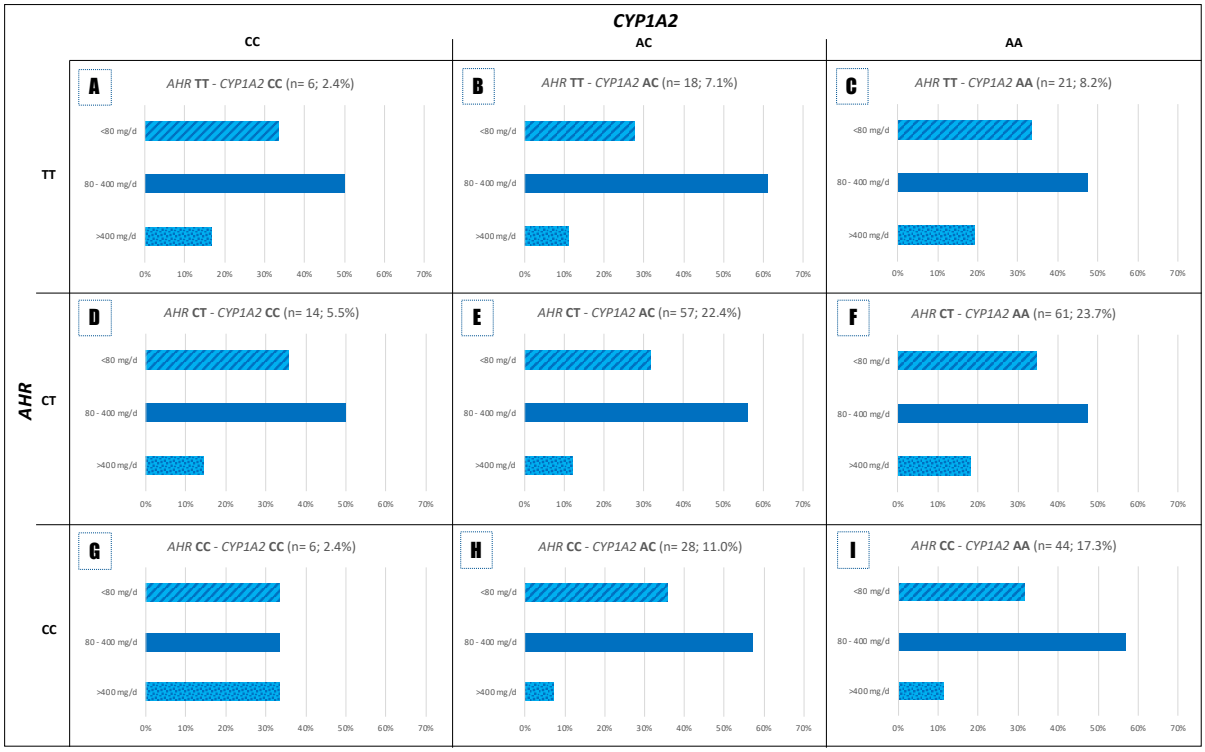

Fig. 3.3 Daily caffeine intake of participants by genotype combination *AHR* and *CYP1A2*. A demonstration of the impact of caffeine-metabolism rate and regulation of *CYP1A2* expression on caffeine intake

Each bar in each graph A - I represents the percentage of participants within the named genotype combination. Caffeine intake <80 mg/d - low intake; 80 - 400 mg/d - moderate intake; >400 mg/d - high intake.

Prediction of caffeine-related behaviour based on literature: A - Slow metaboliser; lowest caffeine consumer (Predicted overall lowest intake group). B - Intermediate metaboliser; lowest caffeine consumer. C - Fast metaboliser; lowest caffeine consumer. D - Slow metaboliser; moderate caffeine consumer. E - Intermediate metaboliser; moderate caffeine consumer. F - Fast metaboliser; moderate caffeine consumer. G - Slow metaboliser; highest caffeine consumer. H - Intermediate metaboliser; highest caffeine consumer. I - Fast metaboliser; highest caffeine consumer (Predicted overall highest intake)

3.3.3 Perceived "Adverse Symptoms" Post Consumption of Caffeine

Perceived "adverse symptoms" were categorised into three groups of interest: "unable to sleep"; "fast or uneven heartbeat"; and "indicators of anxiety". Nearly two thirds (n= 163; 63.9%) of participants reported at least one of these symptoms, while 19.6% (n= 32) reported all three groups of symptoms. Of these, half (n= 14; 50.0%) carry *ADORA2A* CT, 40.6% (n= 13) carry the CC allele, and 15.6% (n= 5) carry the TT allele. No association was found between perceived "adverse symptoms" and *ADORA2A* genotypes (**Supplementary Table 6**). **Figure 3.4** shows the distribution and frequency of participants who reported "unable to sleep" by *ADORA2A* genotype. **Figures 3.5** and **3.6** show the distribution and frequency of participants who reported the symptoms "fast and uneven heartbeat" and "indicators of anxiety", respectively.

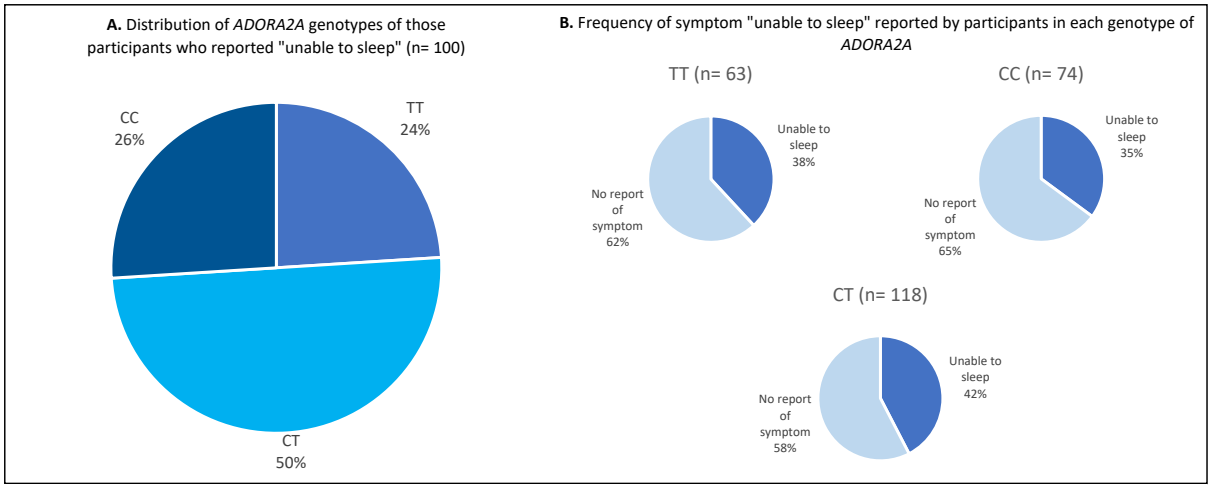

Fig. 3.4 Distrubution and frequency of adverse symptom "unable to sleep" by *ADORA2A* genotype
A. Demonstrates the distribution of *ADORA2A* genotypes of the 100 participants who reported experiencing the symptom "unable to sleep".
B. Demonstrates the frequency of participants who reported experiencing the symptom "unable to sleep" in each *ADORA2A* genotype.

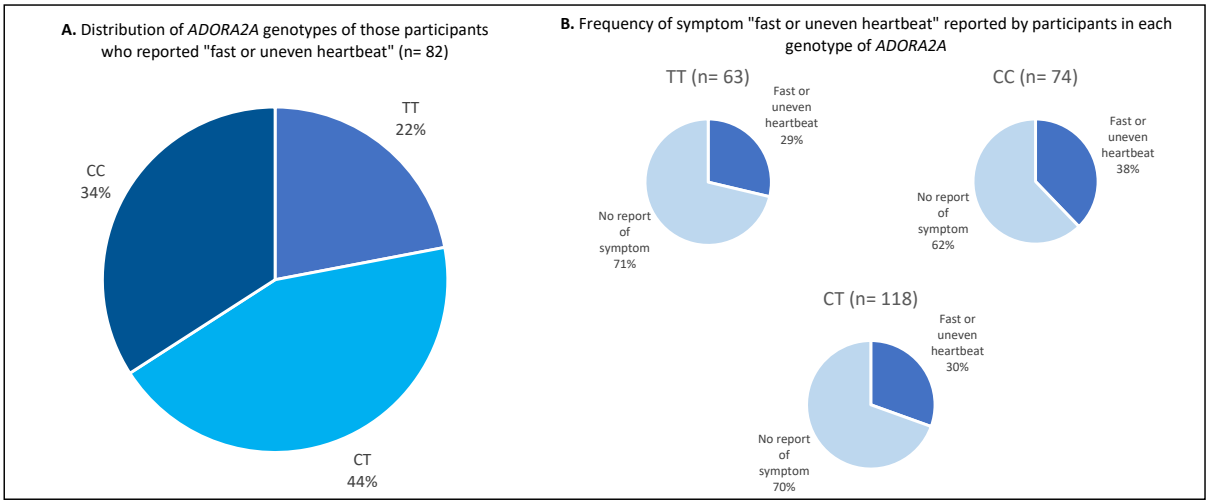

Fig. 3.5 Distrubution and frequency of adverse symptom "fast or uneven heartbeat" by *ADORA2A* genotype
A. Demonstrates the distribution of *ADORA2A* genotypes of the 82 participants who reported experiencing the symptom "fast or uneven heartbeat".
B. Demonstrates the frequency of participants who reported experiencing the symptom "fast or uneven heartbeat" in each *ADORA2A* genotype.

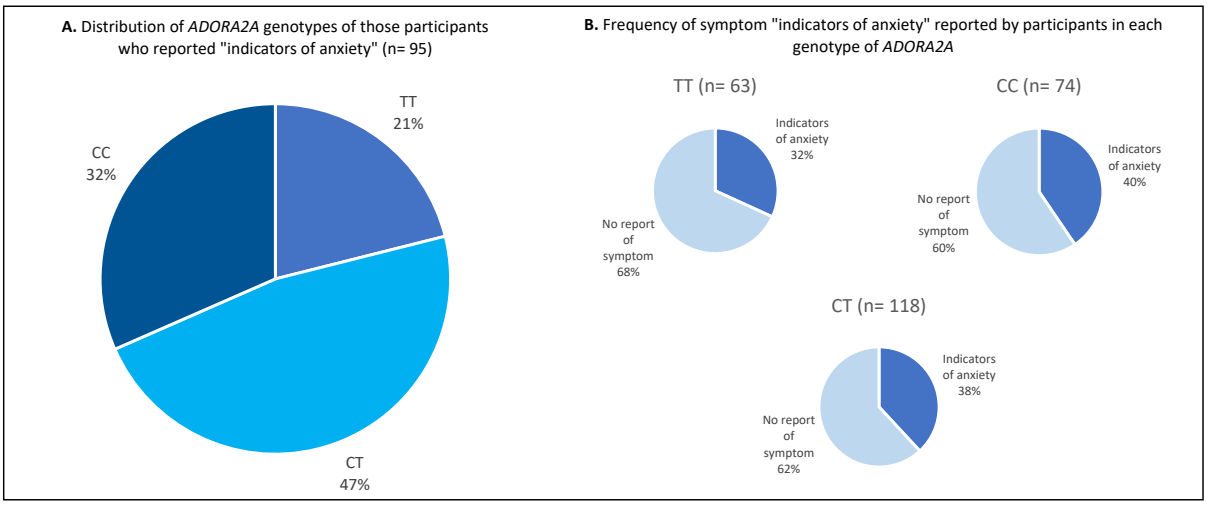

Fig. 3.6 Distrubution and frequency of adverse symptom "indicators of anxiety" by *ADORA2A* genotype
A. Demonstrates the distribution of *ADORA2A* genotypes of the 95 participants who reported experiencing the symptom "indicators of anxiety".
B. Demonstrates the frequency of participants who reported experiencing the symptom "indicators of anxiety" in each *ADORA2A* genotype.

The impact of the rate of caffeine metabolism on perceived “adverse symptoms” is shown in **Figure 3.7 (A-I)**, by combining participants into their genotypes for *ADORA2A* and *CYP1A2* into nine carrier groups. No association was found between perceived “adverse symptoms” and the combined genotype groups of *CYP1A2* and *ADORA2A* (**Supplementary Table 7**). Participants most vulnerable to anxiety (*ADORA2A* TT) across groups A (*ADORA2A* TT / *CYP1A2* CC), B (*ADORA2A* TT / *CYP1A2* AC), and C (*ADORA2A* TT / *CYP1A2* AA) reported experiencing the symptom “indicators of anxiety” at frequencies of 30.0%, 28.6%, and 34.4%, respectively; “fast” metabolisers reporting the symptom at higher frequencies than “ultra-slow” metabolisers. Participants most vulnerable to sleep impairment (*ADORA2A* CC) across groups G (*ADORA2A* CC / *CYP1A2* CC), H (*ADORA2A* CC / *CYP1A2* AC), and I (*AODRA2A* CC / *CYP1A2* AA) reported experiencing the symptom “unable to sleep” at frequencies of 28.6%, 38.3% and 33.3% respectively; “slow” and “fast” metabolisers reporting the symptom at higher frequencies than “ultra-slow” metabolisers. The symptom “fast or uneven heartbeat” was reported in the highest frequency in groups A (*ADORA2A* TT / *CYP1A2* CC; 50%) and H (*ADORA2A* CC / *CYP1A2* AC; 41.2%).

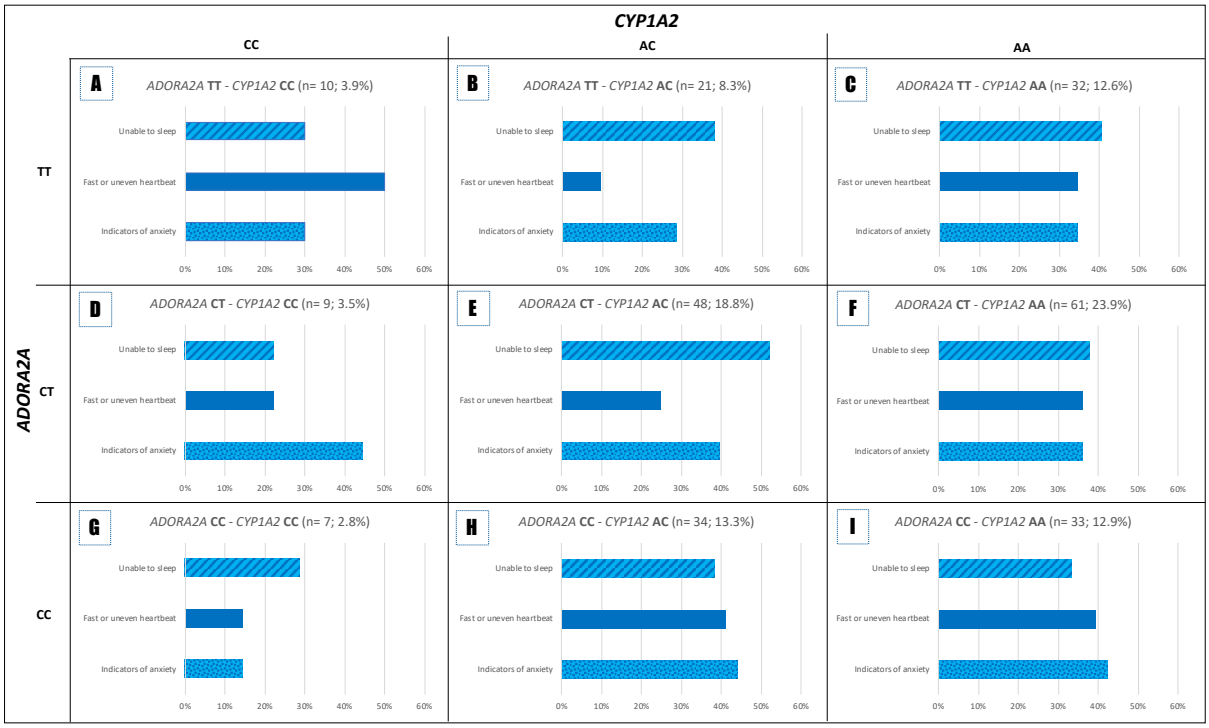

Fig. 3.7 Perceived 'adverse symptoms' reported by participants post caffeine consumption by genotype combination *ADORA2A* and *CYP1A2*. A demonstration of the impact of caffeine-metabolism rate on perceived symptoms
Each bar in each graph A - I represents the percentage of participants within the named genotype combination who reported the 'adverse symptoms'.
'Indicators of anxiety' - Combined symptoms reported: nervous; twitches; jitters.
Prediction of caffeine-related effects based on literature:
A - Slow metaboliser; most vulnerable to caffeine-related anxiety. B - Intermediate metaboliser; most vulnerable to caffeine-related anxiety. C - Fast metaboliser; most vulnerable to caffeine-related anxiety. D - Slow metaboliser; some vulnerability to caffeine-related sleep impairment. E - Intermediate metaboliser; some vulnerability to caffeine-related sleep impairment. F - Fast metaboliser; some vulnerability to caffeine-related sleep impairment. G - Slow metaboliser; most vulnerable to caffeine-related sleep impairment. H - Intermediate metaboliser; most vulnerable to caffeine-related sleep impairment. I - Fast metaboliser; most vulnerable to caffeine-related sleep impairment.

3.4 Discussion

The choice to consume caffeinated products is multifactorial, personal, and constantly changing. One example in the present study was the differential preference for caffeine source held by males and females. Factors influencing this choice were not the focus of this research, however influences come from sociocultural, environmental, and lifestyle factors (Cornelis et al., 2007; Gray, 1998). The focus of this study was to assess the impact of genotypic variations in *CYP1A2*, *ADORA2A*, and *AHR* genes on caffeine consumption habits, and responses post-consumption.

Over half (52.9%) of the participants consumed a “moderate” caffeine intake of 80 - 400 mg/day. With the average cup of instant coffee (the most popular caffeinated beverage choice; Butt & Sultan, 2011) containing 83 mg, this equates to between 1 - 4.8 cups of instant coffee each day, or up to 2 double shot espressos (~210 mg each; Sivakumaran et al., 2017). When analysed by genotype, carriers of *ADORA2A* TT consumed 65 mg/day less caffeine than

carriers of the heterozygote genotype (*ADORA2A* CT), the equivalent of nearly one fewer cups of instant coffee each day (Sivakumaran, Huffman, & Sivakumaran, 2017). This result is consistent with previous research findings that carriers of *ADORA2A* TT consumed less caffeine than C allele carriers (Cornelis et al., 2007).

Based on previous research (Alsene et al., 2003; Childs et al., 2008; Domschke et al., 2012), it was hypothesised that the lowered caffeine consumption in *ADORA2A* TT carriers may be due to an increased prevalence of caffeine-induced anxiety in this genotype. However, among the *ADORA2A* genotypes, only 32% of respondents who carried the *ADORA2A* TT allele reported experiencing "indicators of anxiety", compared to 38% of those that carry the CT genotype. Although this difference was not significant, it suggests that the decreased consumption of caffeine among *ADORA2A* TT carriers was not due to an increased prevalence of anxiety in this population. It has been reported that regular caffeine users (≥40 mg/day) do not experience increased anxiety post consumption, even if they carry the *ADORA2A* TT genotype (Rogers et al., 2010). In the present study, 84% of participants were regular consumers (≥40 mg/day) of caffeine, who therefore may have built up a tolerance to caffeine's effects (Harland, 2000). In this population, the tolerance built may have provided a protective effect from the experience of anxiety, and could explain the muted association of "indicators of anxiety" with the *ADORA2A* TT genotype.

In addition, it was hypothesised that slower metabolisers of caffeine would consume less, particularly when experiencing "adverse symptoms" such as anxiety. Although there was no association of *ADORA2A* TT carriers to "indicators of anxiety", the lowered consumption in carriers of *ADORA2A* TT (compared to CT) was analysed against *CYP1A2*. No clear differences in caffeine consumption were distinguished in "fast" (*CYP1A2* AA) or "ultra-slow" (*CYP1A2* CC) metabolisers who carry *ADORA2A* TT in this population. The potential for both *CYP1A2* and *ADORA2A* to impact on caffeine consumption levels was explored by Cornelis et al. (2007), who identified a link to decreased caffeine consumption in carriers of *ADORA2A* TT. Further work by Cornelis et al. (2011), showed that a different *CYP1A2* SNP (rs2472304) was associated with caffeine consumption, demonstrating the potential for the enzyme activity of CYP1A2 to influence intake. It would be valuable for future research to look into the rs2472304 *CYP1A2* SNP, which was not tested in this study.

Furthermore, when *CYP1A2* genotypes were analysed alone in relation to caffeine consumption, no impact of rate of metabolism (*CYP1A2* variation) on consumption patterns were found, contrasting previous studies which have identified higher caffeine intakes in "fast" metabolisers (carrying *CYP1A2* AA; Denden et al., 2016; Guessous et al., 2012; Rodenburg et al., 2012), particularly in men, those of younger age, and of Caucasian ethnicity. The ethnic disparity in the current study may contribute to the differing results seen. A meta-analysis found an association between the AA genotype and coffee intake in Caucasian subjects (p= 0.07), however not in Asian subjects (p= 0.55; Denden et al., 2016). Only 48% of participants in the present study were of a Caucasian ethnicity, leaving over half of the participants in a category where links may not have previously been noted.

The present study found that median caffeine consumption among carriers of *AHR* CC and TT were similar (131 mg/day and 134 mg/day respectively), whereas heterozygote CT carriers reported slightly higher caffeine intakes of 160 mg/day. Previous studies have shown considerable differences in caffeine consumption between *AHR* genotypes, with individuals carrying the C allele for *AHR* more likely to consume higher caffeine amounts (Cornelis et al., 2011; Josse et al., 2012; Sulem et al., 2011). Although our results are in contrast to this, it is worth noting that all three studies that found this association were in older adult participants. Josse et al. (2012) found a significant relationship in adults aged over 57 years, while Sulem et al. (2011) and Cornelis et al. (2011) had an average participant age of 49 and 54 years, respectively, whereas our study only included five participants over 51 years. Animal studies have shown that AHR activity decreases with age (Harper, Riddick, & Okey, 2006), therefore, this may explain the differential findings, as the effects of genetic variations may become more pronounced with age (Josse et al., 2012).

Being "unable to sleep" was the most reported "adverse symptom" associated with caffeine consumption, reported by 39% of respondents. Experiencing sleep difficulties is consistent with the known pharmacodynamic effects of caffeine through its antagonistic binding to adenosine receptors (Dunwiddie & Masino, 2001; Ribeiro et al., 2002). Previous studies have found the *ADORA2A* CC genotype predisposes individuals to being more sensitive to caffeine-induced sleep disturbances (Bodenmann et al., 2012; Byrne et al., 2012; Rétey et al., 2007). In this study, only 35% of respondents who carried the *ADORA2A* CC genotype reported this

symptom, with higher frequencies being reported by carriers of *ADORA2A* TT and CT genotypes. A possible explanation for the lack of distinct genotypic variance may be the interpretation of this questionnaire item in CaffCo, where respondents were prompted to identify if they were "unable to sleep" *shortly* after caffeine consumption. To distinguish relationships with *ADORA2A* CC and caffeine-induced sleep disturbances (aligning with previous studies), it would be valuable to find out whether respondents experienced being "unable to sleep" after a period of time had passed post consumption (e.g. if caffeine consumed in the morning had an impact on sleep difficulty that night).

Nearly one third (29%) of participants with *ADORA2A* TT genotype, compared to 38% of those who carry *ADORA2A* CC genotype, reported experiencing "fast or uneven heartbeat" following caffeine consumption. Although not a direct measure of blood pressure, the experience of a "fast or uneven heartbeat" may be an indication of changes in blood pressure (Stimulant Drinks Committee, 2002). Genetic variation may affect blood pressure response, with an acute rise in blood pressure post-caffeine consumption previously been found to be more strongly associated with the TT genotype of *ADORA2A* (Renda et al., 2012). Although this study did not show a clear distinction between the genotypes, experiencing a rapid heartbeat post caffeine consumption is a common adverse effect regardless of genotype (Thomson & Schiess, 2011). When gene carriers from *ADORA2A* and *CYP1A2* were grouped and combined, this symptom was reported highest in group with the combination of *ADORA2A* TT / *CYP1A2* CC (50%) and *ADORA2A* CC / *CYP1A2* AC (41%). This could indicate the impact of having a slow caffeine metabolism on experiencing a "fast or uneven heartbeat". Interestingly, "ultra-slow" metabolisers (carrying *CYP1A2* CC) have previously been found to be at a higher risk of hypertension associated with coffee intake (Guessous et al., 2012; Palatini et al., 2009). Given 10.2% of our population were "ultra-slow" metabolisers, these individuals could be at increased risk of developing hypertension if they consume caffeine. Further elucidation of the link between hypertension and *CYP1A2* genotype with caffeine intake may help to guide education on hypertension risk, and the symptom of a "fast or uneven heartbeat" may be a symptom to consider in future research.

A key strength of this research is the use of a novel data analysis technique, grouping genes together to establish links between the genes and caffeine consumption and response. To the

best of our knowledge, this is the first study to investigate these three genes in combination and report results using this visual grid approach. This study has several limitations. Firstly the sample size was too small to observe the combined impacts of the genes, with some gene carrier groups containing fewer than 10 participants. A larger sample size would have provided greater statistical power for more meaningful conclusions. Another consideration is the potential for participant recall error during completion of the CaffCo questionnaire, which is unavoidable when using a questionnaire format (Coughlin, 1990). This error could be compounded by differing interpretations of questions, resulting in the potential for wide variances in data accuracy. Estimation of caffeine intake may hold a further limitation, due to the large variances of caffeine content that can be found both within and between products (Crozier et al., 2012; Sivakumaran et al., 2017). Lastly, the element of *choice* to consume caffeine by the individual is an important variable that is difficult to account for in a cross-sectional study.

Future research is warranted to uncover meaningful relationships between these three genes and caffeine consumption and response. The present study and data analysis technique acts as a pilot and can be built upon with a larger sample size. It would be valuable for future research to consider the impact of age, ethnicity, and gender when looking into relationships with these genes on consumption and response, particularly *CYP1A2* and *AHR*, and take this into account when designing participant recruitment.

In conclusion, this study identified the genetic variation and caffeine consumption habits and responses in a NZ population. Approximately half of our study population consumed 80-400 mg/day caffeine, and our data identified an association between *ADORA2A* TT genotypes and decreased caffeine consumption. This finding is thought to be due to an increased vulnerability to anxiety post consumption, however the data did not reveal this connection. Anxiety experienced post consumption may be muted in the majority of our population due to regular consumption (≥40 mg/day) of caffeine, which may explain the lack of association in this study. These findings are encouraging for ongoing research and ultimately to advance our understanding of the role of dietary and pharmacological caffeine intake for health. From this work we can further delve into the reasons for decreased consumption in *ADORA2A* TT carriers and provide education for carriers as appropriate. Furthermore, providing education

on hypertension risk in “ultra-slow” metabolising (*CYP1A2* CC) caffeine consumers may be appropriate as evidence in this area increases. Continuing advances in knowledge on the impact of our genes on caffeine consumption and response will build towards informing personalised (genotype based) nutrition advice on caffeine use.

Acknowledgements

The study gratefully acknowledges the contribution of all participants. We would also like to thank Massey University for providing funding for the study.

Competing Interests

The authors declare no competing interests.

4 Conclusions and Recommendations

4.1 Aim of the Research

The present study aimed to investigate associations between genotypic variations in the *CYP1A2*, *ADORA2A*, and *AHR* genes on caffeine consumption habits and responses post-consumption, in individuals 15 years and older living in New Zealand. Investigating these relationships will enable a greater understanding of the genetic influences related to caffeine consumption and its effects, and improve understanding of the role of dietary and pharmacological caffeine in health by assessing the intake and post-consumption experiences of participants in our population group. To pursue this aim, participants completed a validated caffeine consumption habits questionnaire (CaffCo), and provided a saliva sample for genetic analysis for the three genes of interest.

4.2 Main Findings of the Study

The present study is the first to identify the occurrence of genotypes in the three genes *CYP1A2* (rs762551), *ADORA2A* (rs5751876), and *AHR* (rs4410790), in a NZ population. Half (49.4%) of the respondents carried *CYP1A2* AA and were classified as "fast" caffeine metabolisers, 40.4% were classified as "slow" metabolisers (CA), and 10.2% were classified as "ultra-slow" caffeine metabolisers (CC) . Within *ADORA2A*, heterozygous CT is most prominent (46.3%), followed by CC (29.0%), and TT (24.7%). Within *AHR*, half (51.8%) of the participants carried heterozygous CT, followed by CC (30.6%), and TT (17.6%) genotypes.

To understand the impact of caffeine consumption on individuals with differing genetic profiles, genotypic variances were first analysed individually relative to CaffCo data, to explore relationships between the genotypes of each of the three SNPs and caffeine intake and response. Both *ADORA2A* and *AHR* were then paired with *CYP1A2* to determine the combined impact of the rate of caffeine metabolism with post-caffeine intake responses and consumption patterns.

The main finding from the present study was the association between participants with the *ADORA2A* TT genotype and lower intake of caffeine compared to other genotypes, which is

consistent with previous research (Cornelis et al., 2007). It could not be demonstrated that the decreased caffeine consumption was due to an increased prevalence of post-consumption anxiety, as there was no association linking the *ADORA2A* TT genotype to prevalence of anxiety versus other *ADORA2A* genotypes (CC and CT), as previously reported (Alsene et al., 2003; Childs et al., 2008; Domschke et al., 2012). In contrast, we observed that "indicators of anxiety" were reported at higher frequencies for the other two *ADORA2A* genotypes (CC and CT). The majority of participants consumed ≥40 mg/day of caffeine, therefore a build-up of tolerance to caffeine's effects may partially explain the lack of an association between *ADORA2A* TT genotype and anxiety in our results (Harland, 2000; Rogers et al., 2010).

Within carriers of the *ADORA2A* TT genotype, no clear trends were observed between "fast" (*CYP1A2* AA) or "ultra-slow" (*CYP1A2* CC) caffeine metabolisers with respect to caffeine intake. There were no trends between caffeine intake and "fast" or "ultra-slow" metabolisers in the *CYP1A2* genotypes alone. While a previous study has reported associations between "fast" metabolisers (*CYP1A2* AA) and an increased caffeine intake among young men of Caucasian ethnicity (Denden et al., 2016), this observation may have been masked by the wide range of ethnicities and ages in the present study.

Being "unable to sleep" was the most common "adverse symptom", reported by 39% of participants post-caffeine consumption. This finding is expected given the antagonistic effect of caffeine on adenosine receptors, regardless of genotype (Ribeiro & Sebastião, 2010). No relationship was found between *ADORA2A* genotypes and sleep difficulties in the current study which contrasts with observations by Byrne et al. (2012) and Rétey et al. (2007) who reported that CC carriers experienced higher vulnerability to sleep difficulties. The phrasing of the CaffCo questionnaire item which asked participants if they had ever experienced being "unable to sleep" *shortly* after consumption may partially explain this lack of association. The interpretation of the "*shortly*" time frame may have differed among the participants, making it challenging to differentiate respondents who experienced being "unable to sleep" for short or extended periods after caffeine consumption.

Experiencing a “fast or uneven heartbeat” was reported mostly by “ultra-slow” or “slow” caffeine metabolisers. Although not a direct measure of blood pressure, experiencing feelings of change in heartbeat may be similar to previous observations where a higher risk of hypertension was associated with coffee intake in “ultra-slow” metabolisers (Palatini et al., 2009), and in keeping with the known rapid heartbeat experienced post caffeine consumption (Thomson & Schiess, 2011).

We found no relationship between caffeine intake and genotype variation in *AHR*, which contrasts with previous studies where an association between the CC genotype of *AHR* and increased caffeine consumption has been observed (Cornelis et al., 2011; Josse et al., 2012; Sulem et al., 2011). These studies included adults 49 years and over and supported observations from animal studies where AHR activity decreases with age (Harper et al., 2006). This suggests that genetic variations related to increased caffeine intake may be more pronounced in older adults (Josse et al., 2012). However, the present study only included five participants over 51 years of age across all genotypes and this may have limited the opportunity to test this association.

A further finding from this study was the differential preference for caffeine sources between gender. Tea, coffee, and chocolate was consumed by a higher proportion of female participants, whereas males were more likely to consume caffeine-containing sports supplements. Although this relationship was not explored in the present study, it is possible that investigating reasons for product choice and how these differ by gender may help direct targeting for caffeine related health messages towards the most appropriate audience.

In summary, the present study was able to identify the genetic variation present in a NZ population for *CYP1A2*, *ADORA2A* and *AHR*. A relationship between decreased caffeine consumption and *ADORA2A* TT genotype was observed. No further clear associations were found between caffeine intake and the genes *CYP1A2* or *AHR* on their own, nor the combined genotypes of *CYP1A2*, *ADORA2A*, and *AHR*. Additionally, no association was observed between “adverse symptom” responses post-consumption and genotype individually, nor paired with *CYP1A2*. Despite this, the findings of this study provide a template for future research to utilise the data analysis techniques adopted in this study (grouping genes together

in a visual grid format), to ascertain meaningful relationships within and between these genes.

4.3 Strengths and Limitations of the Study

4.3.1 Strengths

This was the first study to identify the frequency of genotypes in the three genes *CYP1A2*, *ADORA2A*, and *AHR*, in an NZ population. A second strength is the use of a novel data analysis technique, grouping genes together in a visual grid format, to allow for patterns in consumption and response to be distinguished between genotypes of two genes at once. In the present study, this was utilised in three different ways. Firstly, to view the impact of the rate of caffeine metabolism (*CYP1A2*) on the prevalence of post-consumption response symptoms (*ADORA2A*). Secondly, caffeine consumption data was analysed in this same grid to explore relationships between rate of metabolism, responses post-consumption, and corresponding caffeine intake. Lastly, *CYP1A2* was paired with *AHR* to view the combined impact of caffeine metabolism rate and differing transcriptional activation of *CYP1A2* by AHR on caffeine intake. This grid view technique was particularly valuable given the lack of significant associations in the overall data, allowing for trends and patterns to be observed to inform future research.

A further strength of this research was the methodology for data collection, including the use of the CaffCo questionnaire and saliva sample collection for genetic analysis. The CaffCo questionnaire allowed for a comprehensive analysis of consumption patterns relating to all major caffeine-containing products. This was unique given previous studies which have focused on coffee as a sole caffeine source (Cornelis et al., 2014; Denden, Bouden, Haj Khelil, Ben Chibani, & Hamdaoui, 2016; Guessous et al., 2012; Nordestgaard, Thomsen, & Nordestgaard, 2015; Rodenburg et al., 2012). A large dataset covering many aspects (reasons and environments for consumption, caffeine dependency, withdrawal and intoxication) was obtained from CaffCo and can be used in future research. For genetic analysis, using saliva collection rather than blood sampling is a less invasive technique, less costly, and due to lower participant burden, more people are likely to volunteer for the study.

4.3.2 Limitations

The sample size was a key limitation in this study and results need to be interpreted with caution. Previously, calculations reported in "The Caffeine Study" suggest that at least 382 ± 19 participants are required for adequate statistical power (Stachyshyn, 2017). When pairing the genes to observe the combined impact of caffeine metabolism (*CYP1A2*) on *ADORA2A* and *AHR* into nine groups, some groups in the present study were very small due to the frequency of the minor genotype in each gene (i.e. only 26 participants (10.2%) carry *CYP1A2* CC). Favourably, the information gathered on genetic variation in this study population can now be used to calculate a more accurate statistical power calculation for future research.

Increased specificity in the CaffCo questionnaire may help to overcome limitations in interpretation of questions between participants. A key example arose with differentiating participants' experience of sleep difficulties post consumption. It was expected that many participants would experience sleep difficulties shortly after caffeine consumption (Ribeiro & Sebastião, 2010), which was the phrasing used in CaffCo. However, previous research linked to sleep difficulties in carriers of *ADORA2A* CC genotype has focused on associations with difficulty sleeping for extended time periods post consumption (Byrne et al., 2012; Rétey et al., 2007). Making this distinction in the questionnaire may allow for more significant differentiation between sleep experiences of *ADORA2A* CC carriers compared to TT and CT.

The CaffCo questionnaire was designed to identify patterns and influences for caffeine consumption across various caffeinated products, however in this format there is always the potential for differing interpretations of questionnaire items, an inherent difficulty with subjective responses. This challenge has been previously highlighted by Temple et al. (2015), where subjective responses by gender are observed, with the direction of change being dependent on the question being asked. A further limitation of questionnaires is the potential for participant recall error (Coughlin, 1990). The combination of these limitations results in the potential for variances in data accuracy.

Estimating caffeine intake can be challenging as a result of participant recall and different interpretation of the questionnaire items. Although CaffCo prompts for consumption information on multiple sources and options (e.g. different sized products available), the

necessity for large amounts of detail in the questionnaire may confuse respondents and result in an over or under reporting of caffeine intake. Accurate dietary recall is dependent on many factors, including an individual's mood at the time of questioning, the importance they place on the information, memory, intelligence and age (Krall, Dwyer, & Ann Coleman, 1988). Analysing the resulting participant intake data also poses a challenge owing to the range of caffeine amounts (mg) that can be found in a given product (e.g. caffeine content will vary in a cup of coffee made on a different day or by a different person). The variance between caffeine-containing products (e.g. instant coffee and espresso coffee), also adds to the specificity of the details that participants need to recall for accurate data to be obtained (Crozier et al., 2012; Sivakumaran et al., 2017).

Although each of the major ethnic groups in NZ were represented in the study (European, Māori, Pacific, Asian, Middle Eastern / Latin American / African; Statistics New Zealand, 2014), the results of the present study are not generalisable to the NZ population, as the distribution of ethnicities does not mirror the NZ population (**Supplementary Figure 1**). In addition, previous research has demonstrated differences between ethnicities in caffeine clearance (*CYP1A2*; Denden et al., 2016; Gunes & Dahl, 2008) as well as food and beverage choices (Devine, Sobal, Bisogni, & Connors, 1999). The sample size in this study was not large enough to explore these potential differences between ethnicities, and this would be valuable to investigate in future research.

4.4 Recommendations for Further Research

In order to better understand the impact of *CYP1A2*, *ADORA2A*, and *AHR* on caffeine consumption and responses, future research is warranted. The present study and data analysis technique employed can act as a pilot for future studies with a larger sample size.

Recommendations for future work include:

- Based on genetic variation found in the current study, a new statistical power calculation should be considered to determine optimal participant recruitment size. Within the sample, it would be valuable to consider the specific cohort in relation to previous studies, specifically with regards to age, ethnicity, and gender, in order to

clearly identify results in line with previous research. This is particularly relevant for the *CYP1A2* and *AHR* genes:

  - Caffeine clearance (CYP1A2) has been shown to differ between ethnicities and age, with "fast" metabolisers (*CYP1A2* AA) having increased consumption, particularly in men, those of younger age, and of Caucasian ethnicity (Denden et al., 2016).
  - Animal studies have demonstrated a decline in AHR activity with age (Harper et al., 2006), and previous studies have found significant relationships between *AHR* and caffeine consumption only in older adults (Cornelis et al., 2011; Josse et al., 2012; Sulem et al., 2011).

- Increased data specificity could be gained by making minor adjustments to some questions in the CaffCo questionnaire, particularly with differentiating participants' experiences of sleep difficulties post caffeine consumption. One way to do this may be to include a question asking *"have you attributed difficulty sleeping with caffeine consumed earlier in the day?*" which could lead to a choice of time ranges (e.g. 1-3 hours, 4-6 hours, 7-9 hours, 10 hours +). It would also be beneficial to ask participants who have reported experiencing sleeping difficulty *"do you alter your caffeine consumption to avoid this effect*?" in order to assess the impact on behaviour in response to this effect.
- Genetic variance in other SNPs within the *CYP1A2*, *ADORA2A*, and *AHR* genes could also be explored. For example, *CYP1A2* SNP rs2472297, given the body of evidence that has found links between this SNP and caffeine consumption amounts (Amin et al., 2012; Josse et al., 2012; Sulem et al., 2011).
- Differences in caffeine source preference between genders could also be further explored. The present study found these differences to be statistically significant, but given this was not linked to genetic variations, was not further explored in this study. Investigating the reasons for product choice and consumption may help direct any future public health messages on caffeine to the most appropriate audiences.
- Finally, there is a vast range of data gathered from CaffCo that can be utilised in future research, including data describing both reasons and environments for caffeine

consumption, caffeine dependency, withdrawal and intoxication, as well as further detail on participants' experiences of the effects of caffeine. Exploring this data will further advance our understanding of caffeine-related behaviour and responses in New Zealand.

Further research is needed to build on the findings in this study to advance understandings of the role of dietary and pharmacological caffeine intake on health outcomes. This can better help inform public health messages to enable people to make better informed decisions on their own caffeine intake.

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Appendices

Appendix A: Caffeine Consumption Habits Questionnaire: CaffCo

CaffCo

Start of Block: 1- Introduction

Q1.1
CaffCo -
Caffeine Consumption Habits Questionnaire

Thank you for taking the time to complete this questionnaire.

This questionnaire examines the influences on consumption of various caffeine-containing beverages and foods found in New Zealand. The questionnaire has been designed to be completed by people aged 15 years and over.

Data collected from this questionnaire is confidential.

Further information can be found in the information sheet below; please read this before continuing with the questionnaire.

The questionnaire will take 10-20 minutes to complete.

Q1.2 What age group do you fit into?

- ○ 14 years or under (1)
- ○ 15 years old (2)
- ○ 16-18 years old (3)
- ○ 19-30 years old (4)
- ○ 31-50 years old (5)
- ○ 51-70 years old (6)
- ○ 71 years or over (7)

Q1.3 Click to write the question text

Q1.4 I have read and understand the information sheet provided and agree to participate in the study under the terms laid out in the information sheet.

- ○ Yes (1)
- ○ No (2)

End of Block: 1- Introduction

Start of Block: 2 - 15 yo

Q2.1 Thank you for expressing your interest to participate in this survey.

Due to your age, you will need parental consent to participate in this study.

Please read and sign the forms attached below, then email them back to caffeinestudy@outlook.co.nz; we will then forward you the questionnaire as well as let you know times, locations and dates for saliva collection.

Q2.2 Click to write the question text

Q2.3 Click to write the question text

End of Block: 2 - 15 yo

Start of Block: 3 - ID number block

Q3.1 Please enter your study ID number

_____

Q3.2 Have you provided a saliva sample on location?

- Yes (1)
- No (3)

End of Block: 3 - ID number block

Start of Block: 4- Screening questions

Q4.1 Which of these items do you drink / eat? Include those that you only consume occasionally.

- ☐ Tea (black / green) (1)
- ☐ Coffee (2)
- ☐ Chocolate (3)
- ☐ Kola flavoured drinks (e.g. Coke cola, Pepsi etc) (4)
- ☐ Energy drinks / energy shots (5)
- ☐ Premixed caffeinated alcoholic RTDs with a kola drink base (e.g. rum and kola) or with added caffeine / guarana (6)
- ☐ Caffeinated pre-workout sports supplements and sports gels (7)
- ☐ Caffeine Tablets (e.g. No Doz) (8)
- ☐ ⊗ None one of the above (9)

Q4.2 What is your gender?

- ○ Male (1)
- ○ Female (2)
- ○ Other (3)

End of Block: 4- Screening questions

Start of Block: 5 -Tea

Q5.1 How often do you drink the following types of tea (on average)?

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
Green tea (1 cup) (Q5.1_1)	○	○	○	○	○	○	○	○	○	○
Black tea with or without milk (1 cup) (Q5.1_2)	○	○	○	○	○	○	○	○	○	○
Iced tea (1 glass) (Q5.1_3)	○	○	○	○	○	○	○	○	○	○
Decaffeinated tea (1 cup) (Q5.1_4)	○	○	○	○	○	○	○	○	○	○

Q5.2 Think about **your own reasons** for drinking tea.

Read the following statements about the different reasons for tea consumption and consider whether you 'agree', 'strongly agree', 'disagree', 'strongly disagree'.

I drink tea...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- because it is cheaper than other hot drinks (Q5.2_1)	○	○	○	○
- because it is what I drink with food (Q5.2_2)	○	○	○	○
- to comfort and relax myself (Q5.2_3)	○	○	○	○
- for the warmth (Q5.2_4)	○	○	○	○
- for the taste (Q5.2_5)	○	○	○	○
- with friends (Q5.2_6)	○	○	○	○
- whenever it is offered to me (Q5.2_7)	○	○	○	○
- for mental energy (Q5.2_8)	○	○	○	○
- with family (Q5.2_9)	○	○	○	○
- out of boredom (Q5.2_10)	○	○	○	○
- because I feel I am influenced by peer pressure (Q5.2_11)	○	○	○	○
- out of habit (Q5.2_12)	○	○	○	○
- when I am stressed (Q5.2_13)	○	○	○	○
- because I feel that I am influenced by advertising (Q5.2_14)	○	○	○	○
- because it is easily available (Q5.2_15)	○	○	○	○

- to wake up (Q5.2_16)	○	○	○	○
- because others are drinking it (Q5.2_17)	○	○	○	○
- as my culture influences me to drink it (Q5.2_18)	○	○	○	○
- for energy (Q5.2_19)	○	○	○	○
- when I have had enough coffee for the day (Q5.2_20)	○	○	○	○
- to replace food or meals (Q5.2_21)	○	○	○	○
- while traveling (Q5.2_22)	○	○	○	○
- because I think coffee has too much caffeine in it (Q5.2_23)	○	○	○	○

Q5.3 What time of day do you drink tea? Choose all options that apply to you.

- ☐ Before breakfast (1)
- ☐ At breakfast time (2)
- ☐ Between breakfast and lunch (3)
- ☐ At lunch time (4)
- ☐ Between lunchtime and dinner (5)
- ☐ At dinner time (6)
- ☐ After dinner (7)
- ☐ ⊗ All day (8)
- ☐ ⊗ At no particular time (9)

Q5.4 In which environments do you drink tea? Select all that apply.

- ☐ A home environment (your own or others) (1)
- ☐ A socialising environment (2)
- ☐ A work environment (3)
- ☐ A cafe environment (4)
- ☐ A study environment (5)
- ☐ Other (please specify) (6) _____

End of Block: 5 -Tea

Start of Block: 6- Coffee

Q6.1 How often do you drink the following types of coffee (on average)?

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
Instant coffee (made with 1 teaspoon coffee powder) (Q6.1_1)	○	○	○	○	○	○	○	○	○	○
Plunger / drip coffee (1 medium cup - 250ml) (Q6.1_2)	○	○	○	○	○	○	○	○	○	○
Small espresso coffee (single shot) (Q6.1_3)	○	○	○	○	○	○	○	○	○	○
Large espresso coffee (double shot) (Q6.1_4)	○	○	○	○	○	○	○	○	○	○
Decaffeinated coffee (1 cup) (Q6.1_5)	○	○	○	○	○	○	○	○	○	○
Iced coffee (1 glass) (Q6.1_6)	○	○	○	○	○	○	○	○	○	○

Q6.2 Think about **your o**wn reasons for drinking coffee.

Read the following statements about the different reasons for coffee consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I drink coffee ...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- because it is easily available (Q6.2_1)	○	○	○	○
- out of boredom (Q6.2_2)	○	○	○	○
- as a treat or luxury drink (Q6.2_3)	○	○	○	○
- because it is what I drink with food (Q6.2_4)	○	○	○	○
- to comfort and relax myself (Q6.2_5)	○	○	○	○
- for the warmth (Q6.2_6)	○	○	○	○
- for the taste (Q6.2_7)	○	○	○	○
- with friends (Q6.2_8)	○	○	○	○
- whenever it is offered to me (Q6.2_9)	○	○	○	○
- because others are drinking it (Q6.2_10)	○	○	○	○
- while traveling (Q6.2_11)	○	○	○	○
- with family (Q6.2_12)	○	○	○	○
- when I am stressed (Q6.2_13)	○	○	○	○
- while driving long distances (Q6.2_14)	○	○	○	○
- for physical energy (Q6.2_15)	○	○	○	○
- for mental energy (Q6.2_16)	○	○	○	○

- for energy (Q6.2_17)	○	○	○	○
- because I feel I am influenced by peer pressure (Q6.2_18)	○	○	○	○
- because I feel that I am influenced by advertising (Q6.2_19)	○	○	○	○
- out of habit (Q6.2_20)	○	○	○	○
- as my culture influences me to drink it (Q6.2_21)	○	○	○	○
- to stay awake (Q6.2_22)	○	○	○	○
- to wake up (Q6.2_23)	○	○	○	○
- to replace food or meals (Q6.2_24)	○	○	○	○
- when I am smoking (Q6.2_25)	○	○	○	○

Q6.3 What time of day do you drink coffee? Choose all options that apply to you.

- ☐ Before breakfast (1)
- ☐ At breakfast time (2)
- ☐ Between breakfast and lunch (3)
- ☐ At lunch time (4)
- ☐ Between lunchtime and dinner (5)
- ☐ At dinner time (6)
- ☐ After dinner (7)
- ☐ ⊗ All day (8)
- ☐ ⊗ At no particular time (9)

Q6.4 In which environments do you drink coffee? Select all that apply.

- ☐ A home environment (your own or others) (1)
- ☐ A cafe environment (2)
- ☐ A work environment (3)
- ☐ A study environment (4)
- ☐ A socialising environment (5)
- ☐ A physical exercise environment (6)
- ☐ Other (please specify) (7) _____

End of Block: 6- Coffee

Start of Block: 7- Decaffeinated block

Q7.1 Think about **your own reasons** for drinking **decaffeinated** coffee / tea instead of regular coffee / tea.

Read the following statements about the different reasons for consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I drink decaffeinated coffee / tea ...

	Strongly agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- when I feel that I have had enough regular coffee / tea for the day (Q7.1_1)	○	○	○	○
- because I do not want the caffeine in regular coffee / tea (Q7.1_2)	○	○	○	○
- because it is offered to me (Q7.1_3)	○	○	○	○
- because I can't tolerate the caffeine in regular coffee / tea (Q7.1_4)	○	○	○	○
- for medical reasons (Q7.1_5)	○	○	○	○
- because I prefer the taste of decaffeinated coffee / tea compared to regular (Q7.1_6)	○	○	○	○
Other (please specify) (Q7.1_7)	○	○	○	○

End of Block: 7- Decaffeinated block

Start of Block: 8- Chocolate

X→

Q8.1 How often do you eat the following types of chocolate (on average)?

The pictures below include some examples of products, chose the one closest to what you consume.

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
Milk Chocolate small bar (50g) (Q8.1_1)	○	○	○	○	○	○	○	○	○	○
Milk Chocolate large block (200-250g) (Q8.1_2)	○	○	○	○	○	○	○	○	○	○
Dark Chocolate small bar (50g) (Q8.1_3)	○	○	○	○	○	○	○	○	○	○
Dark Chocolate large block (200-250g) (Q8.1_4)	○	○	○	○	○	○	○	○	○	○
Hot chocolate (1 medium cup) (Q8.1_5)	○	○	○	○	○	○	○	○	○	○

Q8.2 Think about **your own reasons** for eating chocolate.

Read the following statements about the different reasons for chocolate consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I eat chocolate ...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- to comfort and relax myself (Q8.2_1)	○	○	○	○
- for the taste (Q8.2_2)	○	○	○	○
- more when I am on my period (Q8.2_3)	○	○	○	○
- as a treat or luxury food (Q8.2_4)	○	○	○	○
- because I feel that I am influenced by advertising (Q8.2_5)	○	○	○	○
- with friends (Q8.2_6)	○	○	○	○
- with family (Q8.2_7)	○	○	○	○
- because it is already in many of the foods that I eat (Q8.2_8)	○	○	○	○
- for the warmth (drinking chocolate) (Q8.2_9)	○	○	○	○
- because I feel I am influenced by peer pressure (Q8.2_10)	○	○	○	○
- while traveling (Q8.2_11)	○	○	○	○
- to replace other food or meals (Q8.2_12)	○	○	○	○
- whenever it is offered to me (Q8.2_13)	○	○	○	○
- out of boredom (Q8.2_14)	○	○	○	○

- when I am stressed (Q8.2_15)	○	○	○	○
- because others are eating it (Q8.2_16)	○	○	○	○
- out of habit (Q8.2_17)	○	○	○	○
- because it is easily available (Q8.2_18)	○	○	○	○

Q8.3 What time of day do you eat chocolate? Choose all options that apply to you.

- ☐ Before breakfast (1)
- ☐ At breakfast time (2)
- ☐ Between breakfast and lunch (3)
- ☐ At lunch time (4)
- ☐ Between lunchtime and dinner (5)
- ☐ At dinner time (6)
- ☐ After dinner (7)
- ☐ ⊗ All day (8)
- ☐ ⊗ At no particular time (9)

Q8.4 Which pattern of eating chocolate describes your own?

You may choose more than one option

- ☐ I regularly eat a large amount of chocolate at one time (1)
- ☐ I regularly eat small amounts of chocolate (2)
- ☐ I occasionally eat a large amount of chocolate all at one time (3)
- ☐ I occasionally eat small amounts of chocolate (4)
- ☐ Other (please specify) (5) _____

Q8.5 In which environments do you eat chocolate? Select all that apply.

- ☐ A home environment (your own or others) (1)
- ☐ A cafe environment (2)
- ☐ A work environment (3)
- ☐ A socialising environment (4)
- ☐ A study environment (5)
- ☐ Other (please specify) (6) _____

End of Block: 8- Chocolate

Start of Block: 9- Kola Flavoured Drinks

Q9.1 How often do you drink the following types of kola-flavoured drinks (on average)?

This includes brands such as Coca-Cola, Pepsi and other brands of kola-flavoured drinks.

'Diet', 'Zero', 'Max' varieties are included in their own category below ('diet'), rather than with 'regular' kola drinks.

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
1 glass of regular kola drink (250ml) (Q9.1_1)	○	○	○	○	○	○	○	○	○	○
1 can of regular kola drink (355ml) (Q9.1_2)	○	○	○	○	○	○	○	○	○	○
1 small bottle of regular kola drink (600ml) (Q9.1_3)	○	○	○	○	○	○	○	○	○	○
1 glass of DIET / ZERO / MAX kola drink (250ml) (Q9.1_4)	○	○	○	○	○	○	○	○	○	○
1 can of DIET / ZERO / MAX kola drink (355ml) (Q9.1_5)	○	○	○	○	○	○	○	○	○	○
1 small bottle of DIET / ZERO / MAX kola drink (600ml) (Q9.1_6)	○	○	○	○	○	○	○	○	○	○

Q9.2 Think about **your o**wn reasons for drinking kola drinks (both regular and diet).

Read the following statements about the different reasons for coffee consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I drink kola drinks (both regular and diet) ...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- because they are cheaper than other drinks (Q9.2_1)	○	○	○	○
- because is the drink I have with meals (Q9.2_2)	○	○	○	○
- because it is cold and refreshing (Q9.2_3)	○	○	○	○
- for the taste (Q9.2_4)	○	○	○	○
- with friends (Q9.2_5)	○	○	○	○
- out of habit (Q9.2_6)	○	○	○	○
- to replace food or meals (Q9.2_7)	○	○	○	○
- for the bubbles / how it feels in my mouth (Q9.2_8)	○	○	○	○
- while traveling (Q9.2_9)	○	○	○	○
- when I am stressed (Q9.2_10)	○	○	○	○
- whenever it is offered to me (Q9.2_11)	○	○	○	○
- for energy (Q9.2_12)	○	○	○	○
- because they are easily available (Q9.2_13)	○	○	○	○
- out of boredom (Q9.2_14)	○	○	○	○
- instead of coffee when the weather is hot (Q9.2_15)	○	○	○	○
- instead of alcohol (Q9.2_16)	○	○	○	○

- because others are drinking it (Q9.2_17)	○	○	○	○
- with family (Q9.2_18)	○	○	○	○
- as a treat drink (Q9.2_19)	○	○	○	○
- as a mixer for alcohol (Q9.2_20)	○	○	○	○
- with takeaway food (Q9.2_21)	○	○	○	○
- because I feel that I am influenced by advertising (Q9.2_22)	○	○	○	○
- because I feel I am influenced by peer pressure (Q9.2_23)	○	○	○	○

Q9.3 What time of day do you drink kola drinks (both regular and diet)? Choose all options that apply to you.

- ☐ Before breakfast (1)
- ☐ At breakfast time (2)
- ☐ Between breakfast and lunch (3)
- ☐ At lunch time (4)
- ☐ Between lunchtime and dinner (5)
- ☐ At dinner time (6)
- ☐ After dinner (7)
- ☐ ⊗ All day (8)
- ☐ ⊗ At no particular time (9)

Q9.4 In which environments do you drink kola drinks (both regular and diet)? Select all that apply.

- ☐ A home environment (your own or others) (1)
- ☐ A cafe environment (2)
- ☐ A work environment (3)
- ☐ A party environment (4)
- ☐ A study environment (5)
- ☐ A physical exercise environment (6)
- ☐ A bar environment (7)
- ☐ A socialising environment (8)
- ☐ Other (please specify) (9) _____

End of Block: 9- Kola Flavoured Drinks

Start of Block: 10- Energy Drinks / Energy Shots

Q10.1
Energy drinks include brands such as Red Bull, V, Mother, Monster Energy and others.

Q10.2 How often do you drink the following types of energy drinks (on average)?

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
1 energy shot (Q10.2_1)	○	○	○	○	○	○	○	○	○	○
1 small can of energy drink (250ml) (Q10.2_2)	○	○	○	○	○	○	○	○	○	○
1 small bottle of energy drink (350ml) (Q10.2_3)	○	○	○	○	○	○	○	○	○	○
1 large can / bottle of energy drink (500ml) (Q10.2_4)	○	○	○	○	○	○	○	○	○	○

Q10.3 Think about **your o**wn reasons for drinking energy drinks.

Read the following statements about the different reasons for coffee consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I drink energy drinks ...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- because they are cold and refreshing (Q10.3_1)	○	○	○	○
- for the taste (Q10.3_2)	○	○	○	○
- because I feel I am influenced by peer pressure (Q10.3_3)	○	○	○	○
- out of habit (Q10.3_4)	○	○	○	○
- for physical energy (Q10.3_5)	○	○	○	○
- while driving long distances (Q10.3_6)	○	○	○	○
- with family (Q10.3_7)	○	○	○	○
- for energy (Q10.3_8)	○	○	○	○
- whenever one is offered to me (Q10.3_9)	○	○	○	○
- out of boredom (Q10.3_10)	○	○	○	○
- with takeaway food (Q10.3_11)	○	○	○	○
- to improve physical performance (Q10.3_12)	○	○	○	○
- for mental energy (Q10.3_13)	○	○	○	○
- instead of alcohol (Q10.3_14)	○	○	○	○
- as a mixer for alcohol (Q10.3_15)	○	○	○	○

- when I am stressed (Q10.3_16)	○	○	○	○
- because others are drinking it (Q10.3_17)	○	○	○	○
- because I feel that I am influenced by advertising (Q10.3_18)	○	○	○	○
- to replace food or meals (Q10.3_19)	○	○	○	○
- with friends (Q10.3_20)	○	○	○	○
- while traveling (Q10.3_21)	○	○	○	○
- while smoking (Q10.3_22)	○	○	○	○
- with takeaway food (Q10.3_23)	○	○	○	○
- to stay awake (Q10.3_24)	○	○	○	○
- to wake me up (Q10.3_25)	○	○	○	○
- because they are easily available (Q10.3_26)	○	○	○	○
- because it is the drink I have with food (Q10.3_27)	○	○	○	○

Q10.4 What time of day do you drink energy drinks? Choose all options that apply to you.

- ☐ Before breakfast (1)
- ☐ At breakfast time (2)
- ☐ Between breakfast and lunch (3)
- ☐ At lunch time (4)
- ☐ Between lunchtime and dinner (5)
- ☐ At dinner time (6)
- ☐ After dinner (7)
- ☐ ⊗ All day (8)
- ☐ ⊗ At no particular time (9)

Q10.5 In which environments do you drink energy drinks? (Select all that apply)

- ☐ A home environment (your own or others) (1)
- ☐ A cafe environment (2)
- ☐ A work environment (3)
- ☐ A party environment (4)
- ☐ A physical exercise environment (5)
- ☐ A socialising environment (6)
- ☐ A study environment (7)
- ☐ A bar environment (8)
- ☐ Other (please specify) (9) _____

End of Block: 10- Energy Drinks / Energy Shots

Start of Block: 11- RTDs

Q11.1 Caffeinated alcoholic RTDs are premixed alcoholic drinks with either a **kola base** (e.g. Jack Daniels, Jim Beam, Woodstock, Coruba and kola etc) or with **added caffeine or guarana** (e.g. some Smirnoff Ice, Purple Goanna).

The pictures below include some examples of products, however there may be products not pictured. Chose the one closest to what you consume.

Q11.2 How often do you drink caffeinated RTDs (on average)?

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
1 RTD can (250-330ml) (Q11.2_1)	○	○	○	○	○	○	○	○	○	○
1 RTD bottle (330 - 350ml) (Q11.2_2)	○	○	○	○	○	○	○	○	○	○

Q11.3 Think about **your own reasons** for drinking Caffeinated RTDs.

Read the following statements about the different reasons for coffee consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I drink caffeinated RTDs ...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- because they are cold and refreshing (Q11.3_1)	○	○	○	○
- for the taste (Q11.3_2)	○	○	○	○
- for the alcohol content (Q11.3_3)	○	○	○	○
- because I feel I am influenced by peer pressure (Q11.3_4)	○	○	○	○
- out of habit (Q11.3_5)	○	○	○	○
- because I know how much alcohol is in them (Q11.3_6)	○	○	○	○
- whenever one is offered to me (Q11.3_7)	○	○	○	○
- out of boredom (Q11.3_8)	○	○	○	○
- when I am stressed (Q11.3_9)	○	○	○	○
- to replace food or meals (Q11.3_10)	○	○	○	○
- to stay awake (Q11.3_11)	○	○	○	○
- for energy (Q11.3_12)	○	○	○	○
- because I feel that I am influenced by advertising (Q11.3_13)	○	○	○	○
- because others are drinking them (Q11.3_14)	○	○	○	○

- because they are easy to transport (Q11.3_15)	○	○	○	○
- with friends (Q11.3_16)	○	○	○	○
- while traveling (Q11.3_17)	○	○	○	○
- with family (Q11.3_18)	○	○	○	○
- for physical energy (Q11.3_19)	○	○	○	○
- because they are cheaper than other alcoholic drinks (Q11.3_20)	○	○	○	○
- instead of spirits (Q11.3_21)	○	○	○	○
- to comfort and relax me (Q11.3_22)	○	○	○	○

Q11.4 What time of day do you drink RTDs? Choose all options that apply to you.

- ☐ Before breakfast (1)
- ☐ At breakfast time (2)
- ☐ Between breakfast and lunch (3)
- ☐ At lunch time (4)
- ☐ Between lunchtime and dinner (5)
- ☐ At dinner time (6)
- ☐ After dinner (7)
- ☐ ⊗ All day (8)
- ☐ ⊗ At no particular time (9)

Q11.5 In which environments do you drink caffeinated RTDs? (Select all that apply)

- ☐ A home environment (1)
- ☐ A party environment (2)
- ☐ A bar environment (3)
- ☐ A socialising environment (4)
- ☐ Other (please specify) (5)

_____

End of Block: 11- RTDs

Start of Block: 12- Caffeinated Pre-workout Sports Supplements and Sports Gels

Q12.1 How often do you take caffeinated pre-workout sports supplements or sports gels (on average)?

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
Pre-workout sports supplements (Q12.1_1)	○	○	○	○	○	○	○	○	○	○
Sports gels (Q12.1_2)	○	○	○	○	○	○	○	○	○	○

Q12.2 Think about **your own reasons** for using sports supplements.

Read the following statements about the different reasons for sports supplement consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I take sports supplements ...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- for physical energy (Q12.2_1)	○	○	○	○
- because I feel that I am influenced by advertising (Q12.2_2)	○	○	○	○
- because of peer pressure (Q12.2_3)	○	○	○	○
- because of pressure from coaches / trainers (Q12.2_4)	○	○	○	○
- to improve physical performance (Q12.2_5)	○	○	○	○
- as they are convenient to take (Q12.2_6)	○	○	○	○
- as a substitute for illegal drugs (Q12.2_7)	○	○	○	○
- while traveling (Q12.2_8)	○	○	○	○
- for energy (Q12.2_9)	○	○	○	○
- to replace food or meals (Q12.2_10)	○	○	○	○
- because they are easy to transport (Q12.2_11)	○	○	○	○
- because others are using them (Q12.2_12)	○	○	○	○
- out of habit (Q12.2_13)	○	○	○	○
- with friends (Q12.2_14)	○	○	○	○

Q12.3 The following is a list of different types of physical activities. Indicate if you take pre workout supplements or sports gels in any of the following environments (select as many or as little as you like)

	Pre workout supplements (1)	Sports gels (2)	I am not involved in this type of activity / do not use these (3)
Recreational individual sports (Q12.3_1)	☐	☐	☐
Recreational team sports (Q12.3_2)	☐	☐	☐
Competitive individual sports (Q12.3_3)	☐	☐	☐
Competitive team sports (Q12.3_4)	☐	☐	☐
Resistance / weight training (Q12.3_5)	☐	☐	☐
Endurance training (e.g. for triathlons, marathons) (Q12.3_6)	☐	☐	☐
Other (please specify) (Q12.3_7)	☐	☐	☐

Q12.4 In which environments do take caffeinated pre-workout sports supplements or sports gels? (Select all that apply)

- ☐ A party environment (1)
- ☐ A physical exercise environment (2)
- ☐ A socialising environment (3)
- ☐ Other (please specify) (4) _____

End of Block: 12- Caffeinated Pre-workout Sports Supplements and Sports Gels

Start of Block: 13- Caffeine Tablets

Q13.1 Caffeine tablets in include No Doz, Thermo, AllMax, Caffeine Pro, Inner Amour and others.

Q13.2 How often do you take caffeine tablets (on average)?

	Never (1)	Less than once a month (2)	1-3 times a month (3)	Once a week (4)	2-4 times a week (5)	5-6 times a week (6)	Once a day (7)	2-3 times a day (8)	4-5 times a day (9)	6+ times a day (10)
1 caffeine tablet containing 50mg of caffeine (e.g. Pro Plus) (Q13.2_1)	○	○	○	○	○	○	○	○	○	○
1 caffeine tablet containing 100mg of caffeine (e.g. No Doz) (Q13.2_2)	○	○	○	○	○	○	○	○	○	○
1 caffeine tablet containing 200mg caffeine (e.g. Thermo, AllMax, Myprotein Caffeine Pro, Inner Armor etc) (Q13.2_3)	○	○	○	○	○	○	○	○	○	○
Other (please specify) (Q13.2_4)	○	○	○	○	○	○	○	○	○	○

Q13.3 Think about **your own reasons** for using caffeine tablets.

Read the following statements about the different reasons for coffee consumption and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

I take caffeine tablets ...

	Strongly Agree (1)	Agree (2)	Disagree (3)	Strongly Disagree (4)
- for physical energy (Q13.3_1)	○	○	○	○
- because I feel I am influenced by peer pressure (Q13.3_2)	○	○	○	○
- because of pressure from coaches / trainers (Q13.3_3)	○	○	○	○
- as they are convenient to take (Q13.3_4)	○	○	○	○
- to replace food or meals (Q13.3_5)	○	○	○	○
- to wake up (Q13.3_6)	○	○	○	○
- to improve physical performance (Q13.3_7)	○	○	○	○
- for energy (Q13.3_8)	○	○	○	○
- as a substitute for illegal drugs (Q13.3_9)	○	○	○	○
- while traveling (Q13.3_10)	○	○	○	○
- because others are using them (Q13.3_11)	○	○	○	○
- for mental energy (Q13.3_12)	○	○	○	○
- while driving long distances (Q13.3_13)	○	○	○	○
- because I feel that I am influenced by advertising (Q13.3_14)	○	○	○	○

- to stay awake (Q13.3_15)	○	○	○	○
- with friends (Q13.3_16)	○	○	○	○

Q13.4 In which environments do you drink take caffeine tablets?

- ☐ A work environment (1)
- ☐ A party environment (2)
- ☐ A physical exercise environment (3)
- ☐ A study environment (4)
- ☐ A socialising environment (5)
- ☐ Other (please specify) (6) _____

End of Block: 13- Caffeine Tablets

Start of Block: 14- Question on why using food to replace products

Q14.1
When I use these products to replace food or meals, I do it because...

	Tea (1)	Coffee (2)	Drinking chocolate (3)	Eating chocolate (4)	Kola drinks (regular and diet) (5)	Energy drinks (6)	Caffeinated RTDs (7)	Pre-workout supplements / sports gels (8)	Caffeine tablets (9)
I want to lose weight (Q14.1_1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
It is cheaper than food (Q14.1_2)	☐	☐	☐	☐	☐	☐	☐	☐	☐
I did not prepare / organise food (Q14.1_3)	☐	☐	☐	☐	☐	☐	☐	☐	☐
It is more easily accessible than food (Q14.1_4)	☐	☐	☐	☐	☐	☐	☐	☐	☐
I am not hungry or do not feel like eating (Q14.1_5)	☐	☐	☐	☐	☐	☐	☐	☐	☐
I enjoy the product more than food (Q14.1_6)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q14.2 Are there any other reasons that you use these products to replace food or meals?

- ○ No (1)
- ○ Yes (please specify product and reason) (2)

_____

End of Block: 14- Question on why using food to replace products

Start of Block: 15- Withdrawals and Intoxication

Q15.1

Have you ever felt dependent on any of the following products?

For example - you have felt that you needed them to 'feel normal' or to 'get through the day'.

- ☐ Tea (1)
- ☐ Coffee (2)
- ☐ Chocolate (3)
- ☐ Kola-flavoured drinks (both regular and diet) (4)
- ☐ Energy drinks / energy shots (5)
- ☐ Caffeinated RTDs (6)
- ☐ Caffeinated pre workout sports supplements / sports gels (7)
- ☐ Caffeine tablets (8)
- ☐ ⊗ No, I have never felt dependent on any of these products (9)

Q15.2 Think about your consumption of the caffeinated products that have been explored.

Have you ever experienced any of the following symptoms ***within one day of stopping their normal use***?

Please tick all options that apply to you.

- ☐ Headaches (1)
- ☐ Mood changes (e.g.. depressed mood, easily annoyed) (2)
- ☐ Marked tiredness or drowsiness (3)
- ☐ Difficulty concentrating (4)
- ☐ 'Flu like' feelings (e.g.. nausea, vomiting, muscle pain, stiffness) (5)
- ☐ ⊗ No, I have never experienced any of these (6)
- ☐ Other (please specify) (7) _____

Q15.3 With which products did these symptoms occur (when you stopped consuming them)? Select as many options as apply.

	Headaches (1)	Mood changes (2)	Marked tiredness / drowsiness (3)	Difficulty concentrating (4)	'Flu-like' feelings (5)	Other (6)
Tea (Q15.3_1)	☐	☐	☐	☐	☐	☐
Coffee (Q15.3_2)	☐	☐	☐	☐	☐	☐
Chocolate (Q15.3_3)	☐	☐	☐	☐	☐	☐
Kola-flavoured drinks (Q15.3_4)	☐	☐	☐	☐	☐	☐
Energy drinks / shots (Q15.3_5)	☐	☐	☐	☐	☐	☐
Caffeinated RTDs (Q15.3_6)	☐	☐	☐	☐	☐	☐
Caffeinated sports supplements / sports gels (Q15.3_7)	☐	☐	☐	☐	☐	☐
Caffeine tablets (Q15.3_8)	☐	☐	☐	☐	☐	☐

Q15.4 Did these negative effects impact on your social life, work life or cause you any kind of distress?

- ○ Yes (1)
- ○ No (2)

Q15.5 Again, think of your experiences with the caffeinated products that have been explored.

Shortly after consuming them, have you ever felt any of these effects?

Please tick all options that apply to you.

- ☐ Restless (1)
- ☐ Nervous (2)
- ☐ Excited (3)
- ☐ Unable to sleep (4)
- ☐ A hot or red face (5)
- ☐ Needing to pee a lot (6)
- ☐ An upset stomach (7)
- ☐ Twitches (8)
- ☐ Unable to concentrate (9)
- ☐ A fast or uneven heartbeat (10)
- ☐ Feelings of unlimited energy (11)
- ☐ Agitated movements / jittery (12)
- ☐ ⊗ No, I have never felt any of these effects shortly after consuming caffeinated products. (13)
- ☐ Other (please specify) (14) _____

Q15.6 With which products did these symptoms occur?
Select as many options as apply.

	Restless (1)	Nervous (2)	Excited (3)	Unable to sleep (4)	A hot or red face (5)	Needing to pee a lot (6)	An upset stomach (7)	Twitches (8)	Unable to concentrate (9)	A fast or uneven heart beat (10)	Feelings of unlimited energy (11)	Agitated movements (12)	Other (13)
Tea (Q15.6_1)													
Coffee (Q15.6_2)													
Chocolate (Q15.6_3)													
Kola-flavoured drinks (Q15.6_4)													
Energy drinks / shots (Q15.6_5)													
Caffeinated RTDs (Q15.6_6)													
Caffeinated sports supplements / sports gels (Q15.6_7)													

Caffeine tablets (Q15.6_8)	

Q15.7 Did these negative effects impact on your social life, work life or cause you any kind of distress?

- Yes (1)
- No (2)

Q15.8 Have the effects mentioned above ever led to any of the following?

Select as many options as are relevant.

	Restless (1)	Nervous (2)	Excited (3)	Unable to sleep (4)	A hot or red face (5)	Needing to pee a lot (6)	An upset stomach (7)	Twitches (8)	Unable to concentrate (9)	A fast or uneven heart beat (10)	Feelings of unlimited energy (11)	Agitated movements (12)	Other (13)
I have never had concern about these effects (Q15.8_1)	(	(	(	(		(	(	(	⊂	(	(	⊂	
Worry or concern (Q15.8_2)	(	(	(	(		(	(	(	⊂	(	(	⊂	
Talking to someone about these effects (Q15.8_3)	(	(	(	(		(	(	(	⊂	(	(	⊂	
Seeking help to stop these effects (Q15.8_4)	(	(	(	(		(	(	(	⊂	(	(	⊂	
First aid being applied (Q15.8_5)	(	(	(	(		(	(	(	⊂	(	(	⊂	
Hospitalisation (Q15.8_6)	(	(	(	(		(	(	(	⊂	(	(	⊂	

Q15.9 When seeking help to try and stop these effects, who did you contact?

Select as many options as apply.

- ☐ Friends (1)
- ☐ Family (2)
- ☐ Poisons Hot-line (3)
- ☐ Medical professional (4)
- ☐ Other (please specify) (5) _____

Q15.10 Has anyone ever talked to you specifically about your caffeine intake?

- ○ Yes (1)
- ○ No (2)

End of Block: 15- Withdrawals and Intoxication

Start of Block: 16- Why products are not consumed

Q16.1 For the following products, please select the main reasons why you might not consume them.

This includes products that you never consume but also ones that you may consume but not all of the time.

Select the options that apply to you.

	I have never considered taking it (1)	I don't like the flavour (2)	There is too much sugar in it (3)	I don't want to be dependent on it (4)	I react badly to it (5)	It isn't 'good' for me (6)	It has too much caffeine in it (7)	It's too expensive (8)	I don't consume caffeine due to medical reasons (9)
Tea (Q16.1_1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffee (Q16.1_2)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate (Q16.1_3)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kola-flavoured drinks (Q16.1_4)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Energy drinks / energy shots (Q16.1_5)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caffeinated alcoholic RTDs (Q16.1_6)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caffeinated sports supplements / sports gels (Q16.1_7)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caffeine tablets (Q16.1_8)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q16.2 Is there any other reason why you don't consume these products? (Please specify product and reason if applicable)

- ○ No (1)
- ○ Yes (2) _____

End of Block: 16- Why products are not consumed

Start of Block: 17- Social conventions and attitudes

Q17.1
The following are statements on attitudes and behaviors around caffeinated products.

Read the following statements and consider whether you 'agree', 'strongly agree', 'disagree' or 'strongly disagree'.

	Strongly agree (1)	Agree (2)	Unsure (3)	Disagree (4)	Strongly disagree (5)
When someone comes to my house, I should offer them a hot drink (Q17.1_1)	○	○	○	○	○
I give chocolate as a gift (Q17.1_2)	○	○	○	○	○
Sometimes I 'go out for a coffee' but will drink something else that is not coffee. (Q17.1_3)	○	○	○	○	○
It is normal to always have kola-flavoured drinks in the fridge at home (Q17.1_4)	○	○	○	○	○
Kola-flavoured drinks are mainly for special occasions (Q17.1_5)	○	○	○	○	○
Caffeinated RTDs are more socially acceptable way to drink alcohol than spirits (Q17.1_6)	○	○	○	○	○
It is socially acceptable to drink kola drinks and energy drinks in the morning (Q17.1_7)	○	○	○	○	○

Q17.2 Think about the following items.
Which age group that you think of as being the main consumers for each product?

Select as many options as apply.

	14 and under (1)	15-18 (2)	19-30 (3)	31-50 (4)	51-70 (5)	70 and over (6)	All age groups (7)	Unsure (8)
Tea (Q17.2_1)	☐	☐	☐	☐	☐	☐	☐	☐
Coffee (Q17.2_2)	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate (Q17.2_3)	☐	☐	☐	☐	☐	☐	☐	☐
Kola drinks (Q17.2_4)	☐	☐	☐	☐	☐	☐	☐	☐
Energy drinks / energy shots (Q17.2_5)	☐	☐	☐	☐	☐	☐	☐	☐
Caffeinated RTDs (Q17.2_6)	☐	☐	☐	☐	☐	☐	☐	☐
Caffeinated pre-workout sports supplements / sports gels (Q17.2_7)	☐	☐	☐	☐	☐	☐	☐	☐
Caffeine tablets (Q17.2_8)	☐	☐	☐	☐	☐	☐	☐	☐

Q17.3 Think about the following items.
Which gender that you think of as the main consumer for each product?

	Male (1)	Female (2)	Both (3)	Unsure (4)
Tea (Q17.3_1)	○	○	○	○
Coffee (Q17.3_2)	○	○	○	○
Chocolate (Q17.3_3)	○	○	○	○
Kola drinks (Q17.3_4)	○	○	○	○
Energy drinks / energy shots (Q17.3_5)	○	○	○	○
Caffeinated RTDs (Q17.3_6)	○	○	○	○
Caffeinated pre-workout sports supplements / sports gels (Q17.3_7)	○	○	○	○
Caffeine tablets (Q17.3_8)	○	○	○	○

End of Block: 17- Social conventions and attitudes

Start of Block: 18- Demographics

X→

Q18.1 What is your ethnicity?

You may choose as many that apply to you.

- [ ] European (1)
- [ ] NZ European (2)
- [ ] Maori (3)
- [ ] Samoan (4)
- [ ] Cook Islands Maori (5)
- [ ] Tongan (6)
- [ ] Niuean (7)
- [ ] Tokelauan (8)
- [ ] Fijian (9)
- [ ] Southeast Asian (10)
- [ ] Chinese (11)
- [ ] Indian (12)
- [ ] Korean (13)
- [ ] Middle Eastern (14)
- [ ] Latin American (15)
- [ ] African (16)

☐ Other (please specify) (17)
_____

Q18.2 Employment status (choose more than one option if applicable):

☐ Student (1)

☐ Unemployed (2)

☐ Part time worker (3)

☐ Full time worker (4)

Q18.3 Does your job involve any of the following?

	Yes (1)	No (2)
Manual labour (Q18.3_1)	○	○
Driving long distances (Q18.3_2)	○	○
Shift work (Q18.3_3)	○	○

Q18.4 What is your highest level of education?

- ○ Primary school education (1)
- ○ Completed year 11 / 5th form (2)
- ○ Completed year 12 / 6th form (3)
- ○ Completed high school (4)
- ○ Diploma / Certificate (5)
- ○ Bachelors Degree (6)
- ○ Postgraduate degree (7)

Q18.5 Are you involved in Sports? If so, what type?

Select as many as apply

- ☐ Recreational team sports (1)
- ☐ Recreational individual sports (2)
- ☐ Competitive team sports (3)
- ☐ Competitive individual sports (4)
- ☐ Resistance / weight training (5)
- ☐ Endurance training (e.g. triathlons/marathons) (6)
- ☒ No, I am not involved in any type of sporting activity (7)

Q18.6 What is your living situation?

- ○ Living alone (1)
- ○ Living in a family home with others (2)
- ○ Flatting with others (3)
- ○ Other (please specify) (4) _____

Q18.7 Do you smoke?

- ○ Yes (1)
- ○ No (2)
- ○ Occasionally (3)
- ○ Prefer not to answer (4)

X→

Q18.8 Are you currently on any type of oral contraceptive?

- ○ Yes (1)
- ○ No (2)
- ○ Prefer not to answer (3)

Q18.9 How much do you weigh (kg)?

- ○ Kg - (1) _____
- ○ Don't know / prefer not to answer (2)

Q18.10 How tall are you (cm)?

- ○ Cm - (1) _____
- ○ Don't know / prefer not to answer (2)

End of Block: 18- Demographics

Appendix B: Author Guidelines for Literature Review: Psychopharmacology Journal

Review Articles:
Should not exceed 25 pages of text (excluding title page, abstract, references, tables, and figures).

All remaining text formatting, tables, figures, and referencing guidelines apply as per **Appendix C**.

Appendix C: Author Guidelines for Manuscript: Psychopharmacology Journal

Original Investigations
Should not exceed 15 pages of text (excluding title page, abstract, references, tables, and figures). It should be noted that Psychopharmacology does not impose a minimum length on original investigations.

Manuscript structure:

Title page
The title page should include:
- The name(s) of the author(s)
- A concise and informative title (avoid assertive sentences)
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone, and fax numbers of the communicating author
- Acknowledgments of funding and grants and of any conflict of interest or of any circumstances that could be perceived as a potential conflict of interest

Abstract
Each paper should be preceded by a structured Abstract in English of not more than 250 words. Abstracts should contain the following subheadings (in italic type), in the following order: Rationale, Objectives, Methods (if applicable), Results, Conclusions.
The **abstract** should not contain any undefined abbreviations or unspecified references.

Keywords
Up to 10 keywords should be supplied after the Abstract for indexing purposes.

Abbreviations
Abbreviations should be defined at first mention in the abstract and again in the main body of the text and used consistently thereafter. Abbreviations and metric units should conform to the International System of Units (SI).

Text Formatting
Manuscripts should be submitted in Word.
- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.

Appendices

- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 182 kB)

Headings
Please use no more than three levels of displayed headings.

Abbreviations
Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes
Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.
Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments
Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Please note:

- One page equals approximately 4500 characters or 800 words without spaces.
- If you use Word 2007, do not create the equations with the default equation editor but use the Microsoft equation editor or MathType instead.
- Save your file as RTF (Rich Text Format) or Microsoft Word compatible formats. Do not submit docx files.

Citation
Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list
The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.
Reference list entries should be alphabetized by the last names of the first author of each work. Order multi-author publications of the same first author alphabetically with respect to second, third, etc. author. Publications of exactly the same author(s) must be ordered chronologically.

- Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI

  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation

  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

- ISSN LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

- EndNote style (zip, 2 kB)

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Figure Submission

- Name your figure files with "Fig" and the figure number e.g. Fig1.eps

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.

- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- When preparing your figures, size figures to fit in the column width.
- For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.
- For books and book-sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Appendix D: Preservation Buffer and DNA Extraction Protocol

Preservation Buffer Recipe

Preservation buffer (pH 8.7):
0.3M Tris HCl
0.67M Urea
6% SDS
20mM EDTA
0.67 NaOAc
0.1M Ascorbate

DNA Extraction Protocol from Saliva

SALIVA COLLECTION	
STEPS	**NOTES**
Collect 500-1000µl of saliva in a sterile tube.	Do not eat or drink 30min prior to obtaining saliva sample.
Add preservation buffer to the saliva in a 1:1 ratio.	- This can now be stored at room temperature for up to ~28days - See appendix for Buffer formula
DNA EXTRACTION	
Add 250µl of saliva/buffer solution into a 1.5ml centrifuge tube	-Do not include the bubbly/foam saliva as part of the 250µl. - If you are adding more than 300µl of saliva/buffer solution, then you will need to use a larger centrifuge tube
Vortex sample for 10min	- This step breaks open the cells. - Using a table top vortex machine is sufficient enough for this step.
Add 20ul Proteinase K .Incubate at 95°C for 10min	-This will further inactivate nucleases and other proteins that may degrade DNA.
Add 0.68 Volume of 5M KAc, mix and incubate on ice for 10min (Approx. 170ul)	- Potassium acetate is used to precipitate dodecyl sulfate (DS) other and DS-bound proteins, allowing the removal of proteins from DNA. - Dodecyl sulfate is found in the preservation buffer. - the solution will become turbid as inhibitors and impurities are precipitated. - Alternatively you can put samples in the -80 freezer for 5minutes
Centrifuge at 4C for 10 minutes at 15,000.g	
Carefully transfer the clear supernatant with a pipette tip into a fresh centrifuge tube. Discard the pellet containing impurities.	- If the pellet is accidently disturbed then re centrifuge.
To the supernatant add 1.2 volumes of Chilled 95-100% Ethanol (Approx. 520ul) and mix by inverting the tube ~10 times. DONOT Vortex	- You may start to see fine thread like fibres precipitating out of solution- this is the DNA. - If this is not seen, then the DNA will be recovered by carefully following the next steps.
Allow the sample to stand at -70C Overnight	- This allows the DNA to fully precipitate.
Place the tube in the centrifuge in a known orientation. Centrifuge at room temperature for 2-5 minutes at 15,000.g.	- Place the tube into centrifuge with hinge pointing outwards. The DNA pellet (even if not visible to naked eye) will collect on the bottom of the tube wall directly under the hinge side.
Carefully remove the supernatant with a pipette tip and discard. Take care to avoid disturbing the DNA pellet.	- Loss of the pellet results in loss of DNA. - The supernatant contains impurities and should be removed as completely as possible
Carefully add 300µL of 70% ethanol. Vortex sample and Let stand at room temperature for ~1 minute.	- This is the Ethanol wash step.
Centrifuge with hinge pointing outwards at room temperature for 2-5 minutes at 15,000.g	

CAREFULLY remove all ethanol from centrifuge tube without disturbing DNA pellet. Arrange the microcentrifuge tubes upside down and air dry (lids open)	- All ethanol needs to be removed as carryover may impact performance of the assay. - After removing all ethanol, the tube can be pulse spun to further allow ethanol removal. This step helps to ensure all ethanol is removed.
OR Incubate tube at 90°c for 1 minute	This step helps to ensure all ethanol is removed.
Add 50µl of 1x TE (8mM)solution to DNA pellet. Vortex for at least 5 seconds.	- If a higher concentration of DNA is required, then add 50µl of TE
Rehydrate the DNA pellet by incubating at room temperature overnight followed by vortexing	- Alternatively, incubate at 50°c for 1 hour with occasional vortexing.
STORAGE: DNA can be stored at 4°c for up to 2 months or -20°c for long term storage	- To avoid freeze thaw cycles, aliquot DNA into smaller volumes and store in -20°c freezer.
GEL ELECTROPHORESIS	
Make an agarose gel	- 1% Agarose gel is sufficient to view DNA bands. (1g of agarose added to 100ml 1x Sodium Borate or 1X Tris-EDTA buffer). - Microwave solution until agarose is completely dissolved. This usually takes 1-2 minutes. - Once the gel has cooled down enough that you can hold onto the bottle, Pour gel into casting tray and let it set.
Load DNA templates into the wells of the gel	- Place the set gel into electrophoresis tank filled with SB buffer - Mix loading dye (4µl) with DNA product (7-10µl) and load into gel wells. - Make sure to add 1-2 µl of ladder into an empty well
Run Gel electrophoresis	Run gel for ~30minute at 100 -120V or until the dye has migrated at least 2/3rd down the gel.
Stain Gel with Ethidium Bromide	- Place the gel into EtBr tank for ~20min - EtBr intercalates with DNA and allows DNA bands to be visualized under UV light.
De-stain Gel (optional)	- De-stain by placing gel into container of H_20 for ~10minutes.
Visualize Bands	- Place gel under UV imaging system to visualize bands. - A single band at 542bp should be seen. -If non-specific banding occurs then adjust PCR conditions accordingly. - Once a single clear 542bp band is seen, then proceed to digestion step.

Appendix E: Caffeine Study: Standard Operating Procedures

Unique Identifier:

A six-digit numerical figure is used to link together each participants' questionnaire, saliva sample, and contact information. The first two digits are derived manually according to the number of

participants recruited i.e. for the first 100 participants the first two digits =01, the second 100 participants first two digits = 02. For the final four digits of the unique identifier, an online random number generator is used (e.g. StatTrek.com) to generate 100 random four digit numbers at once with no duplicate entries allowed. This technique is used to ensure no duplicate numbers occur when generating codes for additional participants. Enter codes into sticker template. Label as "Participant ID" to avoid confusion with student ID.

Equipment:
- Participant ID stickers
- Participant information sheets
- Parental consent forms
- Participant contact sheets
- Saliva collection tubes
- Tissues
- Buffer (approx. 100mL per day)
- Gloves
- Disposable pipettes
- 4 x fully charged tablets
- Electric drill (to set up stands)
- Biohazard rubbish bags
- Advertisement posters
- Access to Wifi
- Tube stand
- Chilly bin/ box for tube stands and buffer storage

Data Collection:

A data collection stand should be set up for approximately 6 hours and should be located in areas where a large amount of human traffic is expected.

The data collection stand consists of two trestle tables with four tablets (Apple iPad) set up on stands. To set up and man the data collection stand efficiently and effectively, this required at least one researcher and one research assistant.

Questionnaire:

Participants should be given the option of whether to complete their questionnaire on the tablets provided or at home. If they choose the latter, a card should be provided with the link to the questionnaire (study's Facebook page) and a unique identifier code. Explain that the questionnaire may take up to 15 minutes to complete. The screening questions, participant information sheet and informed voluntary consent statement (with yes/no tick box options) are incorporated into the beginning of the questionnaire. Participants under 16 years of age require parental consent before taking part in the study.

The participant is required to enter their unique identifier code before continuing onto the main block of questions.

Participants completing the questionnaire at home should be sent two reminder emails at two-weekly intervals if they have not already completed the questionnaire.

Saliva collection:
Participants must refrain from eating or drinking for 30 min prior to saliva collection. Ask participant to provide at least a 1 mL sample. The collection is carried out by drooling into a sterile tube (with 1 mL interval marked measurements). Provide participant with tissues if required. A researcher (wearing disposable gloves) should then add the preservation buffer into the saliva in a ratio of 1:1

using a disposable pipette. The saliva samples do not have to be kept on ice during collection days. The saliva should then be stored in a -20°C freezer at the end of each collection day or the day following.

Data handling:

Export questionnaire data from Qualtrics into Microsoft Excel and screen for any missing information. The estimated daily caffeine consumption should be calculated for every caffeine-consuming participant. In order to do this, the caffeine concentration data for the various caffeine-containing products (see Table 1) is combined with the consumption frequency data from the CaffCo questionnaire using Microsoft Excel software. The different consumption frequencies are assigned a factor (see Table 2) according to their relationship to daily consumption (e.g. if the consumption frequency was once a week, the factor would be 1/7 = 0.143). If the consumption frequency included a range, the middle value would be used (e.g. 2-3 times a day would be a factor of 2.5). All data should then be entered into IBM SPSS in order to carry out statistical analysis.

Table 1: Caffeine content of food and beverages in New Zealand

Product	**Quantity of product**	**Caffeine content (mg)***
Coffee		
Instant coffee powder	1 teaspoon	~ 83
Decaffeinated instant coffee powder	1 teaspoon	~ 1.9
Plunger/ drip coffee	250 mL	~ 100
Espresso	Single shot	~ 120
	Double shot	~ 210
Tea		
Black tea	250 mL made with 1 teabag	~ 57
Green tea	250 mL made with 1 teabag	~ 31
Decaffeinated black tea	250 mL made with 1 teabag	~ 4.7
Chocolate		
Milk chocolate	100 g	~ 20
Dark chocolate	100 g	~ 60
Cocoa powder	1 teaspoon	~ 2
Kola drinks		
Regular kola	100 mL	~ 11
Diet kola (diet, zero, max etc.)	100 mL	~ 14
Energy drinks	100 mL	~ 31.2
Energy shots	60 mL	~ 162.6
Caffeinated RTDs	100 mL	~ 14.4
Pre-workout	100 g	~ 2110
Sports gel	100 g	~ 77.7
Caffeine tablets	1 tablet	~ 50–200

Table 2: Consumption frequency factors

Consumption Frequency	**Multiplication Factor**
Never	0
Less than once a month	1/30 = 0.0333
1-3 times a month	2/30 = 0.0667
Once a week	1/ 7 = 0.14286
2-4 times a week	3/7 = 0.4286
5-6 times a week	5.5/7 = 0.7857

Once a day	1	
2-3 times a day	2.5	
4-5 times a day	4.5	
6+ times a day	6	

Appendix F: Caffeine Study Information Sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND
School of Food and Nutrition
Massey University
Private Bag 102904
North Shore City 0745, Auckland, New Zealand

Does genetics affect caffeine intake habits of New Zealanders?

PARTICIPANT INFORMATION SHEET

Invitation to participate in research
We are looking for individuals 15 years of age or older to take part in a study looking at caffeine consumption.

Researcher Introduction
Hello, we, Rebecca Tennent and Sophie Turner, are currently studying towards a Master of Science degree in Nutrition and Dietetics at Massey University. We are undertaking this research project as it is a requirement in partial fulfilment of our degree. Our supervisors are Assoc Prof Kay Rutherfurd-Markwick, Dr Ajmol Ali and Assoc Prof Carol Wham. Together, the supervisors have an extensive background of research in the fields of nutrition, biochemistry, physiology and public health.

Project Description
The positive effects of caffeine intake are well known, whereas the negative effects of caffeine intake aren't as widely recognised. Recently it has been found that the risk of side effects has a large genetic basis. One of the most studied caffeine-related genes is CYP1A2 which codes for the enzyme that metabolises caffeine- cytochrome p450. This enzyme is also responsible for the metabolism of multiple other drugs. There are three variations of this gene which determine whether an individual is a slow, intermediate or fast metaboliser of caffeine. Slow metabolisers are considered to have a higher risk of the negative effects of caffeine due to caffeine remaining in the blood stream for a longer period of time. One variant of this gene has been associated with an increased risk of myocardial infarction (heart attack). Another gene with an established relationship to caffeine is the adenosine receptor gene, ADORA2A. A variation of this gene has been found to be associated with Panic Disorder. This same variant has been associated with caffeine-induced anxiety, sleep changes and caffeine sensitivity. There is currently very little information about caffeine intake and the reasons behind the consumption of caffeine in New Zealand. New Zealand has an ever-growing supply of caffeinated products on the market, making this is a very important research area. This study aims to gather information on the caffeine consumption habits, knowledge, beliefs and responses of people living in New Zealand with the use of a questionnaire. In addition, genetic testing will be carried out with the use of saliva samples (to test for caffeine metabolism genes including CYP1A2 and ADORA2A). This information will help to determine groups who are at the most risk of suffering the ill-effects of caffeine consumption.

Participant recruitment and involvement
We are looking for approximately 400 participants to take part in this study in order to obtain sufficient statistical power. As we require a representative, unbiased sample, a range of recruitment strategies will be used. This may include social media (e.g. Facebook), news and print media, on-line

recruitment agencies, posters and flyers at popular venues, interactive displays at university open days, shopping malls, community and church groups and by word of mouth. To take part in this study you must be:
- 15 years of age or older
- Competent in reading English
- Willing to provide a saliva sample
- Willing to complete a questionnaire.

We will invite you to fill out a questionnaire and provide a saliva sample. Completing the questionnaire will take approximately 20 minutes. Providing the saliva sample will take approximately 5 minutes.

At the completion of the study, you will receive a summary of the results and will have the option to receive your caffeine-related genetic information. This will include the caffeine-related genes tested, your particular genotype and an explanation of what this means. The decision whether to receive your genetic information will be made at the time of the completion of the consent form, however we will allow a three-month period from the time of analysis in the case of a change in mind (after the three-month period is over the genetic results will be anonymized and therefore cannot be linked back to the participant). Please contact the researcher if after completing the consent form you have changed your mind in regards to receiving your genetic information.

Project procedures

Screening

Potential participants will receive a hard copy or link to the information sheet and screening questionnaire. The screening questionnaire will determine whether you meet the criteria to participate and whether you would like to complete the questionnaire in hard or soft (online) copy. If you meet the criteria, you will be asked to fill out a consent form before progressing. If you're aged 15-17 years old, you will also need parental consent to take part in the study.

Questionnaire

You will have the choice of whether to fill out a soft (online) or hard copy of the questionnaire. If you choose to complete it online, you will be given a link to the questionnaire and a unique identifier code. The data from the completed questionnaire will be sent directly online to the research team. If you choose to complete the questionnaire in hard copy, it will either be sent to you and returned via regular mail or handed straight to you in person then returned in person.

Saliva collection

A 0.5-1mL saliva sample will be required in order to carry out genetic analysis. We ask that you refrain from eating or drinking for 30 minutes prior to collection. The collection will be carried out by drooling into a sterile tube. A preservation buffer must then be added into the saliva in a ratio of 1:1 to allow the sample to be stable. The saliva sample must have a turnaround time of ~20 days from when the saliva is deposited to when the sample is received in the lab. If you will be collecting the saliva away from the researcher (e.g. at home), the container will be posted to you in a paid-return package and clear instructions will be provided, which must be posted back to the research team.

Data Management

All data and materials will be solely used for this study. Only the researchers and supervisors will have access to the data and consent forms. Hard copies of data will be kept in a locked filing cabinet on campus at Massey University Albany, Oteha Rohe campus. Soft copies will be stored on password-protected computers in password-protected files, where the password is only known to the research team.

In order to maintain confidentiality, a coding system will be used where each participant is given a unique identifier. This code will be used to link together your questionnaire, saliva sample, and

consent form data. This means that although you will not be anonymous (to the research team), all data will be anonymised.
Saliva samples will be analysed and transformed into soft form data at the first chance possible. Any excess saliva will be disposed of as soon as analysis is complete by Assoc Prof Kay Rutherfurd-Markwick. Please note once DNA has been extracted (by Assoc Prof Rutherfurd-Markwick), the analytes will be sent to Australia for genetic assay determination. This could take up to three months after receiving the sample. The completely anonymised raw results data will be kept for 5 years, after which will be disposed of by Dr Ajmol Ali or another member of staff.

Participant's Rights
You are under no obligation to accept this invitation. Should you choose to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study up until submission of the questionnaire;
- ask any questions regarding the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the study findings when research has been concluded;
- If you feel concerned about the possible effects on you of your caffeine consumption, you may request a copy of your genetic information. Note: Before agreeing to this you should be aware that under New Zealand law an insurance company could ask you to disclose such information should you apply for life or health related insurance – such as medical cover. You could be obliged to disclose it even if the insurer does not ask for it expressly. Not disclosing it could result in the insurer not having to pay out under the policy. Should you choose not to receive this information for your protection should the current insurance situation change, the possibility of identifying your genetic information will be removed three months after it becomes available.

If you feel concerned about your caffeine or other food and beverage consumption, please consult with your GP. Otherwise, Samaritans NZ is an organisation available for non-judgemental, confidential support to anyone in distress (04 473 9739). Alcohol Drug Helpline (0800 787 797) is a free, anonymous service available if you have concerns about your alcohol consumption.

Committee Approval Statement
This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 17/01. If you have any concerns about the conduct of this research, please contact Dr Lesley Batten, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 356 9099 x 85094, email humanethicsoutha@massey.ac.nz.

Project Contacts
If you have any questions regarding this project, please contact the student researcher and/or one of the supervisors.
Rebecca Tennent (School of Food and Nutrition)
Sophie Turner (School of Food and Nutrition)
Email: caffeinestudy@outlook.co.nz
Phone: +64 21 223 7093

Dr Ajmol Ali (School of Sport and Exercise)
Email: A.Ali@massey.ac.nz
Phone: +64 (09) 213 6414

Assoc Prof Kay Rutherfurd-Markwick (School of Food and Nutrition)
Email: K.J.Rutherfurd@massey.ac.nz
Phone: +64 (09) 414 0800 ext. 43646

Assoc Prof Carol Wham (School of Food and Nutrition)
Email: C.A.Wham@massey.ac.nz
Phone: +64 (09) 414 0800 ext. 4364

Appendix G: Caffeine Study Consent Form

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

School of Sport, Exercise and Nutrition
Massey University
Private Bag 102904
North Shore City 0745, Auckland, New Zealand

Does genetics affect caffeine intake habits of New Zealanders?

PARTICIPANT CONSENT FORM

- I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.
- I understand that I have the right to withdraw from the study up until submission of the questionnaire.
- I agree to provide information to the researcher on the understanding that my name will not be used without my permission (The information will be used only for this research and publications arising from this research project).
- I understand that any saliva samples collected from me will only be used for this study, and that samples will be analysed and destroyed as soon as possible (up to three months after receiving sample).
- I agree to submit genetic material to the researcher for use only in this study (Genetic material will not be deposited into a gene data bank).
- I understand that if my genetic information obtained by the researcher is disclosed to me, I may have to pass this information to an insurance company should I seek life or health-related insurance cover in the future. I understand that failure to disclose the information could invalidate my insurance policy.
- I agree to participate in this study under the conditions set out in the Information Sheet.

□ I would like to receive my caffeine-related genetic information. Note: We will allow a three-month period from the time of analysis in the case of a change in mind (after the three-month period is over the genetic results will be anonymized and therefore cannot be linked back to you).

Signature:_____ Date: _____

Full name (printed): _____

Phone number: _____ Date of Birth: _____

Address: _____

Email: _____

Are you willing to be contacted regarding future research projects within the School of Sport, Exercise and Nutrition? Your name and email address will be saved in a secure location. You will be sent periodic newsletters regarding research studies within the School. You can opt out of this newsletter at any time.

☐ Tick here if you accept.

Appendix H: Ethical Approval

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date: 03 April 2017

Dear Dr Aj Ali

Re: Ethics Notification - **SOA 17/01** - **Caffeine consumption and the impact of genetic variants on caffeine-related responses in New Zealanders**

Thank you for the above application that was considered by the Massey University Human Ethics Committee: **Human Ethics Southern A Committee** at their meeting held on **Monday, 3 April, 2017**.
On behalf of the Committee I am pleased to advise you that the ethics of your application are approved.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Dr Brian Finch
Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

Research Ethics Office, Research and Enterprise
Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand **T** 06 951 6841; 06 95106840
E humanethics@massey.ac.nz; animalethics@massey.ac.nz; gtc@massey.ac.nz

From: Broad, Patsy
To: Ali, Ajmol
Subject: Southern A Applications SOA 17/01
Date: Friday, 11 May 2018 4:17:00 PM
Attachments: image001.png

HEC: Southern A Application SOA 17/01
Title: Genetic Associations with Caffeine Consumption and Experiences in NZ Adults - Rebecca Tennent
Title: Secondary School Students and Caffeine: Consumption Habits and Experiences - Sophie Turner

Dear Aj

Thank you for your email dated 10 April 2018 outlining the change you wish to make to the above application.

The change to the consent form has been approved and noted to allow for the option of participants to approve of their contact details being maintained for contact regarding future studies.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee. If over time, more than one request to change the application is received, the Chair may request a new application.

Regards
Patsy

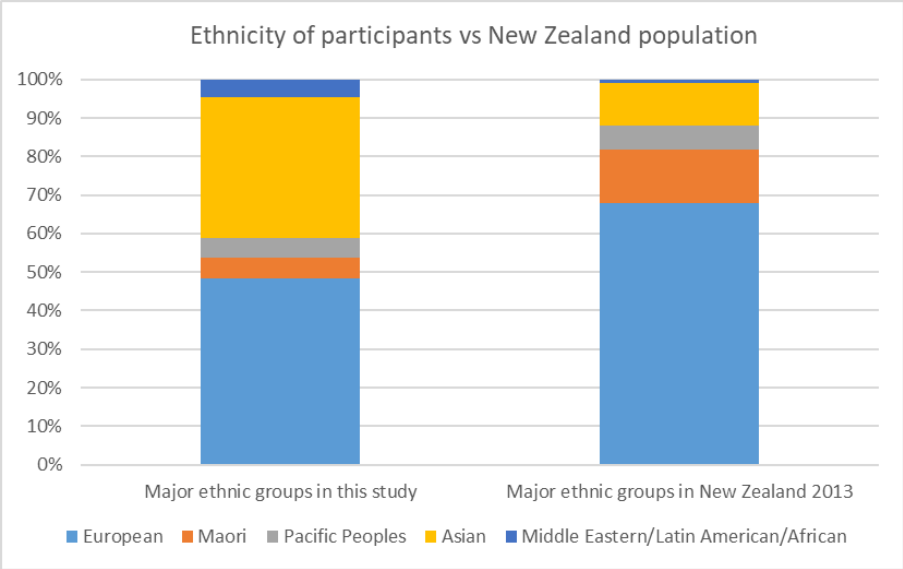
Patsy Broad
Team Leader, Research Ethics
Research Ethics Office
Courtyard Complex, Room 1.23
Manawatu Campus
Massey University/Te Kunenga ki Purehuroa
Private Bag 11222
Palmerston North 4410
NEW ZEALAND

Extension: 83840
Phone (DDI): 06 951 6840
Email: p.l.broad@massey.ac.nz
Fax 06 355 7973
http://rims.massey.ac.nz/rmenet/

Appendix I: Supplementary Results

Supplementary Table 1. Genotype distribution by gender

		Total (%)	Male (%)	Female (%)	*P* value
rs4410790	CC	30.6	28.5	32.6	0.477
	CT	51.8	49.6	53.8	0.505
	TT	17.6	22.0	13.6	0.082
rs5751876	CC	29.0	31.7	26.5	0.363
	CT	46.3	43.1	49.2	0.327
	TT	24.7	25.2	24.2	0.860
rs762551	CC	10.2	10.6	9.8	0.850
	CA	40.4	40.7	40.2	0.936
	AA	49.4	48.8	50.0	0.846



Ethnicity data from 17 chosen ethnicities was categorised into five groups (European, Māori, Pacific Peoples, Asian, Middle Eastern/ Latin American/ African) according to a prioritisation system to streamline results (Statistics New Zealand, 2017). This was compared to the New Zealand population 2013 (Statistics New Zealand, 2014)

Supplementary Fig. 1. Ethnicity of participants compared to New Zealand population 2013 (Statistics New Zealand, 2014; Statistics New Zealand, 2017)

Supplementary Table 2. Caffeine sources consumed by gender

Caffeine source	Male (%) (n= 123)	Female (%) (n= 132)	Pearson Chi-Square value (X^2)	P value
Tea	64.2	78.8	6.662	0.010*
Coffee	69.9	81.1	4.295	0.038*
Chocolate	74.0	90.2	11.452	0.001*
Kola drinks	53.7	48.5	0.682	0.409
Energy drinks	44.7	36.4	1.845	0.174
Caffeinated RTDs	22.8	18.9	0.566	0.452
Caffeine-containing sports supplements	8.9	3.0	4.021	0.045*
Caffeine tablets	2.4	5.3	1.386	0.239[b]
None	1.6	0	2.163	0.141[b,c]

*Significant at the 0.05 level.
[b]Minimum expected count <5
[c]Minimum is less than one. Results may be invalid.

Supplementary Table 3. Number and percentage of participants stratified by consumption groups within rs5751876 ADORA2A genotypes

Genotypes	<80 mg/day (n=84) n (%)	80-400 mg/day (n= 135) n (%)	>400 mg/day (n=36) n (%)
rs5751876 ADORA2A			
CC	27 (36.5)	37 (50.0)	10 (13.5)
CT	30 (25.4)	68 (57.6)	20 (17.0)
TT	27 (42.9)	30 (47.6)	6 (9.5)
Kruskal-Wallis test	0.232	1.124	2.197
(H)	0.891	0.570	0.333
P value			

Supplementary Table 4. Estimated median daily caffeine intake expressed on a per kg body weight basis by genotype

Genotypes	Total intake (mg/kgbw/day)	Kruskal-Wallis test statistic (H)	p value
CYP1A2			
CC	2.50	0.234	0.890
CA	2.02		
AA	2.36		
ADORA2A			
CC	1.71	5.922	0.052
CT	2.78		
TT	1.76		
AHR			
CC	2.02	1.550	0.461
CT	2.49		
TT	1.83		

Supplementary Table 5. Estimated median daily caffeine consumption by genotype groups

ADORA2A/CYP group from Figure 3.2	Frequency (n)	Median (interquartile range)	Test Statistic (H) and *P* value
A	10	120 (64, 332)	H = 11.076 Df = 8 P = 0.197
B	21	102 (49, 165)	
C	32	108 (49, 226)	
D	9	161 (41, 297)	
E	48	186 (81, 340)	
F	61	160 (97, 299)	
G	7	217 (173, 402)	
H	34	112 (64, 258)	
I	33	130 (35, 385)	
AHR/CYP group from Figure 3.3	Frequency (n)	Median (interquartile range)	Test Statistic (H) and *P* value
A	6	297 (265, 345)	H = 4.407 Df = 8 P = 0.819
B	18	119 (90, 167)	
C	21	134 (73, 251)	
D	14	159 (41, 279)	
E	57	154 (70, 288)	
F	61	161 (64, 314)	

G	6	161 (47, 402)
H	28	120 (47, 221)
I	44	130 (58, 298)

Supplementary Table 6. Frequency of "adverse symptom" prevalence by ADORA2A genotype

ADORA2A genotype	Frequency n (%) of participants reporting "Indicators of anxiety"	Test Statistic (H) and *P* value
CC	30 (31.6)	H = 1.194
CT	45 (47.4)	Df = 2
TT	20 (21.0)	P = 0.550
ADORA2A genotype	Frequency n (%) of participants reporting "Unable to sleep"	Test Statistic (H) and *P* value
CC	26 (26.0)	H = 1.33
CT	50 (50.0)	Df = 2
TT	24 (24.0)	P = 0.567
ADORA2A genotype	Frequency n (%) of participants reporting "Fast or uneven heartbeat"	Test Statistic (H) and *P* value
CC	28 (34.1)	H = 1.690
CT	36 (43.9)	Df = 2
TT	18 (22.0)	P = 0.430

Supplementary Table 7. Frequency of "adverse symptom" prevalence by genotype groups

ADORA2A/CYP group from Figure 3.7	Frequency n (%) of participants reporting "Indicators of anxiety"	Test Statistic (H) and *P* value
A	3 (30.0)	
B	6 (28.6)	
C	11 (34.4)	H = 3.99
D	4 (44.4)	Df = 8
E	19 (39.6)	P = 0.858
F	22 (36.1)	
G	1 (14.3)	

H	15 (44.1)	
I	14 (42.4)	
ADORA2A/CYP group from Figure 3.7	Frequency n (%) of participants reporting "Unable to sleep"	Test Statistic (H) and *P* value
A	3 (30.0)	
B	8 (38.1)	
C	13 (40.6)	
D	2 (22.2)	H = 5.564
E	25 (52.1)	Df = 8
F	23 (37.7)	P = 0.696
G	2 (28.6)	
H	13 (38.2)	
I	11 (33.3)	
ADORA2A/CYP group from Figure 3.7	Frequency n (%) of participants reporting "Fast or uneven heartbeat"	Test Statistic (H) and *P* value
A	5 (50.0)	
B	2 (9.5)	
C	11 (34.4)	
D	2 (22.2)	H = 11.981
E	12 (25.0)	Df = 8
F	22 (36.1)	P = 0.152
G	1 (14.3)	
H	14 (41.2)	
I	13 (39.4)	