



A phenome-wide association study of genetically determined nicotine metabolism reveals novel links with health-related outcomes

Jadwiga Buchwald¹ · Terho Lehtimäki^{2,3} · Olli Raitakari^{4,5,6} · Veikko Salomaa⁷ · Jaakko Kaprio¹ · Matti Pirinen^{1,8,9}

Received: 13 September 2024 / Accepted: 23 June 2025
© The Author(s) 2025

Abstract

Faster nicotine metabolism, defined as the nicotine metabolite ratio (NMR), is known to associate with heavier smoking and challenges in smoking cessation. However, the broader health implications of genetically determined nicotine metabolism are not well characterized. We performed a hypothesis-free phenome-wide association study (PheWAS) of over 21,000 outcome variables from UK Biobank (UKB) to explore how the NMR (measured as the 3-hydroxycotinine-to-cotinine ratio) associates with the phenome. As the exposure variable, we used a genetic score for faster nicotine metabolism based on 10 putative causal genetic variants, explaining 33.8 % of the variance in the NMR. We analysed ever and never smokers separately to assess whether a causal pathway through nicotine metabolism is plausible. A total of 57 outcome variables reached phenome-wide significance at a false discovery rate of 5 %. We observed expected associations with several phenotypes related to smoking and nicotine, but could not replicate prior findings on cessation. Importantly, we found novel associations between genetically determined faster nicotine metabolism and adverse health outcomes, including unfavourable liver enzyme and lipid values, as well as increased caffeine consumption. These associations did not appear to differ between ever and never smokers, suggesting the corresponding pathways may not involve nicotine metabolism. No favourable health outcomes were linked to genetically determined faster nicotine metabolism. Our findings support a possibility that a future smoking cessation therapy converting fast metabolizers of nicotine to slower ones could work without adverse side effects and potentially even provide other health-related benefits.

Keywords Nicotine metabolism · Nicotine metabolite ratio · CYP2A6 activity · Smoking cessation · PheWAS · Phenome

Introduction

Smoking remains a leading cause of global morbidity and mortality [1]. While global smoking prevalence has declined due to effective tobacco control policies, the prevalence

has risen or remained stagnant in many countries [2]. Furthermore, the emergence of new nicotine products, such as e-cigarettes, which have aggressively been targeted at the youth, has created a whole new generation of addicted

✉ Jadwiga Buchwald
jadwiga.buchwald@helsinki.fi

¹ Institute for Molecular Medicine Finland (FIMM), Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Helsinki, Finland

² Department of Clinical Chemistry, Fimlab Laboratories, and Finnish Cardiovascular Research Center, Tampere, Finland

³ Faculty of Medicine and Health Technology, Tampere, Finland

⁴ Centre for Population Health Research, University of Turku and Turku University Hospital, Turku, Finland

⁵ Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland

⁶ Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland

⁷ Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Helsinki, Finland

⁸ Department of Mathematics and Statistics, University of Helsinki, Helsinki, Finland

⁹ Department of Public Health, University of Helsinki, Helsinki, Finland

individuals in some countries [2, 3]. Nicotine, a toxic substance in itself [4, 5], is as addictive as cocaine and heroine [6].

The Nicotine Metabolite Ratio, NMR, measured as the 3-hydroxycotinine-to-cotinine ratio (3HC/Cot), is an established biomarker for the rate of nicotine metabolism [7]. Individuals with higher NMR values, reflecting faster nicotine metabolism, typically smoke more [8, 9] and find quitting more challenging [10–12]. Personalizing cessation treatments based on an individual's NMR could improve cessation rates [13]. Another approach would be to develop smoking cessation drugs tailored to modulate metabolization rates.

The NMR is highly heritable [14], and understanding which genetic loci it associates with, can illuminate potential drug targets. The largest genome-wide association study (GWAS) on the NMR to date identified two independent association loci on chromosomes 4 and 19, explaining 1.8 % and 36.4 % of the variation in the NMR, respectively [15]. Notably, the chromosome 19 locus contains *CYP2A6*, which codes for CYP2A6, the main metabolic enzyme for nicotine [16]. Nicotine is metabolized primarily to cotinine (Cot) (~ 75 %), mainly by CYP2A6, and Cot is metabolized primarily to 3-hydroxycotinine (3HC) (~ 55 %), exclusively by CYP2A6 [16]. Thus, their ratio, 3HC/Cot, i.e. the NMR, also reflects CYP2A6 activity [7]. A smaller fraction of nicotine and cotinine is metabolized through other enzymatic pathways [16].

It is also essential to understand what traits or diseases the NMR is associated with. This knowledge is critical for assessing possible side-effects and other opportunities for drug development. A previous study by Chenoweth et al. showed the NMR to be associated with ethnicity, gender, hormonal replacement therapy, BMI, cigarettes smoked per day (CPD), and number of alcohol drinks/week [17]. In that hypothesis-driven study, the variables investigated had been carefully chosen based on previous literature. Therefore, unknown and potentially important associations may have been missed.

Phenome-wide association studies (PheWASs) present a hypothesis-free approach to discover novel associations [18]. PheWASs aim to identify associations with a genetic instrument (e.g. a single nucleotide polymorphism (SNP) or genetic risk score), proxying a given variable of interest, across an array of phenotypes (the phenome). Two PheWASs of CYP2A6 activity have been published to date. The first, assessed 358 traits for nine *CYP2A6* SNPs, and identified an association between one of the SNPs and hearing loss among the nicotine-exposed subgroup but not among the nicotine-unexposed subgroup [19]. The second, was a PheWAS of a genetic score for CYP2A6 activity in UKB [20]. However, they limited their analyses to a total of 1,029

disease endpoints, based on ICD-9 and ICD-10 diagnostic rubrics. They found associations with lung cancer and other known smoking related diseases; no associations were seen among their subsets of former or never smokers.

Our aim was to assess how the NMR is associated with the phenome without limiting ourselves to any specific category of phenotypes by using the UKB data (n = 343,662). Additionally, our objective was to assess ever and never smokers separately in order to distinguish whether the associations reflected a causal pathway through a) the NMR, either directly or through other traits such as amount smoked (effect only seen in ever smokers), b) some other pathway not including the NMR (same effect seen also in never smokers), or c) both (effect only seen in never smokers, or there are quantitative or directional differences in the effect sizes between ever and never smokers). Our findings contribute valuable information for drug development and personalized interventions for treating nicotine addiction, as well as, for example, cancer, given CYP2A6's role in metabolizing various drugs, including the chemotherapeutic agents letrozole and tegafur [21, 22].

Materials and methods

Summary of the study design

We used PHESANT [23] on the UKB data (n = 343,662) to perform a PheWAS of a genetic score for the NMR across 21,000 outcome variables. Importantly, we used the GxE MR-PheWAS approach [24] by running the PheWAS separately for ever and never smokers and comparing the results. The genetic score was based on ten putative causal SNPs, explaining 33.8% of the variance in the NMR. The ten SNPs were identified using FINEMAP [25] on NMR GWAS results from two Finnish data sets, the FINRISK and Young Finns Study.

Study samples

UK Biobank

UK Biobank (UKB) is a population-based prospective study on genetic and non-genetic determinants of diseases of middle and old age. The cohort consists of over 500,000 participants from the UK, aged between 37–73 at recruitment (2006–2010) [26]. The resource comprises imputed genome-wide genotype data from all participants [27], along with a comprehensive range of phenotypic data. This includes data from clinical assessments, questionnaires, sample assays, and health record linkage—many of which

are available from all of the participants (see Sudlow et al. [28]). Of note, the NMR is not available in the UKB data.

We had access to genetic data from 487,235 individuals after excluding withdrawals. We restricted our sample to individuals of self-reported White British ancestry, and performed further quality control measures, resulting in a final sample size of 343,662 individuals. During the quality control process, we ensured the genetic sex corresponded to the reported sex, and that there were no instances of sex aneuploidy. We also verified that our subset did not include any samples that were outliers with respect to genotype heterozygosity or missingness. Furthermore, we only retained one randomly chosen individual from each pair of third-degree or closer relatives.

FINRISK

The National FINRISK Study consists of cross-sectional population-based data on chronic non-communicable diseases in Finland [29]. Data collection was conducted every five years between 1972–2012. Our previous GWAS of the NMR ($n = 5,185$ current smokers from 5 cohorts) included 1,405 current smokers ($\text{Cot} \geq 10$ ng/ml) from the 2007 and 2012 FINRISK data collections [15]. These two cross-sectional studies included independent samples from 25–74 year old Finns (see Buchwald et al. [15], Table S1). Cot and 3HC concentrations were acquired from blood plasma samples using gas chromatography-mass spectrometry. Self-reported variables on smoking behaviour, including the number of factory and self-rolled cigarettes smoked per day (CPD), were obtained from surveys. Genome-wide genotype data were imputed using a Finnish reference panel (see Buchwald et al. [15], Table S2).

Young Finns study

The Young Finns Study (YFS) is a prospective population-based study of cardiovascular risk factors from childhood to adulthood. The initial cross-sectional sample from 1980 was selected so as to be representative of Finnish children aged 3, 6, 9, 12, 15 and 18. The individuals have been followed up at regular intervals (see Raitakari et al. [30]). Our previous GWAS of the NMR included 714 current smokers from the YFS, aged 15–45 (see Buchwald et al. [15], Table S1). For each individual we had chosen the time point for which the NMR, sex, age and BMI were available, Cot was ≥ 10 ng/ml, and for which we had the least amount of missing values concerning the other GWAS variables (CPD, Pack years, alcohol use). Whenever there were ties, the most recent time point had been chosen. Cot and 3HC concentrations were acquired from blood plasma samples using liquid chromatography-tandem mass spectrometry,

and self-reported smoking behaviour variables including CPD were obtained from surveys. Genome-wide genotype data was imputed using the Haplotype Reference Consortium reference panel (see Buchwald et al. [15], Table S2).

Measures

The genetic score for the nicotine metabolite ratio

Using the UKB data we created a genetic score (GS) for the NMR. We created it so that higher values reflect faster nicotine metabolism, and thus, also refer to it in this paper as the genetic score for faster nicotine metabolism. The GS served as the independent variable (exposure) in our PheWAS. We constructed the GS as the weighted sum of the 10 putative causal SNPs highlighted by our FINEMAP analyses (see below). These SNPs together explained 33.8 % of the variance in the NMR. Prior to running the PheWAS, we standardized the GS (zGS) by subtracting the mean and dividing by the standard deviation for a simpler interpretation of the results.

Outcome variables

We used the PheWAS software package PHESANT [23] to preprocess the phenotype data. Of the 5,559 UKB phenotype fields available to us, we included 4,546 in our PheWAS. The excluded phenotype fields were either not listed in the variable information file of PHESANT (252 dropped), were categorized as auxiliary variables in the UKB data (STRATA=Auxiliary) (further 398 dropped) or based on the grouping done by Gibson et al. in their insomnia PheWAS [31] (further 141 dropped), or were marked for exclusion in the PHESANT variable information file (further 60 dropped).

PHESANT uses a rule-based system for preprocessing and deciding which association test to use for each variable. For full details, see [23]. For example, when a phenotype has been measured at multiple time points, PHESANT automatically considers only the first occurrence. For phenotypes measured multiple times at the first occurrence to improve accuracy, PHESANT uses the mean value of those measurements. For multiple choice questions where individuals have ticked all relevant options, PHESANT forms multiple binary variables. For continuous variables, PHESANT inverse rank transforms them to normality (unless, for example, there is a notable portion of some particular value, in which case an ordered categorical variable is created). Out of the 4,546 phenotype fields, PHESANT created 21,094 outcome variables in total, meaning that our initial PheWAS consisted of 21,094 regression analyses. Each

outcome variable served as the dependent variable in its respective regression model.

Covariates

We adjusted our PheWAS for sex and age to increase statistical power and to adjust for potential confounding. We also adjusted for the first ten principal components of genetic structure (1–10 PCs) to control for confounding due to population stratification. We did not include any additional covariates to avoid inducing collider bias.

Ever-never status

We wanted to perform the PheWAS separately for ever and never smokers to distinguish whether the associations were independent of nicotine metabolism or indicative of a possible causal pathway through nicotine metabolism (Fig. 1), an approach described by Millard et al. [24]. To reduce noise, we excluded experimenters and occasional smokers from our Ever and Never subsets. We created a new Ever-Never variable based on two existing variables:

1. Current tobacco smoking ("Do you smoke tobacco now?", UKB field 1239)

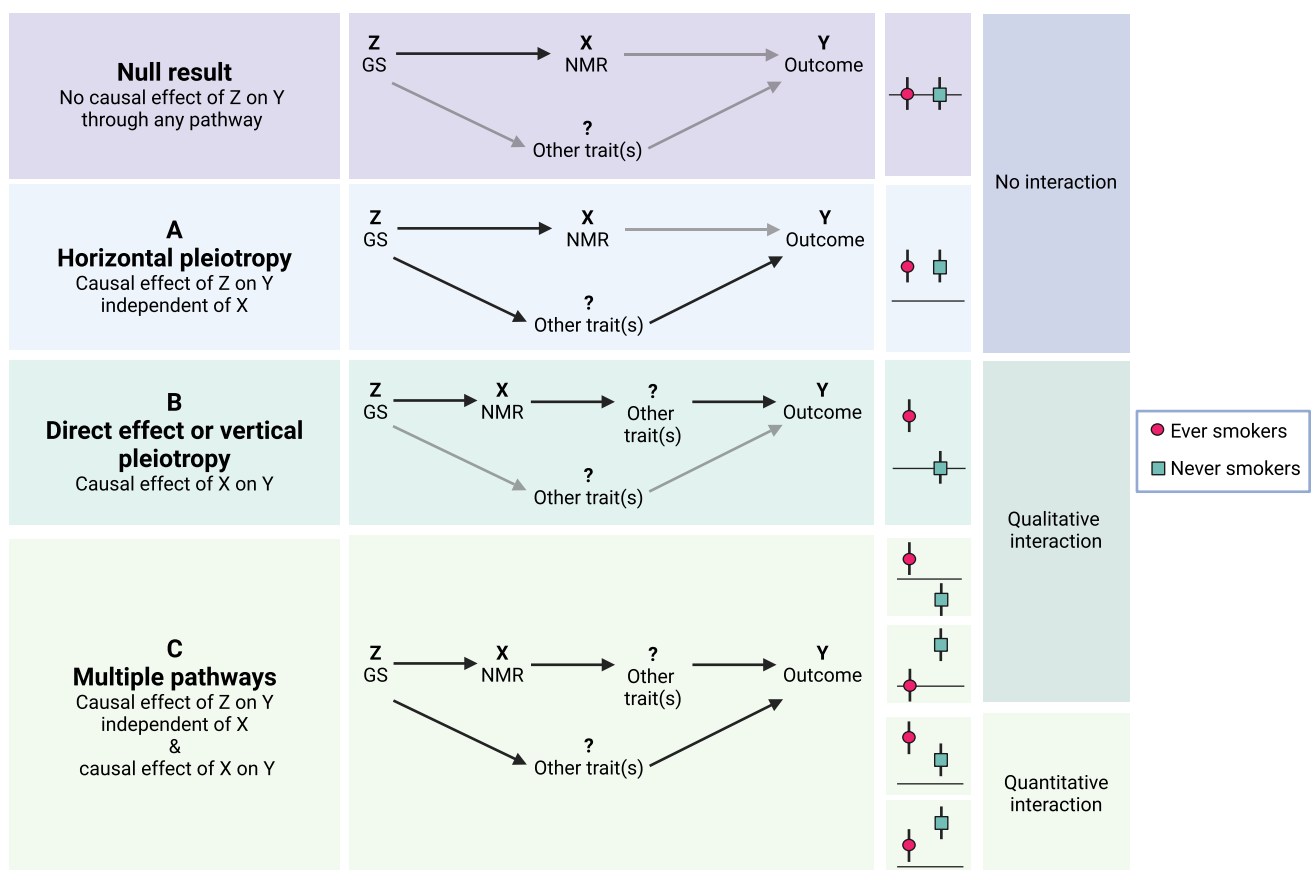


Fig. 1 Interpretations of the possible GxE MR results. The figure illustrates how the different types of results from GxE MR can be interpreted in our case, where the exposure, X, is the NMR, and the genetic instrumental variable, Z, is the genetic score for the NMR. The ever and never results can be categorized in to three types: 1) no interaction, i.e. the effect of Z on outcome Y is not affected by smoking status, 2) qualitative interaction, i.e. the effect of Z on Y is apparent only in one group or the effects are in opposing directions between the groups, and 3) quantitative interaction, i.e. the effects are consistent between the groups but of different magnitude. There are three different interpretations for the associations: **A** if an effect of the same magnitude is apparent in both groups, there is evidence for a causal effect of Z on Y through a pathway excluding X (this is known as

horizontal pleiotropy), **B** if an association is only seen among ever smokers, there is evidence for a causal effect of Z on Y through X, and for X being causally associated with Y, either directly or through another trait/other traits such as amount smoked (the latter is known as vertical pleiotropy), and **C** if the ever and never subsets show opposing effects, effects of different magnitude, or there is only an effect among never smokers, there is evidence for a causal effect of Z both through the NMR (either directly or through vertical pleiotropy) and through another pathway (horizontal pleiotropy). The figure has been modified from Millard et al. Fig. 1 [24] and the figures at: <https://mr-dictionary.mrcieu.ac.uk/term/vertical-pleiotropy/>. The figure was created with BioRender.com

2. Past tobacco smoking ("In the past, how often have you smoked tobacco?", UKB field 1249)

The second question had been asked from all except for those who indicated they currently smoke on "most or all days" in response to the first question. We classified all who answered "most or all days" to either of the two questions as ever smokers. Those who answered "no" to the first question and "never" to the second question, were classified as never smokers. All others were assigned a missing value in our Ever–Never variable, and excluded from the Ever and Never subsets. This included individuals who answered "Occasionally" or "Prefer not to answer" to either question, as well as those who responded with "Tried once or twice" to the second question.

Out of our full sample ($n = 343,662$), we classified 135,890 as never smokers and 110,348 as ever smokers. Of those who dropped out, 99 % had answered "Occasionally" or "Tried once or twice". This group had marginally higher GS values as compared to the Never group (Table S1 & Fig. S2). The GS distribution of the Ever group did not differ from the Never group (Table S1 & Fig. S2).

Statistical analyses

Figure S1 illustrates the main analyses and follow-up analyses performed in this study.

Forming the GS

We previously performed a GWAS meta-analysis of the NMR in European ancestry current smokers ($n = 5,185$) [15]. Our main GWAS model included sex, age, BMI, alcohol use (g/week) and birth year as covariates. We identified two NMR loci, one on chromosome 4 and one on chromosome

19. By fine-mapping these loci using FINEMAP [25] and the datasets YFS and FINRISK ($n = 2,119$), we found there to be only a single putative causal SNP on chromosome 4, explaining 1.7 % of the variance in the NMR, but 13 putative causal SNPs on chromosome 19 explaining 36.7 % of the variance in the NMR.

In the current study, we wanted to use the SNPs and weights obtained by the earlier FINEMAP analyses to construct the GS for the NMR in the UKB data set. Unfortunately, two of the 13 SNPs from chromosome 19 did not pass our quality control in the UKB dataset (both had a Hardy-Weinberg Equilibrium $p < 10^{-6}$). Excluding these two SNPs would have dropped the variance explained in the NMR by the chromosome 19 SNPs from 36.7 % to 23.7 %. Therefore, to gain maximal power, we reran the FINEMAP analyses for the chromosome 19 locus as described previously (see Buchwald et al. [15]) using only SNPs that were available and passed quality control in UKB (See Supplementary information). The new FINEMAP analysis of the chromosome 19 locus resulted in nine SNPs in the top configuration of putative causal SNPs, explaining 32.1 % of the variance in the NMR (Table S2). All the aforementioned estimates of the variance of the NMR explained, are the configuration-specific heritability estimates obtained by FINEMAP.

We then calculated the GS as a weighted sum of these nine chromosome 19 SNPs and a single chromosome 4 SNP, resulting in a GS explaining 33.8 % of the variance in the NMR (Table 1). For both loci, we used the largest possible sample sizes to estimate the effect sizes with the highest possible accuracy. For chromosome 19, we used the joint effect size estimates from FINEMAP as the weights in order to adjust for correlation among these SNPs. As only the datasets YFS and FINRISK were available for fine-mapping, the sample size was $n = 2,119$. For chromosome 4, as

Table 1 The ten SNPs and their weights used for calculating the GS for the NMR

SNP	CHR	BP	EA/NEA	MINOR	MAF _{UKB}	WEIGHT (MAF)
RS36103218	4	69359253	T/C	C	0.3422	-0.175 (MAF _{META} = 0.43)
RS189621498	19	41288136	A/G	A	0.0003	0.6127 (MAF _{FY} = 0.03)
RS74719953	19	41335799	T/C	T	0.0652	-0.3581 (MAF _{FY} = 0.09)
RS34945948	19	41340842	G/A	G	0.1474	1.0276 (MAF _{FY} = 0.14)
RS12985907	19	41343544	A/G	A	0.2307	-0.8003 (MAF _{FY} = 0.28)
RS1801272	19	41354533	T/A	T	0.0271	-1.0103 (MAF _{FY} = 0.02)
RS7250713	19	41355195	C/G	G	0.4386	0.3251 (MAF _{FY} = 0.37)
RS7248187	19	41437426	C/G	G	0.3023	0.3187 (MAF _{FY} = 0.24)
RS116382863	19	41534881	T/C	T	0.1242	0.2212 (MAF _{FY} = 0.13)
RS11466310	19	41861858	T/C	T	0.0244	-0.5414 (MAF _{FY} = 0.02)

For all chromosome 19 SNPs the weight has been obtained from their joint model using FINEMAP in the Finnish data. For the chromosome 4 SNP the weight is the effect size obtained from our previous GWAS [15]. *SNP*, single-nucleotide polymorphism; *CHR*, chromosome; *BP*, base pair position in GRCh37 coordinates; *EA/NEA*, the effect allele/ the non-effect allele; *MINOR*, the less common allele; *MAF*, minor allele frequency; *WEIGHT*, weight used to calculate the GS (reported for the effect allele); *UKB*, UK Biobank; *META*, data used in our previous GWAS meta-analysis ($n = 5,185$) from which the weight for the chr 4 SNP is; *FY*, FINRISK and YFS data ($n = 2,119$) used to obtain the weights for the chr 19 SNPs

there was only one putative causal SNP, and thus no need to adjust for any correlated SNPs, the effect estimate of this single chromosome 4 SNP equals to its marginal effect in the original GWAS ($n = 5,185$). Of note, FINEMAP was not able to distinguish which of the three top GWAS SNPs in the chromosome 4 locus was most likely to be the causal one (the posterior probability for there being one causal SNP in the region was 0.856) [15]. All three SNPs had the same GWAS p-value and were perfectly correlated. Also FINEMAP gave the same posterior inclusion probability (PIP) of 0.050 for all three SNPs. For the GS of the NMR, we chose to use the SNP located in the middle based on base pair position.

To assess the performance and reliability of our GS, we plotted it against the NMR top SNP (rs56113850) in the UKB data. In our previous study, the top SNP alone explained 23 % of the variance in the NMR (see Buchwald et al [15], Table S5). Among current smokers, we also plotted cigarettes smoked per day (CPD) against our GS as CPD has been shown to associate positively with the NMR [8, 9]. Additionally, we tested the association of the GS with CPD with a linear model. We did this by first regressing out sex, age and the first ten principal components of genetic structure, and then inverse normalizing CPD. We used the whole data but also split the data into two: lower and higher ends of the GS. We used 0 as the cut-off point based on the bend apparent in the loess curve of the scatter plot (Fig 2) and to obtain roughly the same sized groups as the mean was close to the median (Table 2). In order to have a reference point, we used our Finnish data to create plots of CPD against the

NMR, as well as Cot+3HC, a biomarker for nicotine intake, against the NMR.

PheWAS

We used the GxE MR-pheWAS approach [24], which allows for hypothesis-free testing of the causal effects of the exposure (in our case, the NMR), while simultaneously examining the presence of association pathways not involving the exposure (horizontal pleiotropy) (See Supplementary Information). The idea is to divide the data in to groups with different levels of the exposure, in our case, ever and never smokers. We assume that the effect of nicotine metabolism only occurs in people who are actually using nicotine, in other words our ever smokers subset. The approach permits us to distinguish whether the PheWAS associations reflect a causal pathway through a) the NMR, either directly or through other traits such as amount smoked (effect only seen in ever smokers), b) some other pathway not including the NMR (same effect seen also in never smokers), or c) both (effect only seen in never smokers, or there are quantitative or directional differences in the effect sizes between ever and never smokers) (see Fig 1).

We began by running PHESANT (version 1.1) on the full sample using the 'save' option to save the variables processed and derived by PHESANT. Out of the 4,546 UKB phenotype fields we had to begin with, PHESANT created a total of 21,094 outcome variables. We then performed the actual PheWAS analyses in two stages.

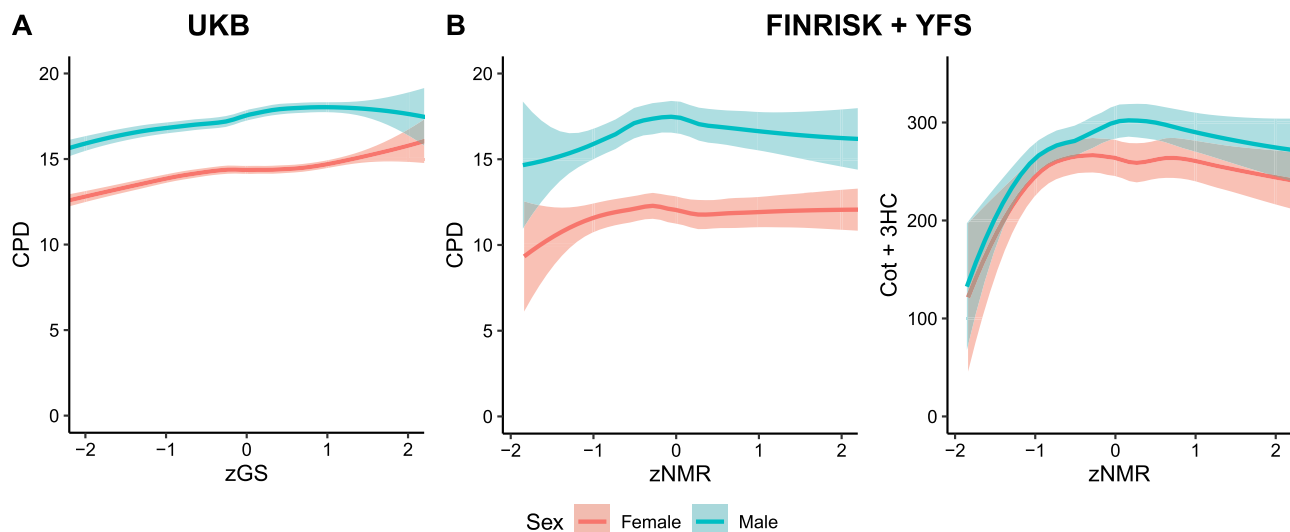


Fig. 2 **A** Loess curve depicting the association between the standardized genetic score (zGS) for the Nicotine Metabolite Ratio (NMR) and cigarettes smoked per day (CPD) in UK Biobank (UKB). **B** Loess curves depicting the associations between the standardized NMR (zNMR) and CPD, as well as zNMR and Cotinine + 3-Hydroxycotinine (Cot + 3HC), a biomarker for nicotine intake, using the combined

data from FINRISK and Young Finns Study (YFS). All plots are for current smokers. The x-axes have been restricted to show only data points within two standard deviations from the mean to highlight the main trends. Full data are shown in Fig. S4. The standardized variables (zGS and zNMR) were calculated by subtracting the mean and dividing by the standard deviation

Table 2 Descriptive statistics for age, sex, the GS for faster nicotine metabolism, and various smoking and alcohol variables in the UKB data

	All <i>n</i> = 343,662	Female <i>n</i> = 184,565(53.7%)	Male <i>n</i> = 159,097(46.3%)
	mean (sd), median [min, max]	mean (sd), median [min, max]	mean (sd), median [min, max]
Age	56.9 (8), 58 [39, 72]	56.7 (7.9), 58 [40, 71]	57.1 (8.1), 59 [39, 72]
GS	1.2 (0.6), 1.2 [−1.7, 2.9]	1.2 (0.6), 1.2 [−1.7, 2.9]	1.2 (0.6), 1.2 [−1.7, 2.8]
zGS	0 (1), 0.1 [−5.2, 3.1] n (%)	0 (1), 0.1 [−5.2, 3.1] n (%)	0 (1), 0.1 [−5.2, 2.9] n (%)
Smoking status			
Never	135,890 (39.7)	81,037 (44.1)	54,853 (34.6)
Experimenter	96,272 (28.1)	52,700 (28.6)	43,572 (27.5)
Ever	110,348 (32.2)	50,217 (27.3)	60,131 (38.0)
Former-Current (Ever subset)			
Former	81,179 (73.6)	36,490 (72.7)	44,689 (74.3)
Current	29,141 (26.4)	13,711 (27.3)	15,430 (25.7)
Wants to quit (Current subset)			
Yes	17,876 (70.3)	8,759 (72.4)	9,117 (68.4)
No	7,543 (29.7)	3,336 (27.6)	4,207 (31.6)
Alcohol intake frequency			
Never	22,315 (6.5)	14,526 (7.9)	7,789 (4.9)
Special occasions only	35,991 (10.5)	25,594 (13.9)	10,397 (6.5)
One to three times a month	38,011 (11.1)	24,161 (13.1)	13,850 (8.7)
Once or twice a week	90,435 (26.3)	48,762 (26.4)	41,673 (26.2)
Three or four times a week	82,961 (24.1)	39,918 (21.6)	43,043 (27.1)
Daily or almost daily	73,707 (21.4)	31,477 (17.1)	42,230 (26.5)
Prefer not to answer	242 (0.1)	127 (0.1)	115 (0.1)
	mean (sd), median [min, max]	mean (sd), median [min, max]	mean (sd), median [min, max]
Age started smoking on most days			
Current (n = 25,522)	17.7 (5.8), 16 [5, 69]	18.1 (5.8), 17 [5, 69]	17.4 (5.7), 16 [5, 65]
Former (n = 80,788)	17.2 (3.6), 17 [5, 63]	17.7 (3.7), 17 [5, 60]	16.8 (3.4), 16 [5, 63]
Cigarettes smoked per day			
Current (n = 23,682)	15.7 (8.4), 15 [1, 140]	14.2 (7.3), 15 [1, 120]	17.4 (9.2), 15 [1, 140]
Former (n = 76,865)	19.3 (10.5), 20 [1, 140]	16.8 (8.4), 15 [1, 100]	21.5 (11.6), 20 [1, 140]
Number of unsuccessful stop-smoking attempts			
Current (n = 2,527)	4.1 (10.1), 3 [0, 200]	4 (10.7), 3 [0, 200]	4.1 (9.7), 3 [0, 200]
Former (n = 74,108)	2.9 (7.1), 2 [0, 200]	2.4 (5.3), 2 [0, 200]	3.2 (8.3), 2 [0, 200]
Age stopped smoking on most days			
Current (n = 3,218)	47.3 (11.6), 48 [12, 70]	47.4 (11.5), 48 [13, 70]	47.1 (11.6), 48 [12, 70]
Former (n = 80,859)	39.5 (11.6), 39 [9, 69]	39.2 (11.5), 38 [12, 69]	39.7 (11.6), 39 [9, 69]

GS, genetic score for the nicotine metabolite ratio; zGS, standardized GS; Ever, individuals indicating smoking on most days at present (UKB Field 1239) or in the past (UKB Field 1249); Never, individuals responding negatively to current (UKB Field 1239) and past (UKB Field 1249) tobacco smoking; Experimenters, individuals indicating occasional or minimal past (UKB Field 1249) or present (UKB Field 1239) tobacco use; Former-Current, subsets of ever smokers differentiating those who had smoked in the past and those who smoke presently based on UKB Field 20116; Wants to quit, derived from the variable “Wants to stop smoking” (UKB Field 3496) with responses grouped into “Yes” (including “Yes, definitely” and “Yes, probably”) and “No” (including “No, probably not” and “No, definitely not”); Alcohol intake frequency, UKB Field 1558; Age started smoking on most days, UKB Field 2867 for former smokers, UKB Field 3436 for current smokers; Cigarettes smoked per day, UKB Field 3456 for current smokers, UKB Field 2887 for former smokers; Number of unsuccessful stop-smoking attempts, all individuals who had indicated that in the past they smoked tobacco on most or all days and that during the time they smoked they stopped for more than 6 months were asked “How many times did you try to give up smoking before you were successful?” (UKB Field 2926); Age stopped smoking on most days, UKB Field 2897

Exploratory PheWAS In the first stage, we used the 21,094 PHEsANT derived outcome variables to run an exploratory PheWAS for the entire sample (All), as well as separately for those who had ever smoked daily (Ever) and those who had never smoked (Never). We adjusted for age, sex and 1-10 PCs. For each PheWAS (All/Ever/Never), we used the 5 % false discovery rate (FDR) level to define statistical significance at the phenome-wide level. We used the Benjamini-Hochberg method to obtain the cut-off points for the 5 % FDR, i.e. the thresholds for phenome-wide significance (TPWSs). Within each PheWAS, we ranked the results based on ascending p-values. Next, for each outcome variable, we calculated the critical value $c_i = 0.05 \times i/n$, where i is the rank and n the total number of tests performed in that PheWAS. The TPWS, was then defined as the c_i corresponding to the outcome with the highest rank to satisfy the condition $p_i \leq c_i$ [32]. We used these TPWSs also in the second stage.

Final PheWAS In the second stage of our analyses, we wanted to confirm our findings. We reran the regression analyses for all the outcomes with phenome-wide significant (PWS) associations in any of the three exploratory PheWASs described above (All/Ever/Never). First, we manually checked the coding and the distributions of these highlighted variables, as well as the appropriateness of the models used by PHEsANT. Where appropriate, we split the variable into multiple variables, adjusted the coding or used a different regression model. We did these changes to obtain results that are more readily interpretable. Additionally, we reran the highlighted linear regression analyses by regressing the covariates (age, sex, 1-10 PCs) out before inverse rank transforming the outcome variables to normality. We did this because many of these continuous outcomes, such as waist circumference, are known to be normally distributed around different values depending on sex (one of our covariates). For the linear models, we didn't adjust for covariates any more in the actual regression analyses. Otherwise, we used the same protocol as PHEsANT for our final analyses of the All, Ever and Never data sets. A detailed description of the second stage outcomes that we derived, recoded or analysed using a different model from the initial exploratory PheWASs can be found in Table S3. For each data set we used the corresponding TPWS obtained previously to determine statistical significance at the phenome-wide level.

Differences between the ever and never smokers

We wanted to assess whether there were any outcome variables for which there was a statistically significant difference

between the effect sizes for the Ever and Never subsets. We did the Ever versus Never analyses in R by deriving p-values for the differences using the function `pchisq(x, df=1, lower=FALSE)`, where

$$x = \frac{(\hat{\beta}_{\text{ever}} - \hat{\beta}_{\text{never}})^2}{\text{se}_{\text{ever}}^2 + \text{se}_{\text{never}}^2}, \text{ and}$$

$$x \sim \chi_1^2 \text{ under the null hypothesis of } \beta_{\text{ever}} = \beta_{\text{never}}.$$

First, we did this for our initial PheWAS results. Of all the 21,094 PHEsANT derived outcomes altogether 15,212 had been analysed for both the Ever and Never subsets using the same regression model. Once again, we used the 5 % FDR level to determine phenome-wide significance, and obtained the TPWS using the Benjamini-Hochberg method. We repeated the Ever versus Never analyses for our outcomes from the second stage of our PheWAS analyses, using this same TPWS to define statistical significance.

Follow-up analyses

Current versus former analysis We followed-up our Ever versus Never analyses by taking all the outcomes with a PWS difference in their effect sizes, and repeated the analysis for current versus former smokers. We did this to obtain a better understanding of the possible causal role of smoking in the association pathway. We constructed the current and former smoker subgroups from our Ever subset, and repeated the second stage PheWAS regression analyses for the chosen outcomes in these two groups.

Follow-up of the GS-cessation association Our PheWAS results suggested a positive association between the GS for faster nicotine metabolism and smoking cessation. To investigate further what could be driving the positive association, we examined the variable "Why did you stop smoking? (You can select more than one answer): Illness or ill health / Doctor's advice / Health precaution / Financial reasons" (UKB Field 6157), and the variable "Number of unsuccessful stop-smoking attempts" (UKB Field 2926) which captures the difficulty of quitting.

As faster nicotine metabolism is thought to associate with a greater amount of cigarettes smoked and more difficulties in quitting smoking successfully (reviewed in [10]), we began by rerunning the logistic regression model for cessation, including CPD as an additional covariate to adjust for possible confounding. In other words, among ever smokers, we ran a logistic regression, where cessation was

the dependent variable, and the zGS, age, sex, 1–10 PCs, and CPD were the predictor variables. Smoking more will increase the risk of severe illnesses and the need for treatments such as surgery that require abstinence. Thus, we also ran this model within a subset of the ever smokers from whom we had excluded those who listed illness and/or doctor's advice as the reason for stopping smoking.

Reasons for stopping smoking were available for those ever smokers who had at least once managed to stop for over six months. We divided the Ever subset into three approximately equal sized groups based on the GS tertiles, and named these groups as slow, medium and fast metabolizers. We then compared, one reason at a time, whether the proportions differed between the slow and fast groups. We used the 2-sample test for equality of proportions using the `prop.test` function in R.

To assess the importance of the different reasons, we reran the cessation models (with and without CPD included as a predictor) among those ever smokers who had at least once managed to stop for over six months. Among this subset, we added all four reasons to the models. We wanted to see how the reasons predicted former status at the time of the questionnaire.

Then, among our former smokers, we ran a negative binomial regression model with "Number of unsuccessful stop-smoking attempts" as the dependent variable and each of the four reasons, the zGS, age, sex, and 1–10 PCs as the predictor variables. We also ran the model including CPD as a confounder.

Follow-up of the liver enzyme associations In our PheWAS, the GS for faster nicotine metabolism was associated with the liver enzymes, alkaline phosphatase and alanine aminotransferase. To ensure these associations were not confounded by alcohol consumption we reran these analyses additionally adjusting for alcohol consumption. We used the variable "Alcohol intake frequency: 1 Daily or almost daily / 2 Three or four times a week / 3 Once or twice a week / 4 One to three times a month / 5 Special occasions only / 6 Never / -3 Prefer not to answer" (UKB Field 1558) which was available for the whole of our sample. Prefer not to answer was treated as missing ($n = 242$).

MRBase and FinnGen PheWASs using the top SNP for the NMR For comparison, we ran PheWASs of the top SNP for the NMR (rs56113850) using GWAS summary data from FinnGen (Data freeze 9, from April 2022) and MRBase (Database version 0.3.0, from 25 Oct 2020). We used the open access web interfaces. The FinnGen Data Freeze 9 has a total sample size of 377,277 and consists of 2,272 disease endpoints from Finnish biobank participants [33, 34]. The

GWAS results from FinnGen were adjusted for sex, age, PC1-10, whether using FINNGEN1 or 2 chip, and legacy genotyping batch [33]. The MRBase comprises GWAS summary data from numerous consortia, including UKB and FinnGen [35, 36]. From MRBase, we got results for 39,105 outcomes. This group of outcomes included expression quantitative trait loci (eQTLs) as well. Unfortunately, we do not know what the GWAS results from MRBase were adjusted for as the results come from multiple different cohorts and studies using different protocols.

In both cases, we defined phenome-wide significance as before, and created tables of the PWS results, i.e. those reaching statistical significance at the 5 % FDR level using the Benjamini-Hochberg method. We used the library `biomaRt` in R to annotate the eQTLs that were highlighted in the MRBase PheWAS.

Assessing sex and ancestry differences We scrutinized our results by exploring possible differences between sexes and among ancestries. First, we repeated the final PheWAS analyses exactly as before, this time stratifying by sex. We then assessed whether the effect sizes differed between the sexes by using the same formula we used for the ever-never comparisons. Second, to assess to which extent our results can be generalized to other ethnic groups, we used the mentioned top SNP for the NMR, rs56113850-allele C (versus T), as a proxy for genetically determined faster nicotine metabolism. This SNP has been shown to replicate in other ancestries [37, 38] (Table S12), and to be an important predictor for the NMR across ancestries [39]. We reran all the 33 continuous phenotypes highlighted as having a PWS association in our final PheWAS, this time stratifying by ancestry and using the top SNP instead of the GS. To create the ancestry groups that we then compared to the White British sample, we used the Top level groupings listed for the UKB field 21000 on showcase: Asian or Asian British / Black or Black British / Chinese / Mixed / Other ethnic group / White British [40]. We focused on the continuous traits, since the sample sizes in the ancestries other than White British were too small for disease studies (Table S13). To maintain reasonable sample sizes, we did not separate ever and never smokers in the ancestry-specific analyses.

Results

Descriptive statistics

For female participants, the smoking status (Ever/Experimenter/Never) category Never was the largest (44 %),

whilst for males the Ever category was the largest (38 %) (Table 2). Among both sexes, over 70 % of the ever smokers were former smokers, and roughly 70 % of the current smokers wanted to stop smoking. The distribution of the GS for the NMR was nearly identical for both sexes (Table 2). When running a linear model of the standardized GS by age, the age variable was not statistically significant ($p = 0.909$).

The correlation between the GS and the top SNP (rs56113850) was 0.73 (Fig. S3). Each standard deviation increase in the GS was associated with an increase of 0.6 cigarettes smoked per day (CPD), after adjusting for age, sex and 1–10 PCs (Table S4). The association seemed to be stronger at the lower end of the GS than at the higher end (Table S4, Fig 2a). This trend could also be seen in our Finnish sample when plotting CPD or Cot+3HC against the NMR (Fig 2b).

Exploratory PheWAS

A total of 61 different outcomes reached phenome-wide significance across the initial All, Ever, and/or Never PheWASs (Fig. S5, Table S5). The exploratory full sample PheWAS resulted in 47 PWS associations (TPWS_{All} : $0.05 \cdot 47/21094 = 1.1e - 04$) (Table S5a). For the Ever subset there were 29 PWS associations (TPWS_{Ever} : $0.05 \cdot 29/16648 = 8.7e - 05$) (Table S5b), and for the Never subset, only two associations were PWS (TPWS_{Never} : $0.05 \cdot 2/16103 = 6.2e - 06$) (Table S5c).

Final PheWAS

The set of 61 outcome variables highlighted in the first stage of our PheWAS analyses was then taken to the second stage. Some of these variables were split into multiple variables to tease out which aspect of the phenotype was driving the association (Table S3). For example, Smoking Status (Never/Previous/Current) was split into Cessation (former vs current smoker) and Initiation (ever vs never smoker), out of which only Cessation showed a PWS association when we reran the analyses (Fig 3).

Our second stage of the PheWAS analyses included 71 outcome variables (Table S6). Of these, 57 showed a PWS association in at least one of the three data sets based on the data specific TPWSs (Fig 3, Fig 4). We had 45 PWS associations among the full sample, 26 among the Ever subset and three among the Never subset (Fig 3, Table S6b–d).

For 14 outcomes, we did not observe a PWS association with the GS for faster nicotine metabolism in any of the three groups (All, Ever or Never). This set of non-significant associations included five continuous variables that had reached the TPWS in at least one of the data sets in the exploratory PheWASs (when the covariates had not been adjusted for before normalizing the outcome). The rest of the non-significant results included the derived Smoking Initiation and Occasional vs Daily Smoker variables, as well as some of the derived tea and coffee variables.

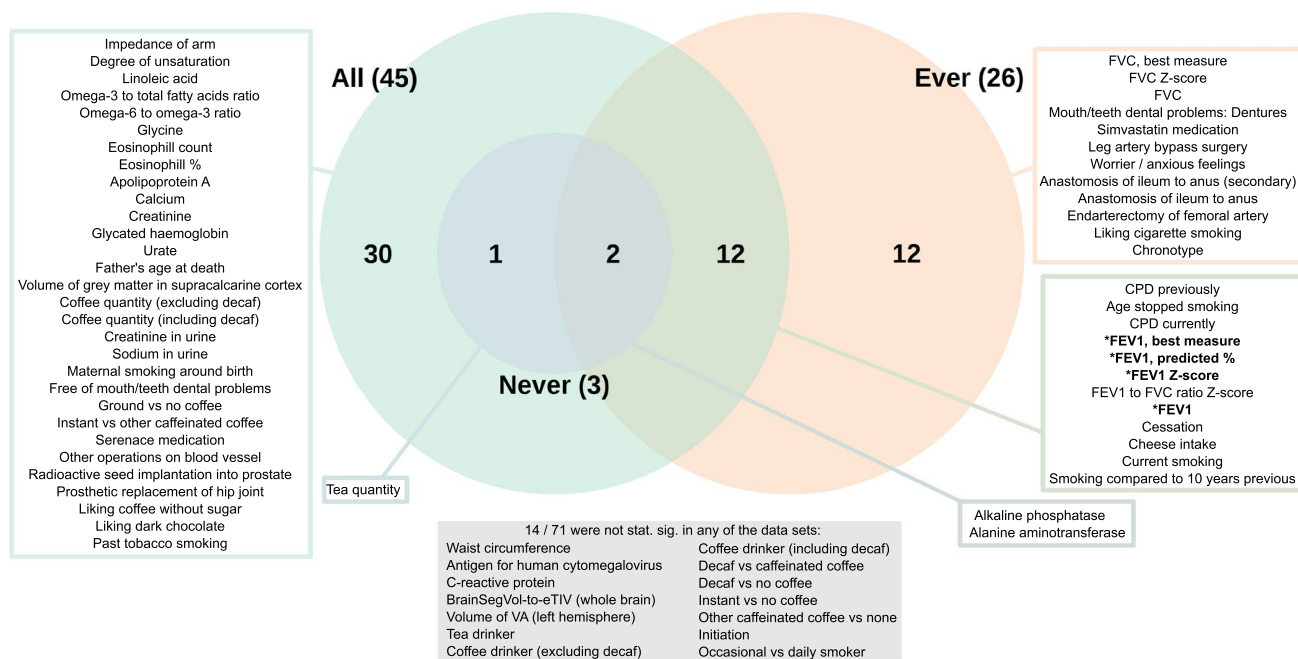
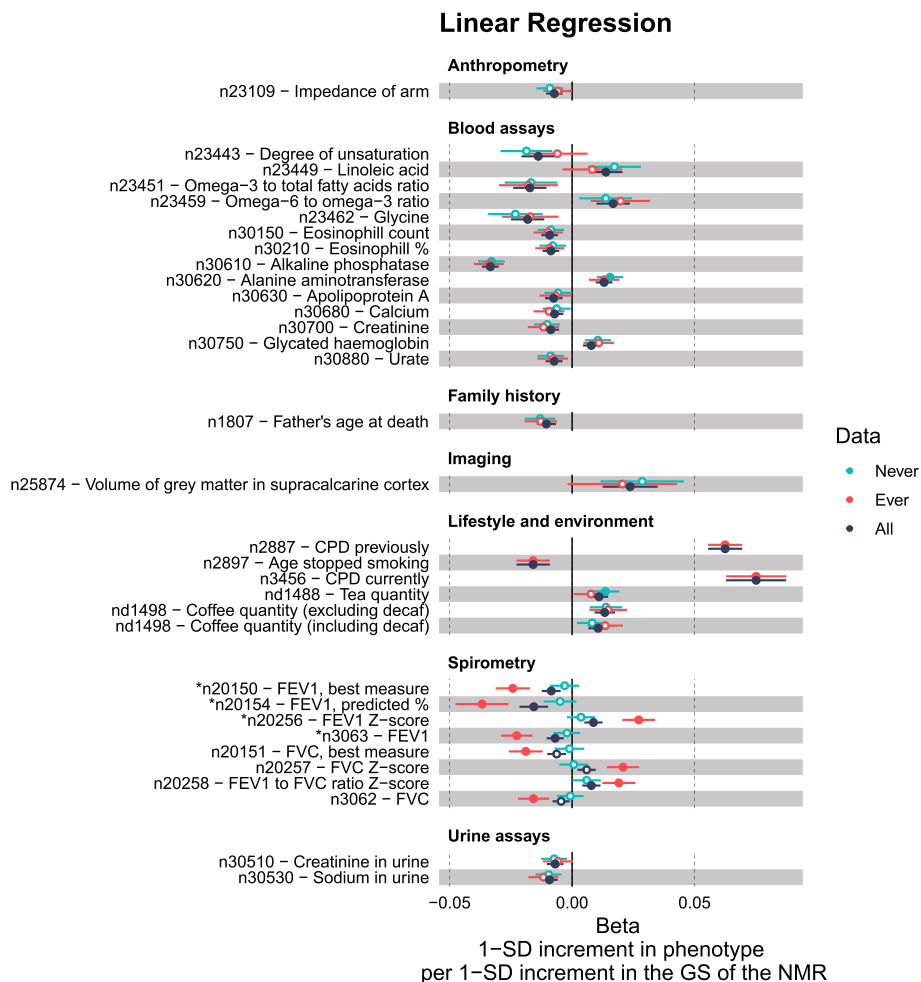


Fig. 3 Venn diagram of the results of the final 71 variables. Out of these variables, 57 had a phenome-wide significant (PWS) association in at least one of the data sets (All / Ever / Never). Those with a PWS

difference in their effect sizes between the ever and never smokers have been indicated with a star and bolded text

Fig. 4 Results of the 71 variables from the final PheWAS analyses. The results of those variables ($n = 57$) that were phenome-wide significant (PWS) (solid circles) in at least one of the data sets (All / Ever / Never) are shown. *, there was a PWS difference between the effect sizes of the ever and never smokers; n , normalised after covariates had first been regressed out; d , derived from the original UKB phenotype; c , coding corrected to be more intuitive



Ever versus never analyses

In the first stage of our PheWAS, a total of 15,212 outcome variables had been analysed for both the Ever and Never subsets using the same regression model. Of these, only two variables showed a PWS difference in their effect sizes between the ever and never smokers (Table S5d). The TPWS was thus at $0.05 \cdot 2/15212 = 6.6e - 06$. Both of these were lung capacity variables and were included among the variables highlighted in the exploratory All and Ever PheWASs (Fig. S5). There was no association among the Never subset while among the Ever and All groups higher values of the GS predicted worse lung capacity.

Of the 71 outcome variables included in the second stage of our PheWAS analyses, 58 were available for our Ever versus Never analysis. Of these, four showed a PWS difference between ever and never smokers in their effect sizes ($p < 6.6e - 06$) (Table S6a, Fig 4). All four were lung capacity measures. For all four, there was no evidence of association among never smokers but a PWS association among ever smokers, suggesting a causal pathway through

smoking. The results suggested that only among ever smokers higher values of the GS predicted worse lung capacity.

Never smokers: associations with liver enzymes and tea quantity

Among never smokers, a higher GS for faster nicotine metabolism was associated with decreased alkaline phosphatase and increased alanine aminotransferase, both of which are liver enzymes (Table S6d). These associations were also seen among the Ever and All groups (Fig 3, Fig 4), and were PWS for all three groups already in our exploratory PheWASs (Fig. S5).

Among never smokers, there was also a PWS association with increased tea consumption. This was PWS in the All group too, but not among the ever smokers subset. Apart from these three outcomes, none of the other outcomes showed a PWS association in the never smokers subset. The direction and magnitude of association was nevertheless similar to those of the other groups for many variables such as for the other blood and urine assay variables and variables related to coffee (Fig 4).

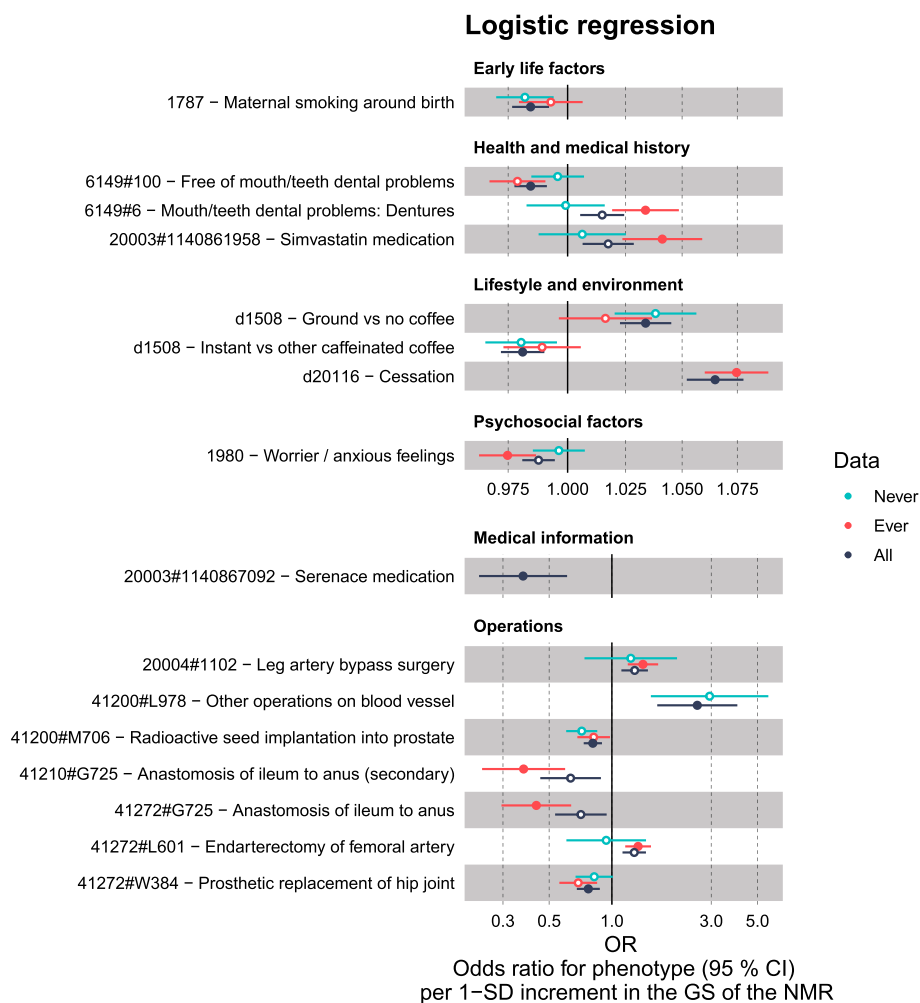


Fig. 4 (continued)

Ever smokers: associations with liver enzymes and smoking related variables

Among ever smokers, 26 of the final second stage outcome variables showed PWS associations (Fig 3, Table S6c). In addition to the liver enzymes, a higher GS for faster nicotine metabolism was associated with a greater number of cigarettes smoked per day, an increased likelihood of having quit smoking, decreased lung capacity, an increased likelihood of taking cholesterol medicine (Simvastatin), being more of a morning person than an evening person, quitting smoking at a younger age, an increased likelihood of having dentures, increased smoking compared to 10 years ago, decreased consumption of cheese, a decreased likelihood of being a worrier, decreased liking for cigarette smoking, an increased likelihood of leg artery operations, and a decreased likelihood of an anastomosis of ileum to anus.

The GS for faster nicotine metabolism was also associated with an increased likelihood of smoking less at the time of the interview ("Do you smoke tobacco now?: No / Only

occasionally / Yes, on most or all days", UKB field 1239). This negative association with current smoking was driven by the higher odds of quitting. There was no association for the derived Occasional vs Daily outcome, whereas our derived Cessation outcome demonstrated one of the strongest associations (Table S6c). Our ever smokers only contained individuals who smoked daily or had smoked daily in the past. Therefore, we had better statistical power for our Occasional vs Daily phenotype in our full sample. Nevertheless, we saw no association there either ($p = 0.90$, Table S6b).

Full sample: strongest associations with liver enzymes, smoking related variables, coffee, and tea

Among the full sample there were 45 outcome variables that showed a PWS association with the GS for the NMR (Fig 3, Table S6b). The strongest associations were observed for the liver enzymes, CPD, cessation, and coffee and tea consumption. The GS was also associated with 16 biomarkers,

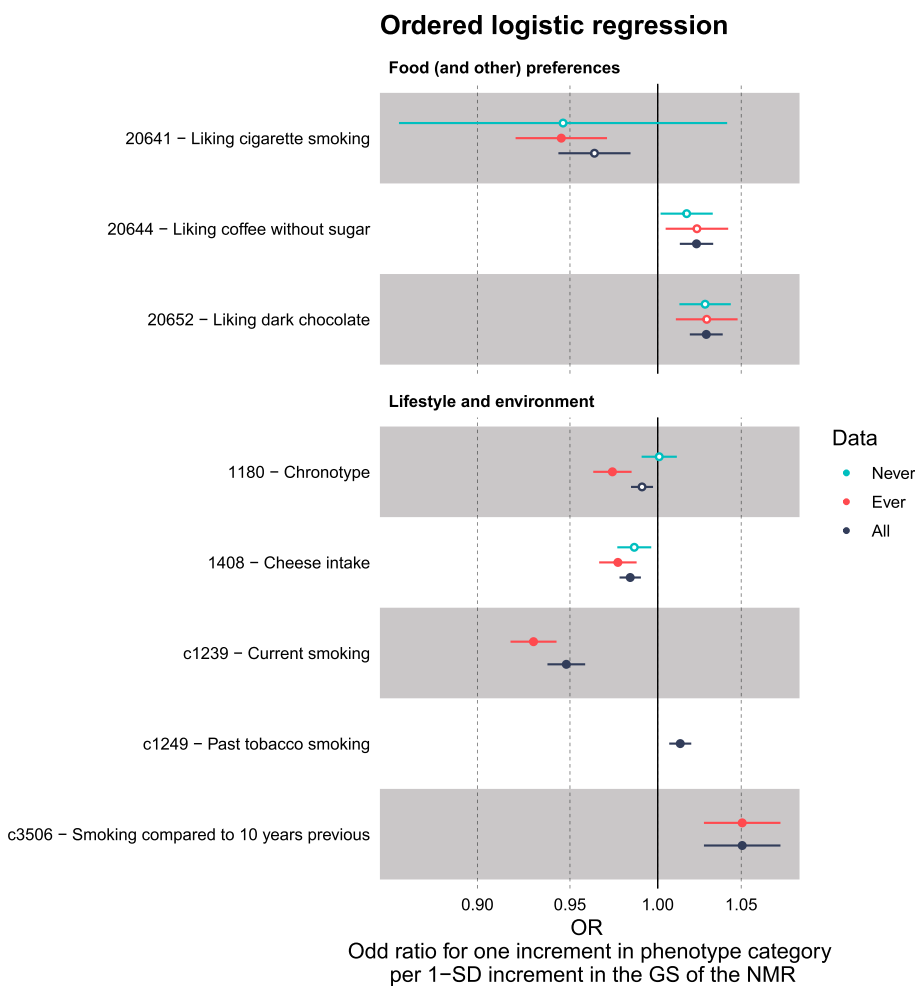


Fig. 4 (continued)

often used to assess overall health. For example, the GS was associated with outcomes related to fatty acids, blood sugar, kidney and liver health, impedance of arm, calcium, and white blood cells. The Never and Ever associations for these biomarkers were in line with each other but neither reached phenome-wide significance, except for the liver enzymes mentioned earlier (Fig 4).

Some additional outcome variables, that had not reached phenome-wide significance in the smaller subsets, were highlighted in the full sample. For example, among the full set, the GS for faster nicotine metabolism was associated with an increased liking for dark chocolate and coffee without sugar, decreased father’s age at death, decreased maternal smoking around birth, increased volumes of grey matter in the supracalcarine cortex, and a decreased likelihood of using haloperidol (Serenace), an anti-psychotic medication. Some outcomes were only analysed in some groups due to a lack of cases, and the use of haloperidol was one of these (only analysed in the full sample). The outcome Past tobacco smoking (Never/Tried once or twice/Occasionally/

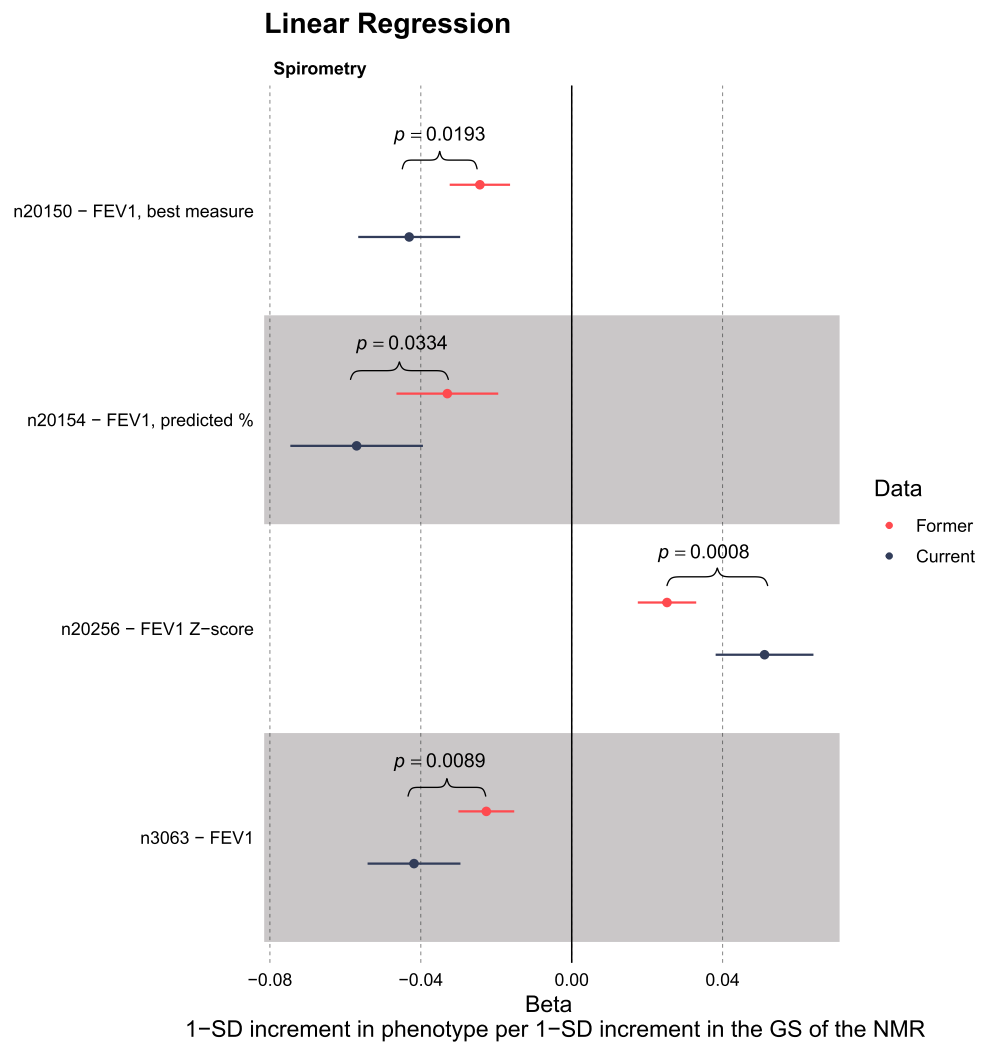
Most days or daily) was only analysed in the All group as it required answers from both ever and never smokers. The GS for faster nicotine metabolism was associated with an increased likelihood of being in a higher past tobacco smoking category.

Follow-up analyses

Comparison of current and former smokers supports the causal role of smoking on worse lung functioning

Our Ever versus Never analyses highlighted four lung capacity measures, suggesting a causal pathway through nicotine metabolism, as the association was only seen in the ever smokers. We followed-up these results by rerunning the analyses for the current and former smoker subsets. The effect sizes for all four lung capacity measures were smaller among former smokers compared to current smokers, though they too remained PWS ($p < TPWS_{Ever} = 8.7e - 05$) (Fig 5, Table S6e).

Fig. 5 Current versus Former smokers comparison for the four lung capacity variables highlighted in the Ever versus Never smoker analysis. For both Current and Former subsets all four variables had a phenome-wide significant (PWS) association with the GS. Differences between these two subsets were not PWS. Nevertheless, the association is visibly attenuated for the former smokers for all four variables. *n*, normalised after covariates had first been regressed out



GS-Cessation association possibly explained by CPD and ill health

In our sensitivity analyses of cessation, the odds ratio of the GS remained positive after adjusting for CPD (Table S7a–b), although it did decrease from 1.07 (95 % CI: [1.06, 1.09]) to 1.05 [1.04, 1.07]. The odds ratio decreased further to 1.01 [0.97, 1.06] and was no longer statistically significant once we excluded individuals who had stopped due to 'Illness or ill health' and/or 'Doctor's advice' ($p = 0.54$) (Table S7c).

When comparing the reasons for stopping smoking (Illness or ill health / Doctor's advice / Health precaution / Financial reasons - participants could choose as many as they wanted), fast metabolizers of nicotine (high GS) seemed to have more incentive to quit than slow metabolizers (low GS), though differences were small (Table S8). Regardless of reason, fast metabolizers had a slightly higher percentage of people selecting it than slow metabolizers. For the more prevalent reasons: 'Health precaution' and 'Financial

reasons', the differences were statistically significant at the 0.05 level (Table S8).

We took a closer look at ever smokers who had managed to quit at least once for over six months, to see whether any of the reasons for stopping smoking were associated with remaining a former smoker still at the time of the questionnaire (Table S7d–e). Listing 'Financial reasons' was positively associated with remaining a former smoker (OR = 1.21 [1.11, 1.31]), while 'Doctor's advice' had a negative association (OR = 0.61 [0.54, 0.69]) (Table S7d). The GS for faster nicotine metabolism showed a positive association (OR = 1.04 [1.005, 1.08]). After adding CPD to the model, the direction of the association remained the same for all reasons, as well as for the GS (Table S7e). Now the reasons with p-values below 0.05 were 'Doctor's advice' (OR = 0.51 [0.45, 0.59]), 'Illness or ill health' (OR = 0.83 [0.74, 0.94]), and 'Health precaution' (OR = 1.08 [1.001, 1.17]). For every additional cigarette the odds of remaining a former smoker was 6 % greater (OR = 1.06 [1.06, 1.07]).

Among former smokers of the Ever subset, the median number of unsuccessful smoking attempts was 2.0, the mean was 2.9 and the standard deviation was 7.1. Once we excluded those listing 'Illness or ill health' and/or 'Doctor's advice', the numbers hardly changed: 2.0, 2.8, and 6.9.

All four reasons were statistically significant at the 0.05 level in explaining the number of unsuccessful stop-smoking attempts in the former smokers subset (Table S9). They were all associated with an increased number of attempts, with 'Health precaution' demonstrating the strongest association. Individuals listing 'Health precaution' had a 39 % higher number of attempts compared to those who had not listed it (IRR = 1.39 [1.36, 1.42]) (Table S9a). Additionally, for every standard deviation increase in the GS for the NMR, there was an expected 1 % increase in the number of attempts (IRR = 1.012 [1.002, 1.022]). However, after including CPD in the model, the GS was not statistically significant any more ($p = 0.24$) (Table S9b). With every additional cigarette, the number of unsuccessful stop-smoking attempts increased by 1.6 % (IRR = 1.016 [1.01, 1.02]).

Liver enzyme associations are likely to be independent of alcohol

Additionally adjusting for alcohol intake frequency did not affect the association between the GS for faster nicotine metabolism and the liver enzymes (Fig. S6, Table S10).

PheWASs in MRBase and FinnGen confirm our findings and highlight lung cancer and lipid outcomes

The NMR top SNP in our previous Meta GWAS of current smokers with European ancestry was rs56113850 on chromosome 19 [15]. Based on our Meta GWAS, the beta for the major allele C (vs. T) was 0.682 (se = 0.02) (C allele frequency was 0.553). Both the FinnGen and MRBase PheWAS results have been presented for the allele C so that the results can be interpreted for genetically determined faster nicotine metabolism.

The FinnGen PheWAS highlighted two PWS associations: both were for lung cancer diagnoses (Table S11a). The associations were positive, so genetically determined faster nicotine metabolism was associated with greater odds of having lung cancer. The MRBase database pinpointed 199 outcomes with a PWS association (Table S11b). However, many of these outcomes overlapped or were highly similar (Table S11c). Most of the top associations were related to smoking, lung cancer, lung functioning, liver enzyme levels, cholesterol and other lipids, as well as gene expression (See annotations for highlighted genes: Table S11d). Our own PheWAS of the UKB had not included some of these

outcomes such as those related to gene expression, but otherwise the MRBase results closely mirrored ours.

Sex and ancestry stratified analyses revealed limited variation

Out of the 71 variables assessed in our sex-stratified analyses, only three showed statistically significant differences between the sexes at a Bonferroni significance level (0.05/71) in at least one of the three data sets (Ever/Never/All) (Fig. S7, Table S14). The genetic score for faster nicotine metabolism was associated with an increased number of cigarettes smoked per day for both sexes but the effect size was greater among males. The GS was negatively associated with glycine. However, this association was absent among male never smokers, while for females, this association was strongest among never smokers. Among current smokers, for smoking compared to 10 years previous (Less nowadays?/About the same?/More nowadays?), there was no association among males but a positive association among females. All variables showing a difference between the sexes that was significant at the 0.05 level have been plotted in Fig. S7, which mainly shows differences in magnitude of effect.

Six of the 33 continuous variables included in our ancestry-stratified analyses were not available for the other ancestry groups (Asian or Asian British / Black or Black British / Chinese / Mixed / Other ethnic group). None of the remaining 27 variables showed differences in effect sizes between the ancestry groups and the White British group at a Bonferroni significance level (0.05/27) (Table S15). All variables showing a difference between any of the other ancestry groups and our White British group at the 0.05 level, or showing an effect reaching the 0.05 significance level in one of the other ancestry groups have been plotted in Fig. S8. For both of the liver enzymes, our findings were replicated in some of the other ancestry groups: the negative association with alkaline phosphatase was replicated in "Black or Black British" and "Other ethnic group", while the positive association with alanine aminotransferase was replicated in the groups "Chinese" and "Mixed".

Discussion

In our study we used a hypothesis-free method, GxE MR-PheWAS [24], to identify novel associations across the phenome with the NMR. Importantly, we conducted separate PheWAS analyses for ever and never smokers. This enabled us to identify which associations might be attributable to a causal effect through nicotine metabolism. We explored over 21,000 outcome variables, making our PheWAS the

most comprehensive to date on the rate of nicotine metabolism. Notably, our study was the first PheWAS to explicitly focus on the NMR itself, rather than solely on CYP2A6 activity.

We found associations with several smoking related traits and diseases. These were in line with existing literature, with the exception of a positive association between our GS for faster nicotine metabolism and smoking cessation. Additionally, our study unveiled novel associations with measures not previously reported to associate with the NMR, including liver enzymes, lipids, and consumption of coffee and tea.

We did not replicate the hearing loss finding identified by the initial PheWAS of CYP2A6 activity [19]. The other PheWAS published to date on CYP2A6 activity, used the UKB data, but focused only on about 1,000 disease endpoints [20]. They did not replicate the hearing loss finding either. Their findings, limited to tobacco related diseases, were in line with ours, although none of their findings reached genome-wide significance in our study which included over 21,000 outcome variables. Their top finding, lung cancer, ranked 56th in significance in our subset of ever smokers, for which we saw 29 PWS associations. In our secondary analyses, however, we conducted a PheWAS replication study of the top GWAS SNP for the NMR using the FinnGen data. This FinnGen PheWAS consisted of roughly 2,000 disease endpoints, and here, the only two PWS associations were indeed for lung cancer diagnoses (Table S11a).

Faster nicotine metabolism is known to associate with increased smoking [8, 9]. Our PheWAS results align with this established relationship. The GS for faster nicotine metabolism was associated with a greater number of cigarettes smoked per day, increased smoking compared to ten years earlier, and greater smoking in the past (asked from all except daily current smokers).

We found both the GS and the NMR to have a non-linear association with CPD (Fig 2). This non-linear relationship, indicative of a plateau-effect on CPD, aligns with previous findings for cotinine [41], where a plateau-effect was observed on cotinine when modelling it against CPD. However, to our knowledge, this phenomenon has not been previously reported for the NMR. This relationship provides a possible explanation to why in previous studies *CYP2A6* variation has shown a stronger association with lung cancer risk in light smokers ($CPD \leq 20$) than in the total sample [20, 42], and hasn't shown any association among the heavy smokers ($CPD > 20$) [42]. However, it is possible that, as our population-based data relied on voluntary participation from invitees, some subgroups of individuals with ill health, such as those with high GS and high CPD values, were under-represented. More research conducted in a controlled

setting would be warranted to explore this association more comprehensively.

While clinical trials and longitudinal data have shown that faster nicotine metabolism predicts lower quit rates [11, 12], our PheWAS yielded contrasting results. The GS was associated with greater odds of quitting, and quitting at a younger age (Fig 4). This positive association between genetically determined faster nicotine metabolism and cessation was also seen by Loukola et al. in their follow-up analyses of their NMR GWAS [14]. Cessation was not included in our FinnGen and MRBase replication PheWASs which we performed using the top NMR SNP, rs56113850. However, the same positive association was seen for the top NMR SNP allele C count (reflecting faster nicotine metabolism) with cessation in the GSCAN GWAS [43]. They found that with every additional allele C, the odds for being a former smoker (versus current) increased by 6 % (OR = 1.06 [1.05, 1.07], $p = 1.61e - 48$ —calculated from [43] Table S3 to reflect our coding for cessation (1 = former, 0 = current)).

To further investigate the positive relationship between genetically determined faster nicotine metabolism and smoking cessation seen in these population based cross-sectional data, we reran the UKB cessation analyses adding CPD as a covariate to the model. We speculated whether CPD might be acting as a possible mediator for the association. Faster nicotine metabolism is known to be positively associated with CPD. Amount smoked, in turn, impacts one's health and possibly one's inclination to quit due to the realization of adverse health effects or the necessity of abstinence, for example, for consequent surgical procedures. After adding CPD into the model, the association weakened but remained positive (Table S7a–b). Once we additionally excluded individuals who listed ill health and/or doctor's advice as the reason for quitting, the association was no longer statistically significant (Table S7c). Loukola et al. observed similar findings in their follow-up analyses of their NMR GWAS [14].

When we looked at the reasons for quitting more closely, it did appear that fast metabolizers might have had slightly more incentive to quit (Table S8). Additionally, it looked like internal reasons (health precaution and financial reasons) were more likely to result in continued abstinence (remaining a former smoker at the time of the UKB questionnaire) (Table S7d–e). Conversely, having quit because of doctor's advice seemed to decrease the likelihood of remaining a former smoker at the time of the questionnaire.

As mentioned, those with faster nicotine metabolism are thought to have a harder time succeeding at quitting [10]. Among former smokers, the GS had a positive association with the number of unsuccessful quit attempts but the association was no longer statistically significant once we

adjusted for CPD (Table S9). All four reasons increased the number of attempts, as did CPD. Health precaution and ill health however, had considerably stronger associations than doctor's advice and financial reasons.

Our results suggest that several factors are at play when it comes to cessation. Individuals with faster nicotine metabolism may smoke more, spend more money on tobacco, and experience more health problems. They may thus be more motivated to quit but succeeding can possibly be more challenging due to greater nicotine dependence which has been shown to correlate with faster nicotine metabolism by some studies [42, 44]. Regardless of the difficulties, faster metabolizers of nicotine appear more likely to quit long-term (remain former smokers) than slower metabolizers of nicotine. This could be due to their heightened motivation and more frequent quit attempts. Additionally, due to the nature of the UKB dataset, selection bias may be also contributing to this positive association. The UKB dataset is enriched with individuals who are healthier and come from higher socioeconomic backgrounds [45]. Thus those who smoke more and have not quit may be under-represented, potentially affecting the results.

In summary, our findings suggest that health precautionary reasons and higher daily cigarette consumption make quitting more challenging. However, they also make it more likely for the individual to remain a former smoker if they do manage to quit. We believe that the positive association between the GS and cessation may be explained, to some extent, by possible confounding or mediation by CPD and ill health, as well as, various selection biases in UKB.

Our Ever versus Never analyses highlighted four lung capacity measures. The associations were only seen in the ever smokers, providing evidence for a causal pathway through nicotine metabolism (Table S6a, Fig 4). When we divided the ever smokers into subsets of former and current smokers, the effect sizes for all four lung capacity measures were attenuated among the former smokers, giving support for the possible causal role of smoking on worse lung functioning (Fig 5, Table S6e). Our results align with existing research. Smoking is known to accelerate the decline in lung function, which may develop into chronic obstructive pulmonary disease [46, 47]. It is worth noting that, although smoking cessation has been shown to slow down this rate of decline, it does not appear to fully revert the rate of decline to that of never smokers [48].

Beyond variables related to smoking, our study identified several other variables associated with our GS for the NMR. It is noteworthy that many of these associations did not seem to rely on pathways through nicotine metabolism. Interesting associations, where we saw no apparent differences between ever and never smokers, included liver enzymes, lipid measures, and coffee and tea consumption.

In our PheWAS, only two phenotypes, the liver enzymes alkaline phosphatase (ALP) and alanine aminotransferase (ALT), showed associations with the GS that were PWS in both ever and never smokers (Fig 4). These associations ranked among the top findings in all three groups (All, Ever, Never), as well as in the PheWAS results from MRBase (Table S6b–d, Table S11b).

Both ALP and ALT are blood serum liver enzymes that are key biomarkers for assessing the extent and cause of liver damage [49–51]. One cause of liver damage is alcohol use, and alcohol use and smoking are well known to be associated behaviours. However, the associations remained unchanged after we adjusted for alcohol. Additionally, the strengths of the associations did not differ between the ever and never smokers, implying that the associations are not mediated by nicotine metabolism or smoking, and thus most likely not by alcohol either.

Building upon previous PheWAS analyses of these liver enzymes, conducted by Liu et al. [49], our study suggests that a higher GS for faster nicotine metabolism is correlated with less favourable liver enzyme levels with respect to associated diseases. We observed a negative association between the GS and ALP levels. The ALP PheWAS by Liu et al., revealed that lower values of the genetically determined ALP were associated with increased odds of hypercholesterolemia, pulmonary heart disease, as well as phlebitis and thrombophlebitis of lower extremities.

On the other hand, we found a positive association between the GS and ALT levels. For genetically determined ALT levels, Liu et al. found 16 associations in their ALT PheWAS. The strongest associations, based on p-values, highlighted a clear trend: higher values of genetically determined ALT levels were consistently associated with a higher risk of hepatic diseases such as hepatitis, primary liver cancer and non-alcoholic cirrhosis. Additionally, genetically determined ALT was positively associated with Type 2 Diabetes, possibly due to the role ALT plays in insulin resistance [49]. In our PheWAS of the entire sample, we identified a PWS positive association between the GS and glycated haemoglobin, suggesting that higher GS values correlate with poorer blood sugar control. The effect size remained consistent across the Ever and Never subsets but did not reach PWS in either subset. Of the 16 associations reported by Liu et al. for ALT, only three showed an opposite direction of effect: dementias, fracture of hand and wrist, and corneal degenerations. However, all three had p-values that had only just surpassed the 5 % FDR threshold, and for dementias, the direction of the effect varied depending on the Mendelian Randomization analysis method they used.

A substantial portion of our MRBase PheWAS results concerned outcome variables related to cholesterol, fatty acids, and other lipids (Table S11c). Likewise, other outcomes

indicative of cardiovascular health were also highlighted. For instance, genetically determined faster nicotine metabolism (the NMR top SNP, rs56113850, allele C count), was associated with elevated levels of cholesterol, triglycerides, LDL levels, and a higher apolipoprotein B/apolipoprotein A ratio (indicative of poorer cardiovascular health), as well as an increased waist circumference, higher BMI and greater odds of using cholesterol medication. Of note, cholesterol medication was among the top five associations in the PheWAS of the FinnGen data, based on p-values, although it did not reach genome-wide significance.

In our UKB PheWAS, the GS was associated with decreased apolipoprotein A, increased linoleic acid, a higher omega-6 to omega-3 ratio and a decreased omega-3 to total fatty acids ratio (Fig 4). Again, the direction of association was such that higher GS values predicted worse lipid values with respect to cardiovascular health (see e.g. [52]). These results were in line with our findings from the MRBase PheWAS. However, they were PWS only in our All group. Nevertheless, there was no apparent difference between the effect size estimates among the ever and never smokers, alluding to effects independent of nicotine metabolism, and possibly to a shared genetic component between nicotine metabolism and lipid levels. Interestingly, only among ever smokers, we observed PWS positive associations between the GS and the use of cholesterol medication, leg artery bypass surgery, and endarterectomy of femoral artery (Fig 4). This suggests that ever smokers with higher GS values may face a compounded risk for cardiovascular diseases due to their smoking history and genetics, and may thus be more likely to face adverse health effects and require medical interventions.

Moving on to another noteworthy association highlighted in our PheWAS, the GS for faster nicotine metabolism was associated with increased coffee and tea consumption (Fig 4). We observed PWS associations with coffee consumption in the All group and with tea consumption in the All and Never groups. The concordant results between Ever and Never subsets suggest an association pathway distinct from nicotine metabolism.

When decaffeinated coffee was included in the coffee quantity variable, there was a slight attenuation of associations, suggesting that the observed effect is possibly driven by caffeine metabolism. Notably, in a GWAS of caffeine metabolites, multiple SNPs at 19q13.2, including the *CYP2* cluster, *NUMBL*, *ADCK4*, *MIA* and *EGLN2*, showed genome wide significant associations [53]. The *CYP2A6* enzyme is known to play a minor role in caffeine metabolism [53], and it is possible that our results reflect this connection.

Interestingly, our PheWAS also highlighted a positive correlation between the GS and preferences for dark chocolate and coffee without sugar. These associations were

PWS only in the All subset. However, both the Ever and Never results were consistent with those of the All subset. Once again, this would imply that the association pathway is unlikely to involve nicotine metabolism. Like coffee and tea, dark chocolate contains caffeine and tastes bitter. Therefore, these findings suggest that the association with coffee and tea consumption could be mediated through preference for bitter taste in addition to caffeine metabolism. Cornelis and van Dam (2021) found in their study that genetically inferred bitter taste perception indeed did play a role in coffee and tea drinking behaviour but to a lesser extent than genetically inferred caffeine sensitivity [54]. They also found support for conditioned taste preferences where individuals learn to associate the bitter taste with either beneficial or adverse physiological effects of caffeine.

While we did not observe direct evidence of a pathway involving smoking, it is well known that smoking and coffee consumption correlate [55]. The prevailing belief is that tobacco smoke increases an individual's caffeine metabolism and that is why smokers require more caffeine to achieve the desired effect [56]. Once an individual stops smoking, their caffeine metabolism eventually returns to normal. A previous Mendelian randomization study found support for this relationship [57]. They compared current, former and never smokers using a single SNP as a biomarker for CPD [57]. They found no association with coffee consumption among never or former smokers in their meta-analysis of three datasets but did find an association among current smokers.

Our results align with the work of Cornelis and van Dam, suggesting some shared genetics between nicotine metabolism and caffeine metabolism, along with potential connections to bitter taste perception. Both smoking and caffeine impact the metabolism of some medications and affect optimal dosages [56]. Additionally, it has been proposed that smokers attempting to quit, may inadvertently confuse symptoms of caffeine toxicity with nicotine withdrawal symptoms if they fail to adjust their coffee consumption [55]. Thus, understanding the interactions between smoking and caffeine consumption is of great interest.

The main limitation of our study was the potential bias due to UKB's enrichment with healthier individuals. Additionally, we had limited statistical power to study ancestry groups other than the White British group, as the sample sizes are notably smaller. This limits the accuracy of the effect size estimates for the other groups, and thus our power to detect differences with the White British group or to observe replication of our results. Based on our ancestry-stratified follow-up analyses, the other ancestry groups did not seem to notably differ from our White British sample, admittedly possibly due to a lack of statistical power. We were, however, able to replicate our liver enzyme findings in some of the other ancestry groups. Another limitation is that

there may be some overlap between the individuals included in our NMR GWAS and those analyzed in the top NMR SNP (rs56113850) PheWASs from MRBase and FinnGen. Considering that the GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN) ($n = 243,952$) is the most likely cohort in MRBase to have overlap with our data, and that the total sample size for FinnGen was 377,277, our NMR GWAS was conducted with a relatively small sample size of 5,185 individuals (of whom 2,572 were from Finland). Thus, we expect that the overlapping individuals have minimal impact on the estimated effect sizes for most of the outcome variables in the FinnGen and MRBase PheWASs. A further limitation, as discussed by Buchwald et al. [15], was that individual-level genotype and NMR data required for the fine-mapping analyses of the NMR loci were only available from two Finnish datasets. Thus, the weights used for the chromosome 19 SNPs in the genetic score rely solely on these datasets. However, one of our main strengths was the ability to perform FINEMAP analysis: the variance explained by the chromosome 19 FINEMAP top configuration was 32.1 % as opposed to 23 % explained by the top SNP alone. Additionally, apart from having a very strong instrumental variable, another main strength of our study was the implementation of the GxE MR approach, which involved comparing ever versus never smokers and allowed for inferences about the association pathways.

Conclusions

This study presents the first comprehensive PheWAS of the NMR, uncovering novel associations not previously reported in the context of nicotine metabolism. Our results suggest that genetically determined faster nicotine metabolism is associated not only with smoking related traits but also with various adverse health outcomes. Our GS for faster nicotine metabolism was associated with worse liver enzyme and lipid values with respect to associated diseases, as well as increased coffee and tea consumption. Importantly, we saw no evidence of a causal pathway through nicotine metabolism for these associations. Leaning on the assumption that slow metabolizers of nicotine have an easier time quitting, our findings are promising. They support a possibility that a future smoking cessation therapy targeting genes associated with the NMR, to convert fast metabolizers into slower metabolizers, could work without adverse side effects and potentially even provide other health-related benefits. Future research, focusing on these newly highlighted variables using additional data and study designs, involving different sources of potential bias, is warranted to confirm and extend these findings.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10654-025-01270-5>.

Acknowledgements This research has been conducted using the UK Biobank Resource under Application Number 22627. We thank Dr. Samuel Jones for organizing the UK Biobank data files. We want to express our gratitude to all participants in UK Biobank, YFS, FINRISK and FinnGen as well as to all people, institutes and funding bodies that have been involved in the infrastructure surrounding these cohorts, and made these valuable resources possible.

Author contributions Jadwiga Buchwald: Conceived and designed the study; acquired funding; determined the statistical analysis methodology; curated data; conducted the analysis; interpreted and visualized the data; wrote the manuscript. Matti Pirinen: Conceived and designed the study; acquired funding; determined the statistical analysis methodology; curated data; interpreted the data; supervised the project; critically reviewed and revised the manuscript. Jaakko Kaprio: Conceived and designed the study; acquired funding; critically reviewed and revised the manuscript. Terho Lehtimäki: Acquired data. Olli Raitakari: Acquired data. Veikko Salomaa: Acquired data. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Funding Open Access funding provided by University of Helsinki (including Helsinki University Central Hospital). This study has been funded by the Doctoral Program in Population Health and the Doctoral School in Health Sciences from the University of Helsinki, as well as Yrjö Jahnsson Foundation to JB, by the Academy of Finland (grants 338507, 336825 and 352795) and Sigrid Juselius Foundation to MP and JK, and by the Academy of Finland Center of Excellence in Complex Disease Genetics (grants 336823, 352792), and the Finnish Foundation for Cardiovascular Research to JK. VS was supported by the Juho Vainio Foundation. The FINRISK surveys have been mainly funded from budgetary funds of THL. Important additional funding has been obtained from the Academy of Finland and from several domestic foundations. The YFS has been financially supported by the following sources: Academy of Finland (grant numbers 356405, 322098, 286284, 134309 (Eye), 126925, 121584, 124282, 255381, 256474, 283115, 319060, 320297, 314389, 338395, 330809, and 104821, 129378 (Salve), 117797 (Gendi), and 141071 (Skidi)); the Social Insurance Institution of Finland; Competitive State Research Financing of the Expert Responsibility Area of Kuopio, Tampere and Turku University Hospitals (grant X51001); the Juho Vainio Foundation; the Paavo Nurmi Foundation; the Finnish Foundation for Cardiovascular Research; the Finnish Cultural Foundation; the Sigrid Juselius Foundation; the Tampere Tuberculosis Foundation; the Emil Aaltonen Foundation; the Yrjö Jahnsson Foundation; the Signe and Ane Gyllenberg Foundation; the Diabetes Research Foundation of Finnish Diabetes Association; EU Horizon 2020 (grant 755320 for TAXINOMISIS and grant 848146 for To Aition); the European Research Council (grant 742927 for MULTIEPIGEN project); the Tampere University Hospital Supporting Foundation, Finnish Society of Clinical Chemistry; the Cancer Foundation Finland; pBETTER4U_EU (Preventing obesity through Biologically and bEhaviorally Tailored inTERventions for you; project number: 101080117); and the Jane and Aatos Erkkö Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability Access to the UK Biobank data can be applied at <http://ukbiobank.ac.uk/register-apply/>. To apply for access to the YFS data see: <http://youngfinnsstudy.utu.fi/>, and for FINRISK see: <https://thl.fi/en/web/thl-biobank>. All analyses were performed in R and bash. Scripts have been archived in GitHub and Zenodo: <https://doi.org/10.>

5281/zenodo.15824655.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval UKB was approved by the North West Multi-center Research Ethics Committee. The Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District approved the FINRISK surveys (decision number 229/E0/06 in 2007 and 162/13/03/00/11 in 2012). The local University Hospital ethics committees in Turku and Tampere approved the YFS study.

Consent to participate All participants provided written informed consent.

Consent for publication Not applicable.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Murray CJ, Aravkin AY, Zheng P, Abbafati C, Abbas KM, Abbasi-Kangevari M, et al. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396(10258):1223–49.
- Drope J, Hamill S, Chaloupka F, Guerrero C, Lee H, Mirza M, et al. The Tobacco Atlas, Seventh edition. Vital Strategies and Tobacconomics; 2022.
- WHO. WHO global report on trends in prevalence of tobacco use 2000–2025, Fourth edition. World Health Organization; 2021.
- Benowitz NL. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. *Annu Rev Pharmacol Toxicol*. 2009;49:57–71.
- Yuan M, Cross SJ, Loughlin SE, Leslie FM. Nicotine and the adolescent brain. *J Physiol*. 2015;593(16):3397–412.
- Stolerman IP, Jarvis M. The scientific case that nicotine is addictive. *Psychopharmacology*. 1995;117:2–10.
- Dempsey D, Tutka P, Jacob P III, Allen F, Schoedel K, Tyndale RF, et al. Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clin Pharmacol Therapeutics*. 2004;76(1):64–72.
- Benowitz NL, Pomerleau OF, Pomerleau CS, Jacob P III. Nicotine metabolite ratio as a predictor of cigarette consumption. *Nicotine Tobacco Res*. 2003;5(5):621–4.
- Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenet Genomics*. 2004;14(9):615–26.
- Chenoweth MJ, Tyndale RF. Pharmacogenetic optimization of smoking cessation treatment. *Trends Pharmacol Sci*. 2017;38(1):55–66.
- Patterson F, Schnoll R, Wileyto E, Pinto A, Epstein L, Shields P, et al. Toward personalized therapy for smoking cessation: a randomized placebo-controlled trial of bupropion. *Clin Pharmacol Therapeutics*. 2008;84(3):320–5.
- Chenoweth MJ, O'Loughlin J, Sylvestre MP, Tyndale RF. CYP2A6 slow nicotine metabolism is associated with increased quitting by adolescent smokers. *Pharmacogenet Genomics*. 2013;23(4):232.
- Lerman C, Schnoll RA, Hawk LW, Cinciripini P, George TP, Wileyto EP, et al. Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: A randomised, double-blind placebo-controlled trial. *Lancet Respir Med*. 2015;3(2):131–8.
- Loukola A, Buchwald J, Gupta R, Palviainen T, Hällfors J, Tikkanen E, et al. A genome-wide association study of a biomarker of nicotine metabolism. *PLoS Genet*. 2015;11(9):e1005498.
- Buchwald J, Chenoweth MJ, Palviainen T, Zhu G, Benner C, Gordon S, et al. Genome-wide association meta-analysis of nicotine metabolism and cigarette consumption measures in smokers of European descent. *Mol Psychiatry*. 2021;26(6):2212–23.
- Hukkanen J, Jacob P, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev*. 2005;57(1):79–115.
- Chenoweth MJ, Novalen M, Hawk LW Jr, Schnoll RA, George TP, Cinciripini PM, et al. Known and novel sources of variability in the nicotine metabolite ratio in a large sample of treatment-seeking smokers. *Cancer Epidemiol Biomarkers Prevent*. 2014;23(9):1773–82.
- Denny JC, Bastarache L, Roden DM. Phenome-wide association studies as a tool to advance precision medicine. *Annu Rev Genomics Hum Genet*. 2016;17:353–73.
- Polimanti R, Jensen KP, Gelernter J. Phenome-wide association study for CYP2A6 alleles: rs113288603 is associated with hearing loss symptoms in elderly smokers. *Sci Rep*. 2017;7(1):1–7.
- Girattallah H, Chenoweth MJ, Pouget JG, El-Boraie A, Alsaafin A, Lerman C, et al. CYP2A6 associates with respiratory disease risk and younger age of diagnosis: a phenome-wide association Mendelian randomization study. *Hum Mol Genet*. 2023; dda172.
- Yamamiya I, Yoshisue K, Ishii Y, Yamada H, Chiba M. Effect of CYP2A6 genetic polymorphism on the metabolic conversion of tegafur to 5-fluorouracil and its enantioselectivity. *Drug Metab Dispos*. 2014;42(9):1485–92.
- Murai K, Yamazaki H, Nakagawa K, Kawai R, Kamataki T. Deactivation of anti-cancer drug letrozole to a carbinol metabolite by polymorphic cytochrome P450 2A6 in human liver microsomes. *Xenobiotica*. 2009;39(11):795–802.
- Millard LA, Davies NM, Gaunt TR, Davey Smith G, Tilling K. Software application profile: PHESANT: a tool for performing automated phenome scans in UK Biobank. *Int J Epidemiol*. 2018;47(1):29–35.
- Millard LA, Munafò MR, Tilling K, Wootton RE, Davey Smith G. MR-pheWAS with stratification and interaction: searching for the causal effects of smoking heaviness identified an effect on facial aging. *PLoS Genet*. 2019;15(10):e1008353.
- Benner C, Spencer CC, Havulinna AS, Salomaa V, Ripatti S, Pirinen M. FINEMAP: efficient variable selection using summary data from genome-wide association studies. *Bioinformatics*. 2016;32(10):1493–501.
- Biobank Showcase. Field 21022: [Variable description]; n.d. Website. Available from: <https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=21022>.

27. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562(7726):203–9.
28. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779.
29. Borodulin K, Vartiainen E, Peltonen M, Jousilahti P, Juolevi A, Laatikainen T, et al. Forty-year trends in cardiovascular risk factors in Finland. *Eur J Public Health*. 2015;25(3):539–46.
30. Raitakari OT, Juonala M, Rönönen T, Keltikangas-Järvinen L, Räsänen L, Pietikäinen M, et al. Cohort profile: The cardiovascular risk in Young Finns Study. *Int J Epidemiol*. 2008;37(6):1220–6.
31. Gibson MJ, Lawlor DA, Millard LA. Identifying the potential causal role of insomnia symptoms on 11,409 health-related outcomes: a phenome-wide Mendelian randomisation analysis in UK Biobank. *BMC Med*. 2023;21(1):1–15.
32. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc: Ser B (Methodol)*. 1995;57(1):289–300.
33. FinnGen. FinnGen Documentation of R9 release; 2023. Website (accessed July 11, 2023). Available from: <https://finngen.gitbook.io/documentation/v/r9/methods/phewas/logistic-regression#null-models>.
34. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner KM, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;613(7944):508–18.
35. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife*. 2018;7:e34408.
36. Elsworth B, Lyon M, Alexander T, Liu Y, Matthews P, Hallett J, et al. The MRC IEU OpenGWAS data infrastructure. *BioRxiv*. 2020; p. 2020–08.
37. Chenoweth MJ, Ware JJ, Zhu AZ, Cole CB, Cox LS, Nollen N, et al. Genome-wide association study of a nicotine metabolism biomarker in African American smokers: Impact of chromosome 19 genetic influences. *Addiction*. 2018;113(3):509–23.
38. Baurley JW, Edlund CK, Pardamean CI, Conti DV, Krasnow R, Javitz HS, et al. Genome-wide association of the laboratory-based nicotine metabolite ratio in three ancestries. *Nicotine Tobacco Res*. 2016;18(9):1837–44.
39. Baurley JW, Bergen AW, Ervin CM, Park SsL, Murphy SE, McMahan CS. Predicting nicotine metabolism across ancestries using genotypes. *BMC Genomics*. 2022;23(1):663.
40. Biobank Showcase. Variable descriptions; n.d. Website. Available from: <https://biobank.ndph.ox.ac.uk/showcase/search.cgi>.
41. Hovanec J, Weiß T, Koch HM, Pesch B, Behrens T, Kendzia B, et al. Smoking intensity and urinary nicotine metabolites by socioeconomic status in the Heinz Nixdorf Recall study. *BMC Public Health*. 2022;22(1):1–10.
42. Wassenaar CA, Dong Q, Wei Q, Amos CI, Spitz MR, Tyndale RF. Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNA4 variation and smoking behaviors and lung cancer risk. *J Natl Cancer Inst*. 2011;103(17):1342–6.
43. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat Genet*. 2019;51(2):237–44.
44. Sofuoglu M, Herman AI, Nadim H, Jatlow P. Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology*. 2012;37(6):1509–16.
45. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol*. 2017;186(9):1026–34.
46. Fletcher C, Peto R. The natural history of chronic airflow obstruction. *Br Med J*. 1977;1(6077):1645–8.
47. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report. GOLD executive summary. *Am J Respir Crit Care Med*. 2017;195(5):557–82.
48. Oelsner EC, Balte PP, Bhatt SP, Cassano PA, Couper D, Folsom AR, et al. Lung function decline in former smokers and low-intensity current smokers: a secondary data analysis of the NHLBI Pooled Cohorts Study. *Lancet Respir Med*. 2020;8(1):34–44.
49. Liu Z, Suo C, Jiang Y, Zhao R, Zhang T, Jin L, et al. Phenome-wide association analysis reveals novel links between genetically determined levels of liver enzymes and disease phenotypes. *Phenomics*. 2022;2(5):295–311.
50. Kalas MA, Chavez L, Leon M, Taweeseedt PT, Surani S. Abnormal liver enzymes: a review for clinicians. *World J Hepatol*. 2021;13(11):1688.
51. Lala V, Zubair M, Minter DA. Liver function tests. In: StatPearls [internet]. StatPearls Publishing; 2022.
52. Julkunen H, Cichońska A, Tiainen M, Koskela H, Nybo K, Mäkelä V, et al. Atlas of plasma NMR biomarkers for health and disease in 118,461 individuals from the UK Biobank. *Nat Commun*. 2023;14(1):604.
53. Cornelis MC, Kacprowski T, Menni C, Gustafsson S, Pivn E, Adamski J, et al. Genome-wide association study of caffeine metabolites provides new insights to caffeine metabolism and dietary caffeine-consumption behavior. *Hum Mol Genet*. 2016;25(24):5472–82.
54. Cornelis MC, van Dam RM. Genetic determinants of liking and intake of coffee and other bitter foods and beverages. *Sci Rep*. 2021;11(1):23845.
55. Swanson JA, Lee JW, Hopp JW. Caffeine and nicotine: a review of their joint use and possible interactive effects in tobacco withdrawal. *Addict Behav*. 1994;19(3):229–56.
56. Zevin S, Benowitz NL. Drug interactions with tobacco smoking: an update. *Clin Pharmacokinet*. 1999;36:425–38.
57. Bjørngaard JH, Nordestgaard AT, Taylor AE, Treur JL, Gabrielsen ME, Munafo MR, et al. Heavier smoking increases coffee consumption: findings from a Mendelian randomization analysis. *Int J Epidemiol*. 2017;46(6):1958–67.