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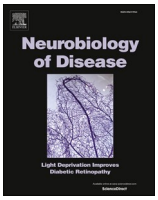

Caffeine consumption outcomes on amyotrophic lateral sclerosis disease progression and cognition

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ABSTRACT

Caffeine consumption outcomes on Amyotrophic Lateral Sclerosis (ALS) including progression, survival and cognition remain poorly defined and may depend on its metabolization influenced by genetic variants. 378 ALS patients with a precise evaluation of their regular caffeine consumption were monitored as part of a prospective multicenter study. Demographic, clinical characteristics, functional disability as measured with revised ALS Functional Rating Scale (ALSFRS-R), cognitive deficits measured using Edinburgh Cognitive and Behavioural ALS

Abbreviations: ADORA2A, Adenosine A2a Receptor; AHR, Aryl Hydrocarbon Receptor; ALS, Amyotrophic Lateral Sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Score-Revised; BMI, Body Mass Index; CYP1A1, Cytochrome P450 Family 1 Subfamily A Member 1; CYP1A2, Cytochrome P450 Family 1 Subfamily A Member 2; ECAS, Edinburgh Cognitive and Behavioural ALS Screen; IQR, Interquartile range; POR, Cytochrome P450 Oxidoreductase; PULSE, Study of Predictive Factors of Progression of Motor Neuron Disease; SNP, Single nucleotide polymorphism; XDH, Xanthine Dehydrogenase.

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Screen (ECAS), survival and riluzole treatment were recorded. 282 patients were genotyped for six single nucleotide polymorphisms tagging different genes involved in caffeine intake and/or metabolism: *CYP1A1* (rs2472297), *CYP1A2* (rs762551), *AHR* (rs4410790), *POR* (rs17685), *XDH* (rs206860) and *ADORA2A* (rs5751876) genes. Association between caffeine consumption and ALSFRS-R, ALSFRS-R rate, ECAS and survival were statistically analyzed to determine the outcome of regular caffeine consumption on ALS disease progression and cognition. No association was observed between caffeine consumption and survival ($p = 0.25$), functional disability (ALSFRS-R; $p = 0.27$) or progression of ALS ($p = 0.076$). However, a significant association was found with higher caffeine consumption and better cognitive performance on ECAS scores in patients carrying the C/T and T/T genotypes at rs2472297 (p-het = 0.004). Our results support the safety of regular caffeine consumption on ALS disease progression and survival and also show its beneficial impact on cognitive performance in patients carrying the minor allele T of rs2472297, considered as fast metabolizers, that would set the ground for a new pharmacogenetic therapeutic strategy.

1. Introduction

Caffeine is the most widely consumed psychoactive agent worldwide via dietary intake from coffee, tea or soda beverages (Fredholm et al., 1999). Coffee consumption has been inversely associated with total and cause-specific mortality (Freedman et al., 2012). Compelling evidence supports acute caffeine's ability to increase/improve wakefulness, alertness and memory (Borota et al., 2014; Cunha, 2016; Van Dam et al., 2020). Multiple epidemiological studies point-out an inverse correlation between regular/chronic caffeine intake and the risk of Parkinson's disease (Chen and Schwarzschild, 2020; Ross, 2000) as well as age-related cognitive decline (Cunha, 2016; Yelanchezian et al., 2022). However, the relationship between caffeine and the third major neurodegenerative disease, amyotrophic lateral sclerosis (ALS), remains poorly understood.

ALS is a neurodegenerative disease characterized by the loss of both lower and upper motor neurons, resulting in muscle progressive paralysis and atrophy, leading, invariably, to the death of patients with a median survival following diagnosis of 2 to 4 years (Feldman et al., 2022). Overall, ALS is a highly heterogeneous disease at multiple levels, including the age of onset, clinical phenotypes (spinal, bulbar or frontotemporal dementia onset) or survival after onset. Disease severity is addressed using the multi-domain Amyotrophic Lateral Sclerosis Functional Rating Score-Revised (ALSFRS-R). While being a predominant motor disorder, cognitive changes occur, even at early stages and before motor impairments, in about half of the patients. They are usually evaluated by the "Edinburgh Cognitive and Behavioural ALS Screen" (ECAS) (Feldman et al., 2022).

Few studies have investigated the relationship between coffee or caffeine consumption and ALS, being essentially related to the impact towards risk and/or survival. Beghi et al. first reported an inverse correlation between coffee consumption and ALS risk, without however addressing the impact of caffeine itself (Beghi et al., 2011). Nevertheless, Fondell et al. did not report a significant association between caffeinated coffee consumption and ALS risk in a meta-analysis of longitudinal studies comprising the participants in five large cohorts (the Nurses' Health Study, the Health Professionals Follow-up Study, the Cancer Prevention Study II Nutrition Cohort, the Multiethnic Cohort Study and the National Institutes of Health-AARP Diet and Health Study) (Fondell et al., 2015). Such lack of association was later confirmed by Pupillo et al. in the Euro-MOTOR population registry (Pupillo et al., 2018). In another meta-analysis of eight prospective cohort studies within the "Pooling Project of Prospective Studies of Diet and Cancer" (Petimar et al., 2019), no impact of caffeine on the survival of ALS patients was identified. Safety of caffeine consumption on disease progression and severity has been partially addressed in cellular or animal models and a clinical study with conflicting results. In cellular models, caffeine improves motor neuron integrity (Zwilling et al., 2020), while in the SOD1-G93A mice, caffeine intake significantly shortened the survival (Potenza et al., 2013). Lastly, a cross-sectional study in a cohort of ALS patients did not support the hypothesis that coffee or tea consumption is associated with ALS progression (Cucovici et al., 2021). Overall, the safety of regular caffeine consumption on disease progression and its effect on the endophenotypes including cognition have not been addressed.

Caffeine is almost entirely metabolized by the cytochrome P450 enzyme system in the liver. This involves different multi-step enzymatic pathways including CYP1A1 enzyme, despite the major enzyme involved is CYP1A2, which is responsible for more than 90% of the primary metabolism of caffeine (Arnaud, 2011). Interindividual variability in the activity of the cytochrome P450 is known to strongly correlates with variability in caffeine metabolism (Nehlig, 2018). According to the genotype of the single nucleotide polymorphism (SNP) rs762551 in the *CYP1A2* gene, population can be divided into rapid versus slow caffeine metabolizers (Sachse et al., 1999). Notably, regardless caffeine intake, this SNP has been associated with ALS risk (Siokas et al., 2021). Similarly, rs2472297 in the *CYP1A1/CYP1A2* locus has been associated with an increased metabolism of caffeine (Cornelis et al., 2016; Yin et al., 2022). Several genome-wide association studies showed that caffeine consumption and metabolism are influenced by SNPs in other genes including rs4410790 in *AHR* gene (Cornelis et al., 2016), rs17685 in *POR* gene (The Coffee and Caffeine Genetics Consortium et al., 2015) and rs5751876 in *ADORA2A* gene (Cornelis et al., 2007). Moreover, although caffeine is mainly metabolized into paraxanthine in the liver, other routes of metabolism, leading rise to theophylline and theobromine, are also involved. These metabolites are taken up by other enzymes such as the xanthine dehydrogenase encoded by the *XDH* gene (Nehlig, 2018). The genetic between caffeine outcomes has been rarely tested in the studies addressing the relationship between caffeine consumption and neurodegeneration and never in ALS.

In the present study, we used data from a large prospective multicentric study, aimed at defining the prognosis factors in ALS patients, to determine the relationship between regular/chronic caffeine consumption and survival as well as disease progression considering common genetic variants involved in caffeine metabolism.

2. Materials and methods

2.1. Participants

This study is ancillary to the Study of Predictive Factors of Progression of Motor Neuron Disease (PULSE). PULSE is an ongoing, prospective, longitudinal multicentric study sponsored by the University Hospital of Lille, approved by the CPP Nord-Ouest-IV Ethical Committee (ID-RCB 2013-A00969–36) and registered on the ClinicalTrials.gov website (NCT0236089) conducted in 16 ALS expert centers from the clinical research networks in France (FILSLAN, ACT4ALS-MND). The present study enrolled patients with probable or definite ALS according to the El Escorial criteria (Brooks et al., 2000) included until end 2023. All participants gave written informed consent prior to participation in the study. The study was conducted with good clinical practice in accordance with the Declaration of Helsinki and local regulations, and data collection was compliant with the general data protection regulation rules. At the time of the baseline visit, for all participants, we

recorded basic demographic data (gender, age, education level, body mass index, daily alcohol consumption, number of cigarettes/day), clinical characteristics (clinical subtype, time since the first symptoms to the time of diagnosis, dysphagia), riluzole treatment. The clinical evaluation included ALSFRS-R (0 to 48 with higher scores for no handicap (Cedarbaum et al., 1999), the progression rate defined by the slope on the ALSFRS-R (calculated as [48-ALSFRS-R score]/time from symptoms onset, in months) and cognitive assessment using the ECAS (0 to 136, with higher score for no cognitive impairment (Abrahams et al., 2014). Survival time was also recorded. At inclusion, the daily intake of caffeine containing items (coffee, tea, chocolate, cola) was assessed using an in-housed validated questionnaire (Simonin et al., 2013) (**Supplementary Material 1**), filled together by the patient and the caregiver. Fig. 1 illustrates the study flowchart of the study.

2.2. DNA sample collection and molecular analysis

Genomic DNA was isolated from peripheral blood mononuclear cells according to standard procedures. Patients were genotyped for six SNPs tagging different genes involved in caffeine metabolism: *CYP1A1* (rs2472297) (Sulem et al., 2011), *CYP1A2* (rs762551) (Sachse et al., 1999), *AHR* (rs4410790) (Cornelis et al., 2016), *POR* (rs17685) (The Coffee and Caffeine Genetics Consortium et al., 2015), *XDH* (rs206860) and *ADORA2A* (rs5751876) (Cornelis et al., 2007) genes. The genotyping was performed using the three dedicated TaqMan™ genotyping assay (Applied Biosystems, Villebon-sur-Yvette, France) on a QuantStudio™ 7 Flex Real-Time PCR System (ThermoFisher Scientific, Illkirch-Graffenstaden, France) according to the according to the manufacturer's instructions.

2.3. Statistical analysis

Categorical variables are expressed in terms of frequency and percentage. Quantitative variables are expressed as means ± standard deviation in the case of normal distribution or medians (interquartile range, IQR) otherwise. Normality of distributions was checked graphically and using the Shapiro-Wilk test. Patient's survival was estimated by the Kaplan-Meier method. Caffeine consumption was compared between spinal and bulbar patient's using Mann-Whitney *U* test. Impact of caffeine consumption on ECAS score, ALSFRS-R and ALSFRS-R rate was assessed using a linear regression model (on log-transformed data for ALSFRS-R rate) and using an ANCOVA (analyze of covariance) when adjusting on predefined confounding factors (age, body mass index: BMI, gender, time from first symptoms to inclusion, education level, dysphagia as well as consumptions of tobacco, alcohol, riluzole and psychotropic); regression coefficient and their 95% confidence interval were derived from models as effect size. Impact of caffeine consumption on patient's survival was assessed using a Cox proportional hazard model without and with adjustment on predefined confounding factors; hazard ratios and their 95% confidence intervals were derived from models as effect size. Association between caffeine consumption and each SNPs was assessed using Mann-Whitney U test. Heterogeneity of the association between caffeine and clinical outcomes (ECAS, ALSFRS-R, ALSFRS-R rate and survival) according to each SNP (*CYP1A1*, *CYP1A2*, *AHR*, *POR*, *XDH* and *ADORA2A*) was tested by adding an interaction term to the previous models (without and with adjustment on predefined confounding factors). Statistical testing was conducted at the two-tailed α-level of 0.05. Data were analyzed using the SAS software version 9.4 (SAS Institute, Cary, NC).

3. Results

3.1. Demographic and clinical characteristics

All demographic and clinical variables are shown in Table 1. The median caffeine consumption was of 250.6 mg/day (Interquartile range or IQR: [110.1; 430.0]). There was no significant difference in caffeine consumption between patients exhibiting spinal (median: 266.6 [138.4; 456.0] mg/day) or bulbar (median: 236.4 [93.5; 380.7] mg/day) phenotypes ($p = 0.17$). Most of the patients were non-smokers (325 out of

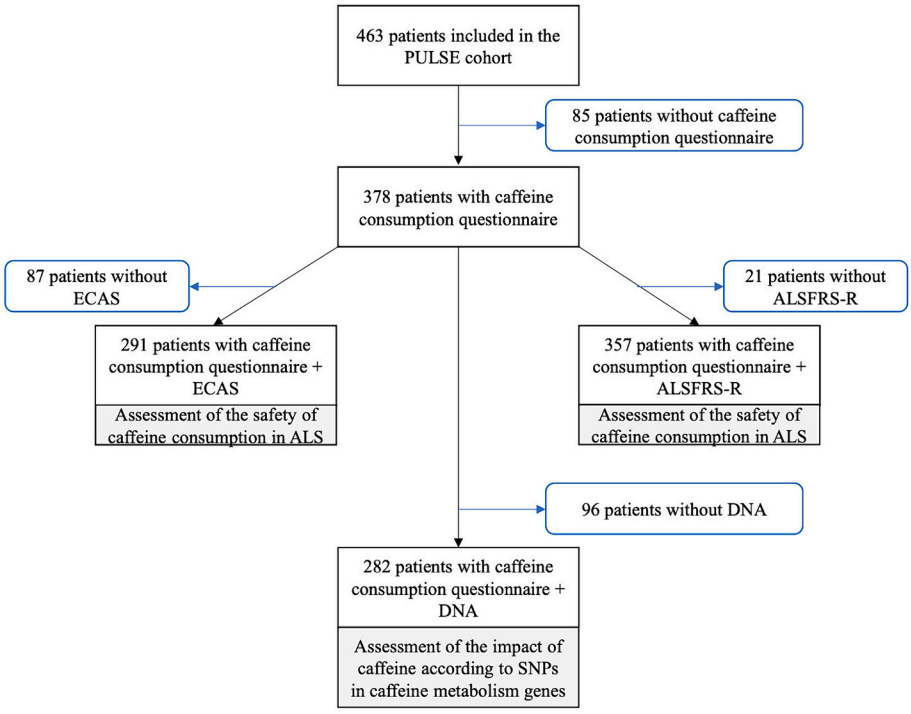

Fig. 1. Flowchart of the study. ALS = Amyotrophic Lateral Sclerosis; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Score -Revised; ECAS = Edinburgh Cognitive and Behavioural ALS Screen; PULSE = Study of Predictive Factors of Progression of Motor Neuron Disease; SNP = Single Nucleotid Polymorphism.

Table 1
Patients' characteristics and outcomes description.

	N	Value
Population characteristics		
Male gender	376	229 (60.9)
Age (years)	376	62.2 ± 10.8
Caucasian (%)	374	361 (96.5)
Body Mass Index (BMI)	370	25.1 ± 4.4
Alcohol consumption (number of glass/day)	378	0.1 (0.0 to 1.1)
Tobacco consumption (number of cigarette/day)	378	0 (0 to 0)
Education level (years)	372	12.4 ± 3.0
Riluzole treatment	372	290 (78.0)
Time from first symptoms to inclusion (years)	340	13.0 (8.0 to 23.0)
Dysphagia	358	63 (17.6)
Spinal phenotype	364	269 (73.9)
Bulbar phenotype	364	89 (24.5)
Fronto-lobar degeneration phenotype	364	6 (1.6)
Caffeine consumption at inclusion (mg/day)	378	250.6 (110.1 to 430.0)
Caffeine consumption duration (years)	272	43 (34 to 51)
Clinical outcomes		
ALSFRS-R (/48)	357	38.3 ± 6.0
ALSFRS-R rate	323	0.6 (0.3 to 1.1)
ECAS (/136)	291	104.6 ± 17.2

Values are expressed as number (percentage) for qualitative variables and mean ± standard deviation or median (1st quartile to 3rd quartile) for quantitative variables. Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ECAS = Edinburgh Cognitive and Behavioural ALS Screen.

378). The median consumption period was 43 years [34 to 51]. The median survival after diagnosis was 1.9 years [1.1 to 4.5]. The survival curve of patients is shown in **Supplementary Fig. 1**.

3.2. Impact of regular caffeine consumption on ALS disease progression and cognition

After adjustment for the predefined confounding factors (age, BMI, gender, time from first symptoms to inclusion, education level, dysphagia as well as consumptions of tobacco, alcohol, riluzole and psychotropic drugs), we did not observe any association between caffeine consumption and patient's survival ($p = 0.25$), ALSFRS-R ($p = 0.27$) as well as ALSFRS-R rate ($p = 0.076$). In addition, we found no association between caffeine consumption and cognitive performance at the ECAS ($p = 0.22$) (Table 2).

3.3. Impact of regular caffeine consumption on ALS according to gene polymorphisms involved in caffeine metabolism

Among the 378 patients, 282 were genotyped for six SNPs in *CYP1A1*, *CYP1A2*, *AHR*, *POR*, *XDH* and *ADORA2A* genes (**Supplementary Table S1**). All SNPs were in Hardy-Weinberg equilibrium. As the SNPs rs2472297, rs4410790, rs17685, rs5751876 respectively in the *CYP1A1*, *AHR*, *POR* and *ADORA2A* genes have been associated with caffeine consumption (Cornelis et al., 2016, 2007; Sulem et al., 2011; The Coffee and Caffeine Genetics Consortium et al., 2015), we first assessed whether, in our cohort, the caffeine consumption differed according to the genotypes of the six SNPs tested. No difference was observed (**Supplementary Table S2**). After adjustment, SNPs in *CYP1A2*, *AHR*, *POR* and *XDH* had no significant impact on the relationship between caffeine and ALSFRS-R, ALSFRS-R rate and survival. The SNP rs2472297 in *CYP1A1* gene was the only one affecting the relationship between caffeine consumption and ECAS score (p-het = 0.004). Indeed, a higher consumption of caffeine was associated with a higher ECAS score in the (C/T + T/T) genotypes ($N = 64$; estimate for a 300 mg/day increase: 6.92 [95%CI: 2.42 to 11.42]; $p = 0.003$), whereas there was no association with the C/C genotype ($N = 127$; $p = 0.81$) (**Supplementary Table S3** and Fig. 2).

Table 2
Association between caffeine and clinical outcomes.

	Unadjusted			Adjusted		
	N	Effect size (95% CI)	p-value	N	Effect size (95% CI)	*p*-value
Survival	377	0.89 (0.79 to 1.00)	0.047	326	0.93 (0.81 to 1.06)	0.25
ALSFRS-R	357	0.10 (−0.45 to 0.64)	0.72	312	−0.33 (−0.91 to 0.26)	0.27
ALSFRS-R rate	319	−0.01 (−0.10 to 0.08)[1]	0.831	308	0.07 (−0.01 to 0.14)[1]	0.0761
ECAS	292	1.21 (−0.51 to 2.94)	0.17	255	1.07 (−0.65 to 2.79)	0.22

Values are expressed as number (percentage) for qualitative variables and mean ± standard deviation or median (1st quartile to 3rd quartile) for quantitative variables. Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ECAS = Edinburgh Cognitive and Behavioural ALS Screen.
[1] Calculated on log-transformed data.

4. Discussion

Our study supports the safety of regular caffeine consumption in ALS patients, with no detrimental impact on survival and clinical endophenotypes, providing an answer to a legitimate dietary question. The regular/chronic caffeine consumption of our population has a median similar as those reported in other epidemiological studies (Fondell et al., 2015; Fredholm et al., 1999). However, conversely to most previous studies addressing consumption as the number of cups or units, our survey allowed a precise determination of caffeine intake per patient.

In the present study, we evaluated the precise consumption of caffeine at the time of inclusion, i.e. at the time of the diagnosis and recorded the number of years of their regular/chronic caffeine intake before this announcement. This may be a limitation as we cannot rule out that caffeine intake measured might be different from the consumption the previous 10 or 20 years and that related association with SNPs might have been different, because consumption might change during life or be dependent of life-threatening conditions. At this stage, answering that question would not be retrospectively manageable considering the short median survival of included ALS patients (1.9 years). Large-scale prospective study monitoring nutrition and disease development, including caffeine and ALS will be thus needed to precisely assess the role of long-term caffeine intake. However, we assume that the amount we measured likely reflects long-term intake of patients rather than actual intake. Indeed, the intake is measured at the time of diagnosis, a very early pathological stage, and the consumption measured reflects most likely patients' usual consumption before the disease. Further, the median caffeine consumption of the patient' French population we measured at inclusion (250 mg/d) does not differ significantly when compared to a previous evaluation reported within the French general population (239 mg/d; (Fredholm et al., 1999)). We therefore consider that the effects observed are rather ascribed to long-term caffeine intake. Another limit of our study may be the impact of caffeine on the different comorbidity frequently associated with ALS, such as depression (Thakore and Pioro, 2016) itself strongly associated with cognitive abilities (Rock et al., 2014) a point that we did not address. Further studies are therefore warranted to evaluate the impact of caffeine on anxiety or depression in ALS patients and the presumable link to cognition, especially when refereed to the genetic association with the *CYP1A1* gene uncovered in the present study.

We studied six SNPs tagging different genes involved in caffeine metabolism or intake. In particular, we performed the genotyping of the SNPs rs2472297, rs4410790, rs17685, rs5751876 respectively in the *CYP1A1*, *AHR*, *POR* and *ADORA2A* genes. These SNPs have been associated with caffeine consumption (Cornelis et al., 2016, 2007; Sulem et al., 2011; The Coffee and Caffeine Genetics Consortium et al., 2015). In our cohort, possibly due to the relatively small size and to the small effect originally reported, we found no association with caffeine

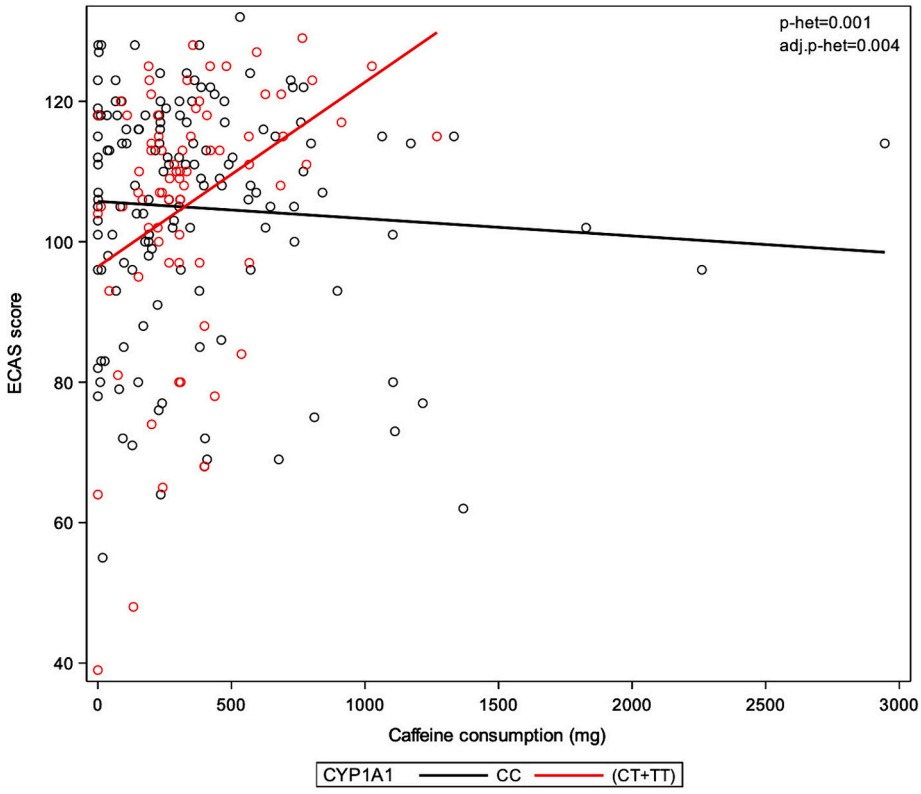

Fig. 2. Impact of caffeine on ECAS score according to rs2472297 in *CYP1A1* gene. Abbreviations: ECAS = Edinburgh Cognitive and Behavioural ALS Screen.

consumption. We found no association of the *AHR*, *POR* and *ADORA2A* genotypes with survival and clinical scores of ALS patients.

Interestingly, we found that caffeine consumption was significantly correlated with the ECAS score in the patients with rs2472297 (*CYP1A1*) C/T and T/T genotypes. To our knowledge, this is the first demonstration of a beneficial effect of caffeine on cognition in ALS patients, according to *CYP1A1/CYP1A2* genotype. The SNP rs2472297 is in the intergenic region between *CYP1A1* and *CYP1A2* genes on chromosome 15q24. The closest gene is *CYP1A1* at ~10 kb. It is not reported to be an expression quantitative locus for *CYP1A1* or *CYP1A2* genes in the Genotype-Tissue Expression database (https://gtexportal.org/home/snp/rs2472297). However, rs2472297 tags an intergenic region including a bidirectional promoter of both *CYP1A1* and *CYP1A2* genes and multiple xenobiotic response elements, which could regulate the transcription of both genes (Corchero et al., 2001). Several genome-wide association studies showed that the minor allele T of rs2472297 is associated with an increased metabolism of caffeine (Cornelis et al., 2016; Yin et al., 2022) that could thus be assimilated to a "faster caffeine metabolizer" phenotype as compared to the major allele C. Noteworthy, the T allele of rs2472297 was previously associated with an increased caffeine consumption of coffee or tea (Said et al., 2020; Zhong et al., 2019) even though the effect was quite low (Sulem et al., 2011) and has not been observed here. This finding might have clinical consequences. Indeed, it has been described that cognitive symptoms have a profound impact on the management of ALS, affecting treatment compliance, decision-making, social interactions and thus the prevention of life-threatening complications (Caga et al., 2019). Accordingly, other studies have demonstrated a strong correlation between cognitive performance and the severity of the disease progression (Elamin et al., 2013; Massman et al., 1996). Therefore, the impact of caffeine consumption on the cognition of patients carrying the T allele of the *CYP1A1* gene may have larger clinical consequences. We did not observe any significant impact on patients' survival or disease progression, but this may be due to insufficient statistical power and needs to be tested in a larger number of subjects with stratification according to genotypes.

In this study, due to the size of our cohort, we focused on (i) SNPs the most significantly associated with caffeine metabolism and/or intake in genome wide association studies, (ii) SNPs frequently reported in the literature or (iii) SNP reported as expression quantitative locus (*in cis*) of genes involved in caffeine intake and/or metabolism. This may be considered as a limitation. However, there are other SNPs and genes involved in metabolism or in the pharmacological action of caffeine that should be considered in larger replication studies. Moreover, our French cohort essentially includes Caucasian patients. Of course, the findings might differ when applied to more diverse populations with different genetic backgrounds, lifestyles, or in different geographical regions. Our work should therefore be considered as an exploratory study that will need replication in larger cohorts of patients from different genetic background, lifestyle and environment to allow the generalization of our findings and their application in clinical practice.

Our data suggest an association between cognitive status and an allele predisposing to faster caffeine metabolism and lower plasma concentration, which is singular with regards to the literature (Lefèvre-Arbogast et al., 2024). Interestingly, while studies addressing the association between caffeine consumption and pathological conditions generally reported U- or J-type associations, in favor of a detrimental effect of higher caffeine intake (Poole et al., 2017; Van Dam and Hu, 2005), we observed here, in the rs2472297 C/T and T/T genotypes carriers, a linear relationship between the caffeine intake and the ECAS total score.

Mechanisms underlying the present association between cognitive status and caffeine remain to be clarified. We can however put forward several hypotheses. As abovementioned, caffeine, as a stimulant, may influence anxiety or mood in some ALS patients and indirectly have an impact on the results of cognitive testing. One other possibility is that caffeine interacts with the effect of riluzole, currently the only disease-modifying treatment shown to extend life in patients with ALS (Bensimon et al., 1994). For example, the metabolism of riluzole is mostly

hepatic and consists of cytochrome P450-dependent hydroxylation and glucuronidation. Its clearance is highly variable among individuals and part of the variability observed in the pharmacokinetics of riluzole is explained by CYP1A2 activity (Van Kan et al., 2005). In our cohort, it remains thus possible that high caffeine intake may have prevented the neutralization of riluzole treatment by the cytochrome P450 enzymatic activity, in the fast metabolizer population i.e. rs2472297 C/T and T/T carriers. One of riluzole's mode of action is thought to be linked to an inhibition of glutamatergic release and *N*-methyl-D-aspartate receptors-related function (NMDAR) (Bryson et al., 1996; Miller et al., 2012). Caffeine has a complex action regarding NMDAR activity (Martins et al., 2020). Interestingly, although the results were not replicated (Kim et al., 2018), caffeine action was originally suggested to interact with polymorphisms in a gene coding a NMDA receptor subunit in Parkinson's disease (Hamza et al., 2011) thereby modifying riluzole action in ALS patients. Another, non-mutually exclusive possibility is that, more than caffeine itself, the beneficial outcome on cognition relies on the effects of caffeine metabolites. These possibilities deserve closer attention since they could lead to the development of new therapies and/or the adaptation of patients' diets. Finally, it might also be useful to test cytochrome P450 enzymatic activity in ALS patients using caffeine as a test drug (Faber et al., 2005). Fast metabolizers could be offered personalized medicine with adaptation of riluzole treatment with the addition of caffeine.

5. Conclusion

In conclusion, our data are in favor of a safety of caffeine consumption in ALS patients. Combining our epidemiological study on ALS endophenotypes with genetics allowed us to uncover a significant association between ECAS score and rs2472297 (*CYP1A1*) (C/T + T/T) genotypes. Further work is needed to better understand the outcomes of caffeine and underlying genetics in ALS.

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CRediT authorship contribution statement

Vincent Huin: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **David Blum:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Violette Delforge:** Formal analysis, Investigation, Methodology. **Emeline Cailliau:** Formal analysis. **Sofia Djeziri:** Formal analysis. **Kathy Dujardin:** Conceptualization. **Alexandre Genet:** Data curation. **Romain Viard:** Data curation. **Shahram Attarian:** Data curation. **Gaelle Bruneteau:** Data curation. **Julien Cassereau:** Data curation. **Steeve Genestet:** Data curation. **Anne-Laure Kaminsky:** Data curation. **Marie-Hélène Soriani:** Data curation. **Mathilde Lefilliatre:** Data curation. **Sophie Pittion-Vouyovitch:** Data curation. **Florence Esselin:** Data curation. **Elisa De La Cruz:** Data curation. **Nathalie Guy:** Data curation. **Ivan Kolev:** Data curation. **Philippe Corcia:** Data curation. **Pascal Cintas:** Data curation. **Claude Desnuelle:** Data curation. **Luc Buée:** Writing – review & editing. **Véronique Danel-Brunaud:** Data curation. **David Devos:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Anne-Sophie Rolland:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The authors have declared that no conflict of interest exists.

Data availability

Data supporting our study findings are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

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References

Abrahams, S., Newton, J., Niven, E., Foley, J., Bak, T.H., 2014. Screening for cognition and behaviour changes in ALS. Amyotroph. Lateral Scler. Front. Degener. 15, 9–14. https://doi.org/10.3109/21678421.2013.805784.

Arnaud, M.J., 2011. Pharmacokinetics and metabolism of natural Methylxanthines in animal and man. In: Methylxanthines, Handbook of Experimental Pharmacology. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 33–91. https://doi.org/10.1007/978-3-642-13443-2_3.

Beghi, E., Pupillo, E., Messina, P., Giussani, G., Chiò, A., Zoccolella, S., Moglia, C., Corbo, M., Logroscino, G., 2011. Coffee and amyotrophic lateral sclerosis: a possible preventive role. Am. J. Epidemiol. 174, 1002–1008. https://doi.org/10.1093/aje/kwr229.

Bensimon, G., Lacomblez, L., Meininger, V., 1994. A controlled trial of Riluzole in amyotrophic lateral sclerosis. N. Engl. J. Med. 330, 585–591. https://doi.org/10.1056/NEJM199403033300901.

Borota, D., Murray, E., Keceli, G., Chang, A., Watabe, J.M., Ly, M., Toscano, J.P., Yassa, M.A., 2014. Post-study caffeine administration enhances memory consolidation in humans. Nat. Neurosci. 17, 201–203. https://doi.org/10.1038/nn.3623.

Brooks, B.R., Miller, R.G., Swash, M., Munsat, T.L., 2000. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 1, 293–299. https://doi.org/10.1080/146608200300079536.

Bryson, H.M., Fulton, B., Benfield, P., 1996. Riluzole: a review of its Pharmacodynamic and pharmacokinetic properties and therapeutic potential in amyotrophic lateral sclerosis. Drugs 52, 549–563. https://doi.org/10.2165/00003495-199652040-00010.

Caga, J., Hsieh, S., Lillo, P., Dudley, K., Mioshi, E., 2019. The impact of cognitive and behavioral symptoms on ALS patients and their caregivers. Front. Neurol. 10, 192. https://doi.org/10.3389/fneur.2019.00192.

Cedarbaum, J.M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B., Nakanishi, A., 1999. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. J. Neurol. Sci. 169, 13–21. https://doi.org/10.1016/S0022-510X(99)00210-5.

Chen, J.-F., Schwarzschild, M.A., 2020. Do caffeine and more selective adenosine A2A receptor antagonists protect against dopaminergic neurodegeneration in Parkinson's disease? Parkinsonism Relat. Disord. 80, S45–S53. https://doi.org/10.1016/j.parkreldis.2020.10.024.

Corchero, J., Pimprale, S., Kimura, S., Gonzalez, F.J., 2001. Organization of the CYP1A cluster on human chromosome 15: implications for gene regulation. Pharmacogenetics 11, 1–6. https://doi.org/10.1097/00008571-200102000-00001.

Cornelis, M.C., El-Sohemy, A., Campos, H., 2007. Genetic polymorphism of the adenosine A2A receptor is associated with habitual caffeine consumption. Am. J. Clin. Nutr. 86, 240–244. https://doi.org/10.1093/ajcn/86.1.240.

Cornelis, M.C., Kacprowski, T., Menni, C., Gustafsson, S., Pivin, E., Adamski, J., Artati, A., Eap, C.B., Ehret, G., Friedrich, N., Ganna, A., Guessous, I., Homuth, G., Lind, L., Magnusson, P.K., Mangino, M., Pedersen, N.L., Pietzner, M., Suhre, K., Völzke, H., Swiss Kidney Project on Genes in Hypertension (SKIPOGH) team, Bochud, M., Spector, T.D., Grabe, H.J., Ingelsson, E., 2016. Genome-wide association study of caffeine metabolites provides new insights to caffeine metabolism and dietary caffeine-consumption behavior. Hum. Mol. Genet. ddw334 https://doi.org/10.1093/hmg/ddw334.

Cucovici, A., Ivashynka, A., Fontana, A., Russo, S., Mazzini, L., Mandrioli, J., Lisnic, V., Muresanu, D.F., Leone, M.A., 2021. Coffee and tea consumption impact on amyotrophic lateral sclerosis progression: a multicenter cross-sectional study. Front. Neurol. 12, 637939 https://doi.org/10.3389/fneur.2021.637939.

Cunha, R.A., 2016. How does adenosine control neuronal dysfunction and neurodegeneration? J. Neurochem. 139, 1019–1055. https://doi.org/10.1111/jnc.13724.

Elamin, M., Bede, P., Byrne, S., Jordan, N., Gallagher, L., Wynne, B., O'Brien, C., Phukan, J., Lynch, C., Pender, N., Hardiman, O., 2013. Cognitive changes predict functional decline in ALS: a population-based longitudinal study. Neurology 80, 1590–1597. https://doi.org/10.1212/WNL.0b013e31828f18ac.

Faber, M.S., Jetter, A., Fuhr, U., 2005. Assessment of CYP1A2 activity in clinical practice: why, how, and when? Basic Clin. Pharmacol. Toxicol. 97, 125–134. https://doi.org/10.1111/j.1742-7843.2005.pto_973160.x.

Feldman, E.L., Goutman, S.A., Petri, S., Mazzini, L., Savelieff, M.G., Shaw, P.J., Sobue, G., 2022. Amyotrophic lateral sclerosis. Lancet 400, 1363–1380. https://doi.org/10.1016/S0140-6736(22)01272-7.

Fondell, E., O'Reilly, Éi J., Fitzgerald, K.C., Falcone, G.J., Kolonel, L.N., Park, Y., Gapstur, S.M., Ascherio, A., 2015. Intakes of caffeine, coffee and tea and risk of amyotrophic lateral sclerosis: results from five cohort studies. Amyotroph. Lateral Scler. Front. Degener. 16, 366–371. https://doi.org/10.3109/21678421.2015.1020813.

Fredholm, B.B., Bättig, K., Holmén, J., Nehlig, A., Zvartau, E.E., 1999. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 51, 83–133.

Freedman, N.D., Park, Y., Abnet, C.C., Hollenbeck, A.R., Sinha, R., 2012. Association of Coffee Drinking with Total and cause-specific mortality. N. Engl. J. Med. 366, 1891–1904. https://doi.org/10.1056/NEJMoa1112010.

Hamza, T.H., Chen, H., Hill-Burns, E.M., Rhodes, S.L., Montimurro, J., Kay, D.M., Tenesa, A., Kusel, V.I., Sheehan, P., Eaaswarkhanth, M., Yearout, D., Samii, A., Roberts, J.W., Agarwal, P., Bordelon, Y., Park, Y., Wang, L., Gao, J., Vance, J.M., Kendler, K.S., Bacanu, S.-A., Scott, W.K., Ritz, B., Nutt, J., Factor, S.A., Zabetian, C. P., Payami, H., 2011. Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. PLoS Genet. 7, e1002237 https://doi.org/10.1371/journal.pgen.1002237.

Kim, I.Y., O'Reilly, É.J., Hughes, K.C., Gao, X., Schwarzschild, M.A., McCullough, M.L., Hannan, M.T., Betensky, R.A., Ascherio, A., 2018. Interaction between caffeine and polymorphisms of glutamate ionotropic receptor NMDA type subunit 2A (*GRIN2A*) and cytochrome P450 1A2 (*CYP1A2*) on Parkinson's disease risk. Mov. Disord. 33, 414–420. https://doi.org/10.1002/mds.27279.

Lefèvre-Arbogast, S., Helmer, C., Berr, C., Debette, S., Samieri, C., 2024. Habitual coffee consumption and risk of dementia in older persons: modulation by CYP1A2 polymorphism. Eur. J. Epidemiol. 39, 81–86. https://doi.org/10.1007/s10654-023-01060-x.

Martins, R.S., Rombo, D.M., Gonçalves-Ribeiro, J., Meneses, C., Borges-Martins, V.P.P., Ribeiro, J.A., Vaz, S.H., Kubrusly, R.C.C., Sebastião, A.M., 2020. Caffeine has a dual influence on NMDA receptor–mediated glutamatergic transmission at the hippocampus. Purinergic Signal 16, 503–518. https://doi.org/10.1007/s11302-020-09724-z.

Massman, P.J., Sims, J., Cooke, N., Haverkamp, L.J., Appel, V., Appel, S.H., 1996. Prevalence and correlates of neuropsychological deficits in amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 61, 450–455. https://doi.org/10.1136/jnnp.61.5.450.

Miller, R.G., Mitchell, J.D., Moore, D.H., 2012. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.CD001447.pub3.

Nehlig, A., 2018. Interindividual differences in caffeine metabolism and factors driving caffeine consumption. Pharmacol. Rev. 70, 384–411. https://doi.org/10.1124/pr.117.014407.

Petimar, J., O'Reilly, É., Adami, H.-O., Van Den Brandt, P.A., Buring, J., English, D.R., Freedman, D.M., Giles, G.G., Håkansson, N., Kurth, T., Larsson, S.C., Robien, K., Schouten, L.J., Weiderpass, E., Wolk, A., Smith-Warner, S.A., 2019. Coffee, tea, and caffeine intake and amyotrophic lateral sclerosis mortality in a pooled analysis of eight prospective cohort studies. Eur. J. Neurol. 26, 468–475. https://doi.org/10.1111/ene.13840.

Poole, R., Kennedy, O.J., Roderick, P., Fallowfield, J.A., Hayes, P.C., Parkes, J., 2017. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. BMJ j5024. https://doi.org/10.1136/bmj.j5024.

Potenza, R.L., Armida, M., Ferrante, A., Pèzzola, A., Matteucci, A., Puopolo, M., Popoli, P., 2013. Effects of chronic caffeine intake in a mouse model of amyotrophic lateral sclerosis. J. Neurosci. Res. 91, 585–592. https://doi.org/10.1002/jnr.23185.

Pupillo, E., Bianchi, E., Chiò, A., Casale, F., Zecca, C., Tortelli, R., Beghi, E., For Slalom, Parals, and for Slap Groups, 2018. Amyotrophic lateral sclerosis and food intake. Amyotroph. Lateral Scler. Front. Degener. 19, 267–274. https://doi.org/10.1080/21678421.2017.1418002.

Rock, P.L., Roiser, J.P., Riedel, W.J., Blackwell, A.D., 2014. Cognitive impairment in depression: a systematic review and meta-analysis. Psychol. Med. 44, 2029–2040. https://doi.org/10.1017/S0033291713002535.

Ross, G.W., 2000. Association of Coffee and Caffeine Intake with the risk of Parkinson disease. JAMA 283, 2674. https://doi.org/10.1001/jama.283.20.2674.

Sachse, C., Brockmöller, J., Bauer, S., Roots, I., 1999. Functional significance of a C→a polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br. J. Clin. Pharmacol. 47, 445–449. https://doi.org/10.1046/j.1365-2125.1999.00898.x.

Said, M.A., Van De Vegte, Y.J., Verweij, N., Van Der Harst, P., 2020. Associations of observational and genetically determined caffeine intake with coronary artery disease and diabetes mellitus. J. Am. Heart Assoc. 9, e016808 https://doi.org/10.1161/JAHA.120.016808.

Simonin, C., Duru, C., Salleron, J., Hincker, P., Charles, P., Delval, A., Youssov, K., Burnouf, S., Azulay, J.-P., Verny, C., Scherer, C., Tranchant, C., Goizet, C., Debruxelles, S., Defebvre, L., Sablonnière, B., Romon-Rousseaux, M., Buée, L., Destée, A., Godefroy, O., Dürr, A., Landwehrmeyer, B., Bachoud-Levi, A.-C., Richard, F., Blum, D., Krystkowiak, P., 2013. Association between caffeine intake and age at onset in Huntington's disease. Neurobiol. Dis. 58, 179–182. https://doi.org/10.1016/j.nbd.2013.05.013.

Siokas, V., Karampinis, E., Aloizou, A.-M., Mentis, A.-F.A., Liakos, P., Papadimitriou, D., Liampas, I., Nasios, G., Bogdanos, D.P., Hadjigeorgiou, G.M., Dardiotis, E., 2021. CYP1A2 rs762551 polymorphism and risk for amyotrophic lateral sclerosis. Neurol. Sci. 42, 175–182. https://doi.org/10.1007/s10072-020-04535-x.

Sulem, P., Gudbjartsson, D.F., Geller, F., Prokopenko, I., Feenstra, B., Aben, K.K.H., Franke, B., Den Heijer, M., Kovacs, P., Stumvoll, M., Mägi, R., Yanek, L.R., Becker, L. C., Boyd, H.A., Stacey, S.N., Walters, G.B., Jonasdottir, A., Thorleifsson, G., Holm, H., Gudjonsson, S.A., Rafnar, T., Björnsdottir, G., Becker, D.M., Melbye, M., Kong, A., Tönjes, A., Thorgeirsson, T., Thorsteinsdottir, U., Kiemeney, L.A., Stefansson, K., 2011. Sequence variants at CYP1A1–CYP1A2 and AHR associate with coffee consumption. Hum. Mol. Genet. 20, 2071–2077. https://doi.org/10.1093/hmg/ddr086.

Thakore, N.J., Pioro, E.P., 2016. Depression in ALS in a large self-reporting cohort. Neurology 86, 1031–1038. https://doi.org/10.1212/WNL.0000000000002465.

The Coffee and Caffeine Genetics Consortium, Cornelis, M.C., Byrne, E.M., Esko, T., Nalls, M.A., Ganna, A., Paynter, N., Monda, K.L., Amin, N., Fischer, K., Renstrom, F., Ngwa, J.S., Huikari, V., Cavadino, A., Nolte, I.M., Teumer, A., Yu, K., Marques-Vidal, P., Rawal, R., Manichaikul, A., Wojczynski, M.K., Vink, J.M., Zhao, J.H., Burlutsky, G., Lahti, J., Mikkilä, V., Lemaitre, R.N., Eriksson, J., Musani, S.K., Tanaka, T., Geller, F., Luan, J., Hui, J., Mägi, R., Dimitriou, M., Garcia, M.E., Ho, W.-K., Wright, M.J., Rose, L.M., Magnusson, P.K.E., Pedersen, N.L., Couper, D., Oostra, B.A., Hofman, A., Ikram, M.A., Tiemeier, H.W., Uitterlinden, A.G., Van Rooij, F.J.A., Barroso, I., Johansson, I., Xue, L., Kaakinen, M., Milani, L., Power, C., Snieder, H., Stolk, R.P., Baumeister, S.E., Biffar, R., Gu, F., Bastardot, F., Kutalik, Z., Jacobs, D.R., Forouhi, N.G., Mihailov, E., Lind, L., Lindgren, C., Michaëlsson, K., Morris, A., Jensen, M., Khaw, K.-T., Luben, R.N., Wang, J.J., Männistö, S., Perälä, M.-M., Kähönen, M., Lehtimäki, T., Viikari, J., Mozaffarian, D., Mukamal, K., Psaty, B. M., Döring, A., Heath, A.C., Montgomery, G.W., Dahmen, N., Carithers, T., Tucker, K.L., Ferrucci, L., Boyd, H.A., Melbye, M., Treur, J.L., Mellström, D., Hottenga, J.J., Prokopenko, I., Tönjes, A., Deloukas, P., Kanoni, S., Lorentzon, M., Houston, D.K., Liu, Y., Danesh, J., Rasheed, A., Mason, M.A., Zonderman, A.B., Franke, L., Kristal, B.S., International Parkinson's Disease Genomics Consortium (IPDGC), North American Brain Expression Consortium (NABEC), UK Brain Expression Consortium (UKBEC), Karjalainen, J., Reed, D.R., Westra, H.-J., Evans, M.K., Saleheen, D., Harris, T.B., Dedoussis, G., Curhan, G., Stumvoll, M., Beilby, J., Pasquale, L.R., Feenstra, B., Bandinelli, S., Ordovas, J.M., Chan, A.T., Peters, U., Ohlsson, C., Gieger, C., Martin, N.G., Waldenberger, M., Siscovick, D.S., Raitakari, O., Eriksson, J.G., Mitchell, P., Hunter, D.J., Kraft, P., Rimm, E.B., Boomsma, D.I., Borecki, I.B., Loos, R.J.F., Wareham, N.J., Vollenweider, P., Caporaso, N., Grabe, H.J., Neuhouser, M.L., Wolffenbuttel, B.H.R., Hu, F.B., Hyppönen, E., Järvelin, M.-R., Cupples, L.A., Franks, P.W., Ridker, P.M., Van Duijn, C.M., Heiss, G., Metspalu, A., North, K.E., Ingelsson, E., Nettleton, J.A., Van Dam, R.M., Chasman, D.I., 2015. Genome-wide meta-analysis identifies six novel loci associated with habitual coffee consumption. Mol. Psychiatry 20, 647–656. https://doi.org/10.1038/mp.2014.107.

Van Dam, R.M., Hu, F.B., 2005. Coffee consumption and risk of type 2 diabetes: a systematic review. JAMA 294, 97. https://doi.org/10.1001/jama.294.1.97.

Van Dam, R.M., Hu, F.B., Willett, W.C., 2020. Coffee, caffeine, and health. N. Engl. J. Med. 383, 369–378. https://doi.org/10.1056/NEJMra1816604.

Van Kan, H.J.M., Groeneveld, G.J., Kalmijn, S., Spieksma, M., Van Den Berg, L.H., Guchelaar, H.J., 2005. Association between CYP1A2 activity and riluzole clearance in patients with amyotrophic lateral sclerosis. Br. J. Clin. Pharmacol. 59, 310–313. https://doi.org/10.1111/j.1365-2125.2004.02233.x.

Yelanchezian, Y.M.M., Waldvogel, H.J., Faull, R.L.M., Kwakowsky, A., 2022. Neuroprotective effect of caffeine in Alzheimer's disease. Molecules 27, 3737. https://doi.org/10.3390/molecules27123737.

Yin, B., Wang, X., Huang, T., Jia, J., 2022. Shared genetics and causality between decaffeinated coffee consumption and neuropsychiatric diseases: a large-scale

genome-wide cross-trait analysis and Mendelian randomization analysis. Front. Psych. 13, 910432 https://doi.org/10.3389/fpsyt.2022.910432.
Zhong, V.W., Kuang, A., Danning, R.D., Kraft, P., Van Dam, R.M., Chasman, D.I., Cornelis, M.C., 2019. A genome-wide association study of bitter and sweet beverage consumption. Hum. Mol. Genet. 28, 2449–2457. https://doi.org/10.1093/hmg/ddz061.
Zwilling, M., Theiss, C., Matschke, V., 2020. Caffeine and NAD+ improve motor neural integrity of dissociated wobbler cells in vitro. Antioxidants 9, 460. https://doi.org/10.3390/antiox9060460.