Causal role of IL-33/ST2 in dementia: A Mendelian randomisation study

Abstract

Background: Experimental, genetic and clinical studies have implicated a regulatory role of the IL-33/ST2 axis in the pathenogenesis of Alzheimer's disease (AD), potentially through prevention of beta-amyloid plaques accumulation and inflammation reduction.The role of such axes in AD initiation and progression could be key to the development of preventive and therapeutic measures. In this study, we investigated the possible role of the IL-33/ST2 pathway in all types of dementia, and in AD in particular, using Mendelian randomisation (MR) methods.
Methods: We used data from the White European participants in the UK BioBank and applied an exhaustive classification of dementia cases to conduct two-stage individual-level, and subsequently, summary-level MR. For the individual-level model, polygenic risk scores (PRS) for IL-33 and ST2 were constructed by genetic instruments, using UK BioBank and external genome-wide association studies (GWAS). MR evaluated the causal effect between these putative risk factors and dementia. Summary level MR was also conducted using external GWAS including a larger pool of AD cases.
Results: Our ST2 PRS was significantly association with serum level of sST2, while the serum level is also positively associated with dementia cases in the UK BioBank. However, neither individual nor summary-level MRs were able to establish a positive link between IL33 or ST2 PRS and the risk of dementia, although there was a statistically non-significant but consistent trend between ST-2 26IV and AD. The results were the same when conditioned for both cytokines and in gender-stratified models.
Conclusion: Our study highlights the relevance of the IL-33/ST2 axis in dementia including AD, but fails to establish a genetic control over the risk of developing the disease.

Introduction

Dementia is a significant global health challenge, defined as ongoing cognitive decline and impairment of memory[12]. Currently, there is over 55 million people living with dementia worldwide [13], with approximately one million diagnosed cases in the United Kingdom. Subsequently, this poses a significant burden to the individuals, their families and healthcare systems across the world. Alzheimer's disease (AD), vascular dementia (VD) and frontotemporal dementia are the main types of dementia [12];therefore, dementia is a general term used to describe the group of similar diseases [17]. The underlying aetiology of most cases is multifactorial, involving intricate interactions between genetic predisposition and environmental factors. AD is characterised by the gradual decline in a person's memory and cognitive functions [1]. This is the result of the build-up of misfolded beta-amyloid (Aβ) in the brain tissue, which clumps together and eventually forms plaques. The plaques can block essential cell signalling pathways and stimulate the activation of immune system cells that signal inflammatory responses that damage neurons [1].

Specific gene variants have been associated with an enhanced risk of developing AD [1]; Currently, Apolipoprotein E (APOE) has shown to be the most widely understood risk variant associated with AD [18]. Specifically, carriers of the APOE-ϵ4 allele have been associated with a heightened risk of developing dementia [14]. Multiple studies have indicated the polygenic nature of AD, suggesting that multiple variants contribute to increased susceptibility to developing the disease[20]. Recent research conducted in 2022 by Karlsson et al. using polygenetic risk score (PRS) analysis on twins suggested a polygenetic contribution to AD susceptibility. The study found a 71% total genetic contribution to the risk of developing AD, and the remaining 29% was accounted for by unique environmental factors experienced by each twin[6].

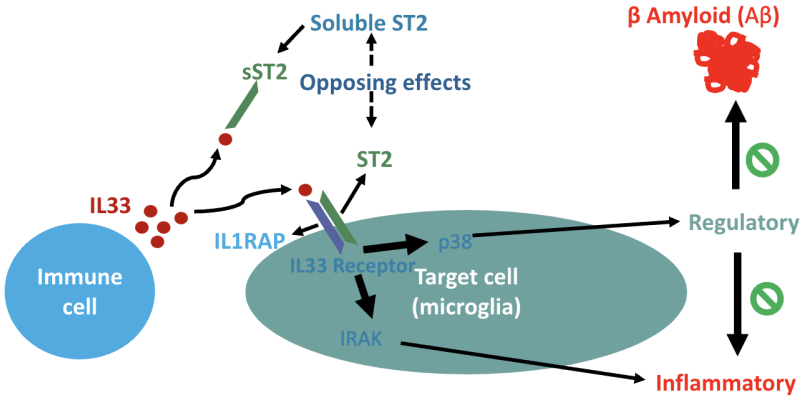

Figure 1: Hypothesised IL-33/sST2 axis and its effect on the beta-amyloid protein in dementia.

Multiple molecular pathways have been hypothesised to be associated with AD parthenogenesis; experimental and observational studies over the last decade have highlighted the role of the interleukin-33 (IL-33)/ST2 pathway and its differential activation in the development of AD. IL-33 was initially identified as a candidate gene of AD by Chapuis et al. in 2009; further analysis found three relevant single-nucleotide polymorphisms (SNP) within IL-33[3] [5] .

This research was followed in 2016 with research suggesting an impairment of the IL-33/ST2 signalling pathway could contribute to the development of AD; this was associated with an observed increase in serum levels of soluble ST2 (sST2) in patients with mild cognitive impairment (preclinical AD) when compared to controls[4]. A significant decrease in IL-33 and an increase in sST2 was observed in AD patients when compared to healthy controls, suggesting a dis-regulated pathway[15]. AD is defined as the extracellular deposit of misfolded beta-amyloid (Aβ); this initiates microglia migration towards the misfolded Aβ primed for their phagocytosis[9]. As AD progresses, the microglial cells become dysregulated and clearance of Aβ plaques is diminished[9]. Lau et al. (2022) observed that an injection of IL-33 into a transgenic mice model of AD reduced Aβ pathology as a result of the microglia epigenetic and transcriptomic profiles being reprogrammed to increase the phagocytotic activity of Aβ plaques [9]. Fu et al (2016) findings were supported in 2022 as higher levels of sST2 were found associated with AD progression in females; additionally, genetic variant rs1921622 was linked to reduced levels of sST2 and subsequent lower AD risk in women carrying the APOE4 genotype. Furthermore, analysis of immunohistochemical and transcriptome studies found a mechanistic link between rs1921622/sST2 levels regulating AD pathology[5].

We therefore aimed to determine if there is a causal effect between dementia cases and IL33/ST2 axis, by employing polygenic risk score (PRS) in Mendelian Randomisation (MR). To our knowledge, this is the first MR study investigating all types of dementia and the IL33/ST2 axis.

Methods

Workflow

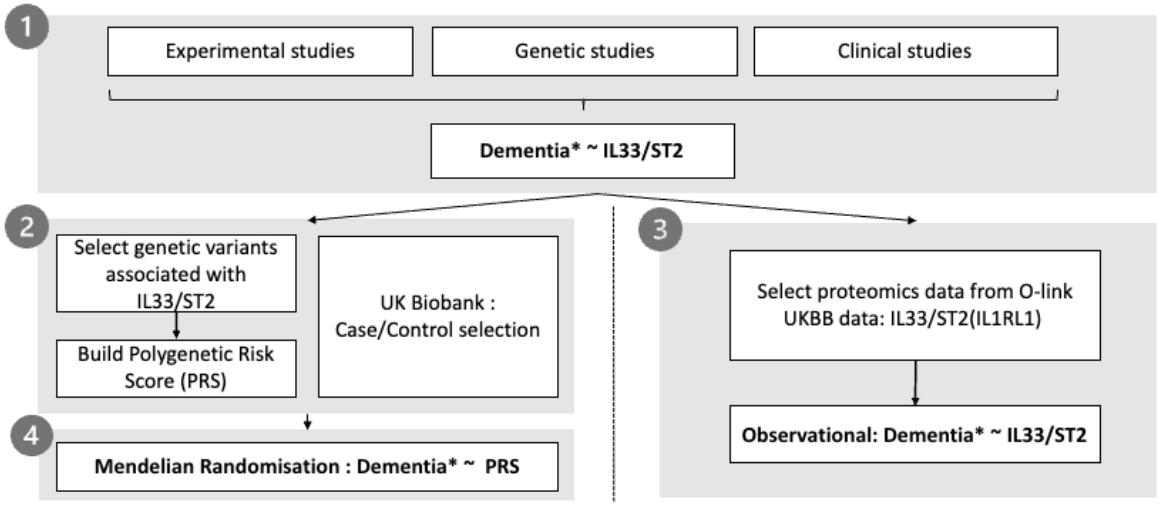

Figure 2: Flowchart representing the study process.

Data Definition and Processing

The cohort for the study was derived from the UK BioBank, which follows the health of 500,000 volunteer participants[10]. An exhaustive classification of dementia cases was used to establish three categories within the dataset: Alzheimer's disease, vascular dementia and other dementia. We also create a collective variable including all types of dementia. Within the analysis, the inclusion of cases reported in Hospital Inpatient Data based on International Classification of Disease 9th (ICD9) and 10th (ICD10) codes and Death Registry Data encoded in ICD10 codes (ICD codes for each category are listed in appendix). Additionally, prevalent cases of dementia (diagnosed dementia in the UK Biobank before the recruitment of the study) were included in the sample. Self-reported cases of dementia and non-white ethnicity were both excluded from the study sample.

Proteomic data was obtained from O-Link UK BioBank data. There are 88% missing for IL-33 due to being below detection limits and 12% missing for ST2 due to no data recorded. Thus, the quantile regression method and K-Nearest Neighbors (KNN) method are employed to impute IL-33 and ST2 respectively, using R package `impute`.

Mendelian Randomisation

Unlike observational risk factors, genetic variants are not typically associated with a wide range of another factors, and are believed not associated with other variants, except those with which they are in linkage disequilibrium (assumption follows from the law of independent assortment). They are less likely to be confounded as observational risk factors and can be used as a proxy for risk factors to estimate effect size unbiased from any unobserved confounding[16]. Therefore, combining the association summarised by the Genome-Wide Association Study (GWAS), this method (MR) allows us to mimic randomised controlled trials by using the genetic instrument variables (genetic IVs) and then estimate the causal effect between risk factors and outcomes.
We conducted MR studies at both individual-level, (genetic data, risk factors and outcomes disease all at the individual-level granularity) and summary-level (using reported summary statistics from GWAS of risk factors, IL-33/ST2, and dementia outcome).

Individual-level MR

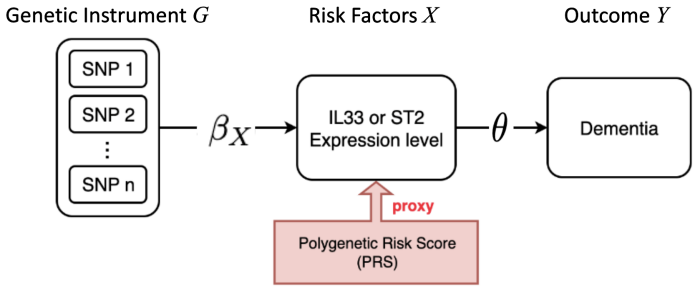

Figure 3: The directed acyclic graph (DAG) of individual-level MR

For individual-level MR, a two-stage method using a polygenic risk score (PRS) as a proxy for risk factors is employed. The two stages are a linear approximation of risk factors and a regression from risk factor to outcome.

$$X = f(G) = \beta_X G + \epsilon_X$$
$$\text{PRS} \propto \hat{X} = \beta_X G$$

The first stage (linear approximation) is defined by the equations shown above, where β_X is the association between genetic IVs G and risk factors X, and θ is the causal effect from risk factor X to outcome Y. G were selected and then used to construct PRS which is a proxy of risk factors (IL-33/ST2 expression level). The genetic IVs selection and definition of PRS construction will be explained in the following sections. β_X was obtained from the GWAS of IL-33 or ST2, while individual-level genotype data G and dementia outcomes Y were obtained from UK BioBank.

$$\begin{aligned} Y &= h(\hat{X}) \\ &= logit(p_i) \\ &= \hat{\theta}_{2SLS} \cdot PRS + \epsilon_Y \end{aligned}$$

The second stage (regression), we estimated the causal effect between risk factors and dementia by regressing the outcome against PRS. Logistic regression is used in the second stage due to the binary nature of our outcome. Since the GWAS of ST2 is also conducted on UK Biobank data, essentially we conducted a one-sample two-stage MR; whereas GWAS of IL-33 is conducted on other sample populations so we performed a two-sample two-stage MR. $\hat{\theta}_{2SLS}$ is the estimated causal effect obtained from individual-level MR.

Summary-level MR

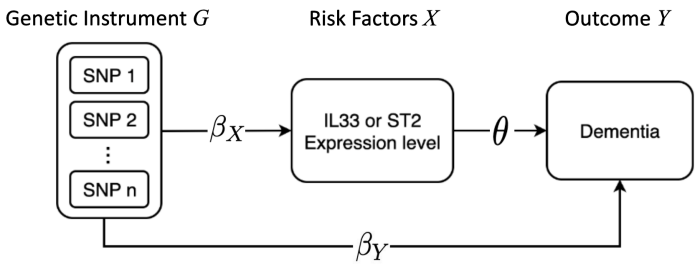

Figure 4: The directed acyclic graph (DAG) of summary-level MR

Due to the limited number of cases for each type and all types of dementia, we also conducted a summary-level MR for sensitivity analysis. Using the GWAS results of AD provides us with a larger sample size of cases. The first stage is the association β_X between genetic IVs G and risk factors X. The GWAS used and the genetic IV selection are the same as individual-level MR. The second stage is the association β_Y between genetic instrument variables G and outcome Y, obtained from the GWAS of AD [8]. The causal effect θ was estimated using four methods: MR-IVW, MR-Egger, simple median and weighted median.

MR Assumptions

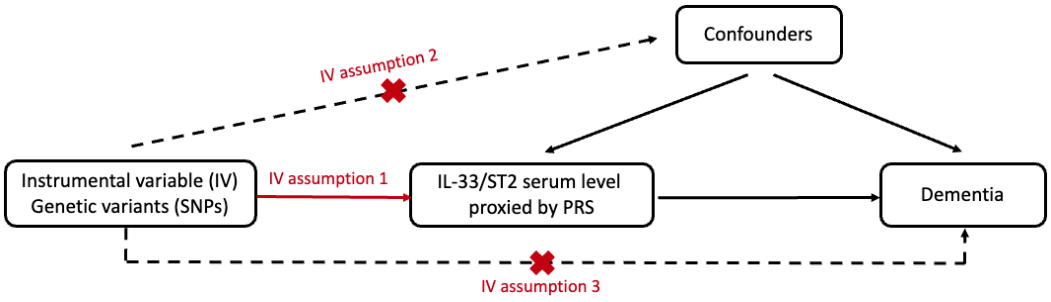

Figure 5: The directed acyclic graph (DAG) of the whole MR framework showing assumptions

For both our MR methods, three assumptions were considered [2]. Since the second two assumptions are difficult to verify, we only examined the first assumption.

1. **Relevance**: Therefore, genetic IVs should be associated with the risk factors X, and only strongly associated IVs should be included. Using a conventional genome-wide significance level can filter many weak instruments, but this can also be verified using the F-statistic as a measure of the strength of the association between the IVs and the risk factors. This assumption was validated by checking if the F-statistics from a linear

regression between PRS and risk factors (IL-33 and ST2 expression level) is larger than 10.

2. **Exchangeability**: This assumption require the genetic IVs independent of other factors which affect the outcome, which is also know as independence assumption. This assumption is hard to verify, but it could be violated if subgroups in the study population have both different genotype frequencies and different distributions of the outcome (population stratification).

3. **Exclusion restriction**: This assumes that the genetic IVs is associated with the outcome through the studied risk factor only. This is hard to verify, and could be violated if the genetic IVs has multiple (pleiotropic) effects or if a nearby variant with which it is in linkage disequilibrium affects the outcome in other ways than through the exposure of interest.

Instrument variable (IV) selection

GWAS summary statistics were used to select genetic IVs for both IL-33 and ST2. IL-33 was investigated using a European population (Sweden, Denmark, U.K., Germany, Estonia, Croatia)(N=11,793) using imputed data from Affymetrix, Illumina [19]. ST2 used a European population (N=21,758) using imputed data from Affymetrix, Illumina[14]. Significant single nucleotide polymorphisms (SNPs) were selected on two specified p-value thresholds, a conventional genome-wide significance level $p < 5e \times 10^{-8}$ and a more loosen $p < 10^{-6}$ since IL-33 has no SNPs selected at the more stringent threshold. We identified 10 SNPs for IL-33 with a p-value threshold of $p < 10^{-6}$. ST2 had two p-value thresholds $p < 5e \times 10^{-8}$ and $p < 10^{-6}$ which resulted in 390 and 1280 SNPs respectively. A clumping of data was conducted to identify independent signals among correlated SNPs. After clumping IL-33 SNPs using $R = 0.01$, 2 SNPs were obtained. ST2 after clumping at $R = 0.01$ resulted in 2 and 26 SNPs were found for each threshold. To account for genotype missingness within our dataset, we excluded individuals with both SNPs missing from our sample size (under 2 variants) and individuals with 26 SNPs missing (under 26 variants).

For summary-level MR, Alzheimer's disease(AD) was investigated using a European population (N=21,982) using imputed data from Affymetrix, Illumina [8]. The summary statistics of GWAS of AD were then merged with the selected IVs of risk factors (IL-33 or ST2). One thing to note is that after merging the 26 genetic IVs selected for ST2 and for outcome Y, we resulted in only 25 SNPs due to one SNP missing in the GWAS of AD.

Polygenetic Risk Score (PRS) construction

Each individual in our study was computed with a PRS using the following equation (1) to calculate the weighted sum of the selected SNPs.

$$\text{PRS} = \frac{\sum_{i=1}^{N} \beta_{Xi} G_i}{N} \tag{1}$$

$\beta_{Xi} \in \mathbb{R}$ is the effect size of i-th SNP from GWAS of IL-33 or ST2. $G_i \in 0, 1, 2$ is the individual's genotype data, which means the number of effect alleles for the i-th SNP in each

individual, obtained from UK BioBank. N is the number of non-missing SNP.

IL33 PRS included 2 selected SNPs (see above) which hereafter is referred to as IL-33 2IV. For ST-2 two PRS were constructed with 2 or 26 SNPs (ST2 2IV and 26IV), selected as described above.

PRS Validation - Positive Control and Negative Control

To examine the validity and strength of the PRS, we identified asthma as highly related to the outcome variable that is a positive control. Asthma is a type 2-driven immune hypersensitivity disorder which is highly associated with IL-33/ST2 axis [7]. ICD codes used for selecting asthma cases are listed in the appendix. We also included an unrelated outcome to IL-33/ST2, hot drink temperature (field 1518) from UK BioBank, as a negative control. This was converted to a binary outcome which resulted in approximately same distribution of cases (4637) and controls.

Analysis Plan

Figure 6 shows the overall regression models included with a specific formula. Dementia includes three types (AD/VD/OD) respectively and all types. PRS includes one PRS for IL-33 and two PRS for ST2. A three-fold model was used for individual-level MR. The first step is the single risk factor regression. To consider the possibility of the effect of one risk factor on the other, the second step, conditioned risk factor regression, where the linear predictors contain one PRS of IL-33 and one PRS of ST2. The third step, stratification by gender, shows a sensitivity analysis.

Figure 6: The overview of all models included in this study.

Step	Analysis performed	Formula
1. Observational study	Logistic regression	Dementia ~ Risk factors
2. PRS strength – relevance assumption	Linear regression	Risk factors ~ PRS
3. Positive control	Logistic regression	Asthma ~ PRS
4. Negative control	Logistic regression	HotDrink ~ PRS
5. Individual-level MR	Logistic regression	Dementia ~ PRS
	Logistic regression (conditioned)	Dementia ~ PRS ST2 + PRS IL33
	Logistic regression stratified by gender	Dementia ~ PRS
6. Summary-level MR	IVW, MR-Egger, Simple median, Weighted median	

- Risk factors: IL-33/ST2 serum protein measurements

Results

Participant Characteristics

Figure 17 in the appendix displays relevant characteristics that were selected as main risk factors for all types of dementia, to extract and characterise relevant data. In summary, there are 7140 cases of all types of dementia. Since there are some patients having multiple diagnoses, there are 3027, 1589, and 5081 cases of Alzheimer's disease, vascular dementia

and other dementia respectively. Females and males are proportionally equally distributed across cases and controls.

PRS Strength and Validation

PRS strength was evaluated by regressing against the serum protein concentration of corresponding putative risk factors, IL-33 and ST2. A positive correlation was observed between both 2IV and 26IV and the serum level of ST2 (Figure 7), with highly significant p-values (p = 2 $\times 10^{-16}$ and p = 2.2 $\times 10^{-16}$ respectively). R^2 values of 0.164 and 0.2582, for ST2 2IV and 26 IV respectively, indicated that up to 25% of variability in serum ST2 can be determined by ST2 PRS, pointing to a strong IV. No association was observed between IL-33 2IV and the serum level of IL-33. Over 88% of test results for serum IL-33 measurements were reported undetectable. This can directly undermine the validity of our linear regression result.

For positive controls, Table 1 shows that both PRS of ST2 present significant results and PRS of IL-33 presents insignificant results; while for negative controls, all PRS are irrelevant to the hot drinks dummy variable (Table 2), which means our PRS was working as expected.

PRS	OR (95% CI)	P-value
ST2 - 26IV	0.181 (0.127, 0.259)	$<2 \times 10^{-16}$
ST2 - 2IV	0.840 (0.811, 0.871)	$<2 \times 10^{-16}$
IL-33 - 2IV	0.944 (0.752, 1.186)	0.622

Table 1: Model results between PRS and positive controls (asthma)

PRS	OR (95% CI)	P-value
ST2 - 26IV	0.460 (0.132, 1.615)	0.225
ST2 - 2IV	0.959 (0.848, 1.084)	0.508
IL33 - 2IV	0.574 (0.261, 1.267)	0.168

Table 2: Model results between PRS and negative controls (hot drinks)

Observational Study

The observational study shows that ST2 is significantly positively associated with all dementia outcomes (all types and each subtypes), while IL-33 is not associated with any dementia diagnosis(Table 3). However, the insignificant results of IL-33 might be caused by the high missingness of the protein expression data, so the association observed is not very convincing.

Individual-level MR results

Single Risk Factor Regression

To identify genetically determined links between IL-33/ST2 PRS and dementia, we conducted two-step MR analyses against all types of dementia, AD, Vascular dementia and other

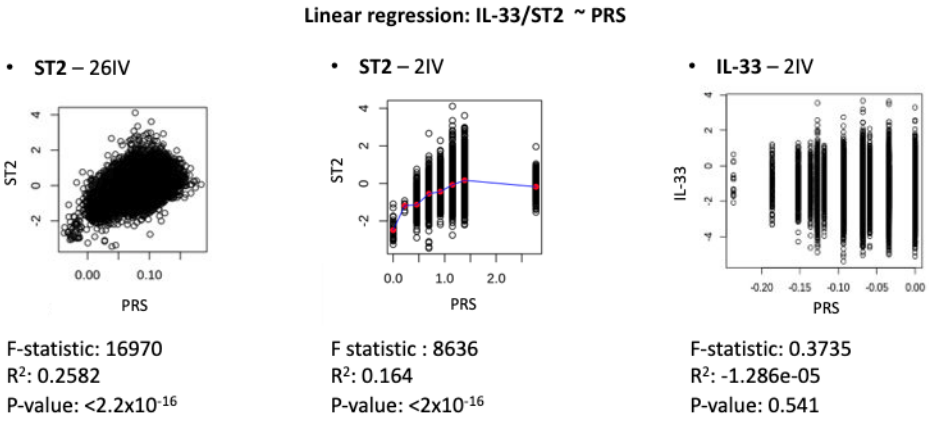

Figure 7: Association between the polygenetic risk score (PRS) and the serum level of proteins under study: ST2 (using two thresholds of p = 5×10^{-8} and p = 10^{-6}) and IL-33 (p = 10^{-6} threshold).

outcome	Risk factor: ST2		Risk factor: IL-33	
	OR (95% CI)	P-value	OR (95% CI)	P-value
All types of dementia	1.563 (1.410, 1.732)	1.64×10^{-17}	0.983 (0.928, 1.041)	0.571
Alzheimer's Disease	1.507 (1.298, 1.748)	6.53×10^{-08}	0.996 (0.916, 1.082)	0.931
Vascular Dementia	1.738 (1.383, 2.179)	1.92×10^{-06}	0.970 (0.853, 1.103)	0.643
Other Dementia	1.500 (1.330, 1.692)	3.85×10^{-11}	1.004 (0.939, 1.074)	0.898

Table 3: Results of the observational studies for ST2 and IL-33 against dementia outcome

types of dementia. As shown in Figure 8, although none of the MRs achieved statistical significance, a trend towards an increase of 3.58 was observed for AD with PRS ST2-26IV. This is consistent with the study hypothesis of higher levels of serum ST2 increasing the odds of dementia.

Conditioned Risk Factor Regression

Both IL-33 and ST2 instruments in combined MRs (IL-33 IV with ST2 2IV or 26IV). However, the results were closely comparable to the unadjusted single cytokine IVs, showing no statistical significance (Figure 9). The same trend in increasing odds of AD with ST2 26IV was seen without achieving statistical significance.

Stratification by Gender

Given a previous MR analysis reporting a genetic link between ST2 levels and the risk of AD in female sub-populations, we extended our study by running our models stratified by gender for all causes of dementia, AD, Vascular Dementia and other dementia. Although ST2 26IV showed an increased trend in odds of disease for most types of dementia, the coefficients had very wide confidence intervals and were not significant (Figure 10). Interestingly, a significant result was observed in females IL-33 2IV (p =0.0387) showing a negative association with other dementia. The OR suggested approximately 1/3 reduction in the odds of

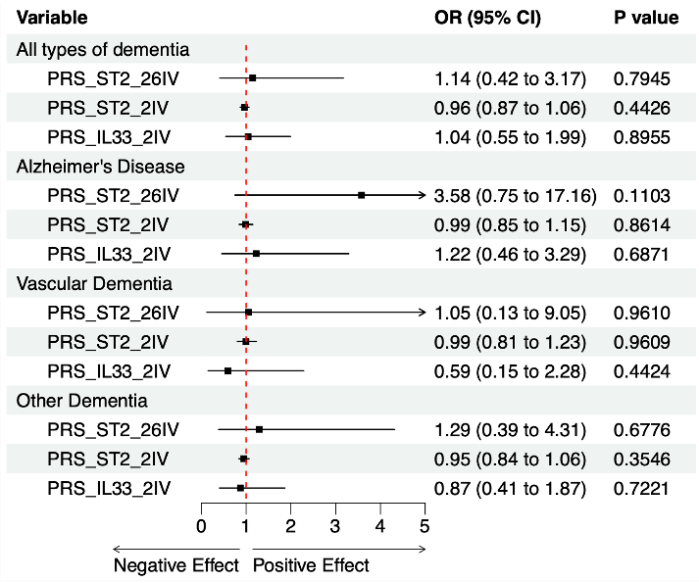

Figure 8: Individual-level two-stage Mendelian randomisation results of the causal link between IL-33 or ST2 and dementia (all types and each type).

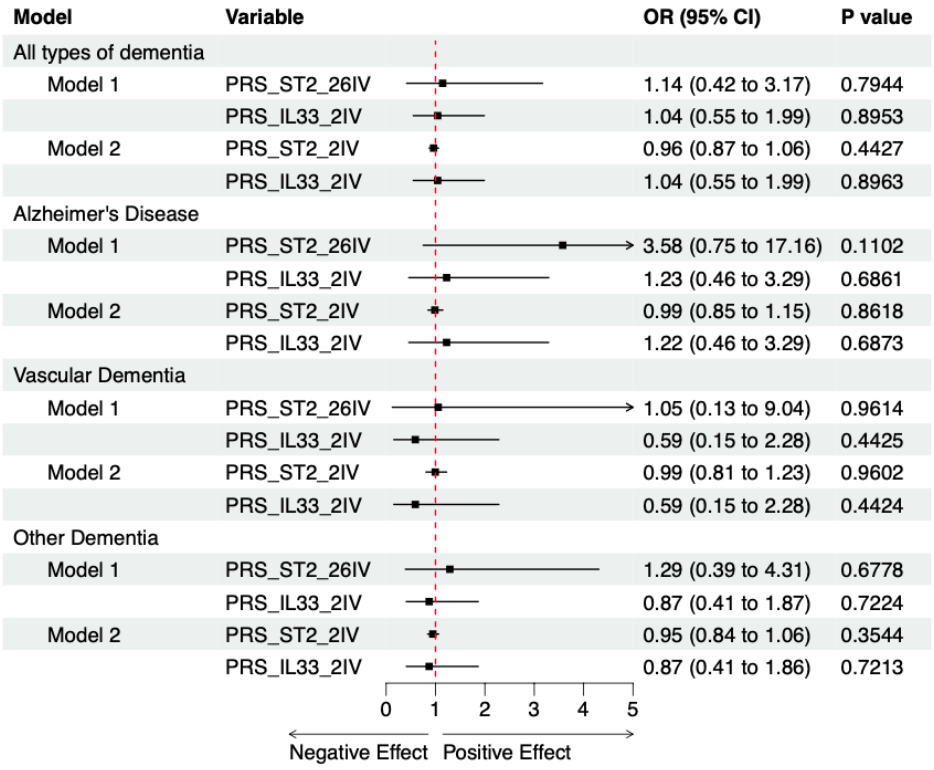

Figure 9: Individual-level two-stage MR results of the causal link between IL-33 or ST2 (conditioned on the other protein) and dementia (all types and each type). Since ST2 has two PRS, there are two combinations of linear predictors (model 1 is IL-33 IV + ST2 26IV, and model 2 is IL-33 IV + ST2 2IV)

disease (OR 0.3, CIs 0.11 - 0.95). As for males, all the MRs stayed insignificant, with only a borderline p-value (0.0578) and OR of 0.86 for PRS ST2 2IV against other dementia.

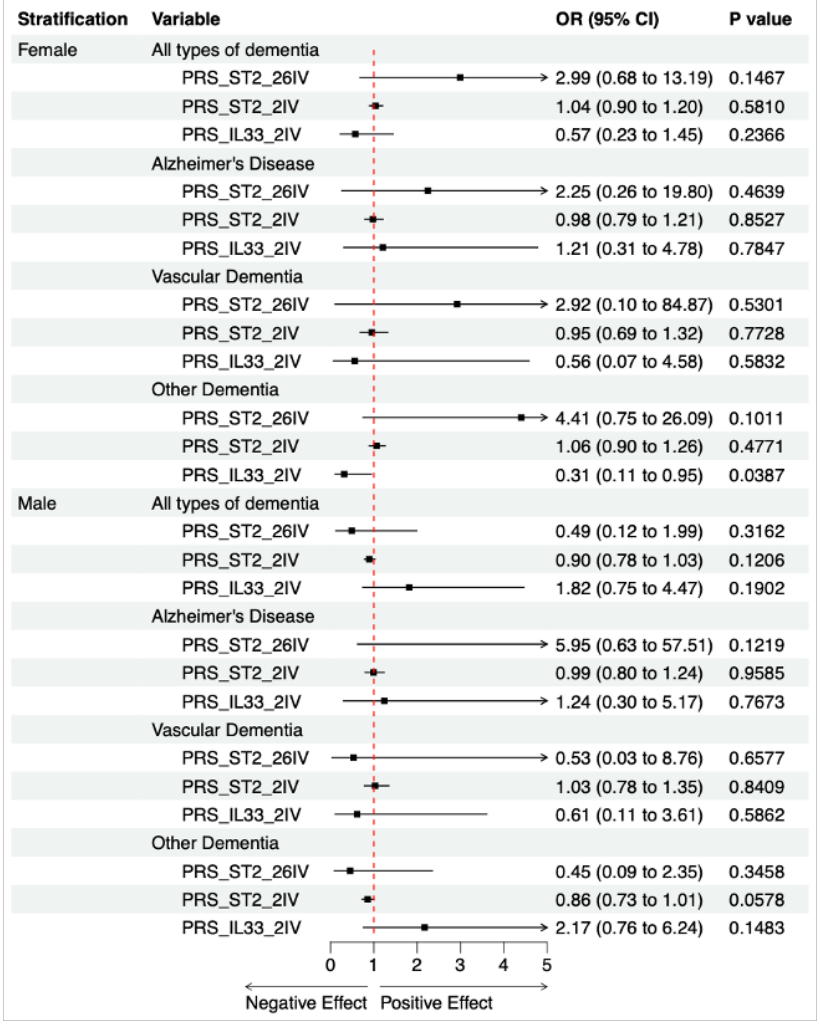

Figure 10: Individual-level two-stage Mendelian randomisation results of the causal link between IL-33 or ST2 and dementia (all types and each type), stratified by gender and dementia subtype.

Summary Level MR

Given the main limitation of our individual-level MR, with the population size of AD cases reaching only over 3000 in the UK Biobank participants at the time of our analysis, we decided to employ summary-level MR to include GWAS with a much bigger sample size with AD cases (21,982, Kunkle et al. 2019). Various MR analyses of ST2 26IV, including Inverse Variance Weighted MR (IVW), MR Egger, and simple and weighted median regression did not show any significant correlation (Table 4). These models showed contrasting slopes, given a few clear outlier SNPs (Figure 16 in Appendix) and not a consistent correlational pattern. As for the other PRSs (ST2 and IL-33 2IV), having only two SNPs, none of the test results were significant or valid.

Table 4: Summary-level MR results.

Instrument variables	Instrument Strength (F)	Method	P-value
IL33 – 2IV	25.15	IVW	0.709
ST2 – 2IV	562.33	IVW	0.281
ST2 – 25IV	1504.52	IVW	0.742
		MR-Egger	0.106
		Simple Median	0.599
		Weighted Median	0.764

Discussion

The results collectively show that although ST2 PRS closely correlates with its serum levels, and despite a positive link between the serum level and increased odds of AD, the MR analyses did not support a genetic determination of AD by the genetic loci relevant to IL-33/ST2 axis studied here.

Upon constructing our PRS, we tested the instrument's strength by regressing it against the serum protein concentration. P-values for the PRS for both ST2-2IV and ST2-26IV were found to be highly significant, accounting for 25% and 16% of the total cytokine serum variation in the study population, respectively (figure 6). This level of genetic control underscored the strength of our ST2 PRS.

We were not able to detect an association with IL-33-2IV PRS (figure 6); however, this result was confounded by the fact that over 88% of the samples from the study population had undetectable levels of IL-33. This is not surprising since the level of this cytokine is generally very low in the serum of individuals without allergic type conditions [7]. Typically, tissue expression (mRNA levels) at the site of inflammation can be a better surrogate marker of IL-33 levels[11]; however, this was beyond the scope of our project. Moreover, we were only able to identify two SNPs, from a single GWAS study available to us, to construct our IL-33 PRS, even after reducing our significance threshold to a p-value of $< 10^{-6}$, which is below the standard practice of $< 10^{-8}$. The unreliability of IL-33 PRS was further highlighted when it failed to associate with asthma, a disease well known to be linked to the pathway [7]. By contrast, both ST2 PRS were highly significant when regressed against asthma.

All MR analyses failed to detect a significant link between ST2 PRS, 2IV and 26IV, and all-cause dementia including AD (figure 8, 9, 10). However, a trend between the ST2 26IV and an enhanced odds of AD was sustained across various MRs, showing up to 5 times increase in odds, although these contained wide confidence intervals which included 1, therefore not statistically significant. The trend was more dominant in male, compared to female subjects, and was not affected by conditioning with IL-33 PRS.

When we stratified our MR model by gender, we observed a statistically significant result ($p = 0.0387$) in females, between IL-33 2IV PRS and other dementia category; displaying a 30% decrease in the odds of disease (figure 9). This reduced risk with each unit increase in PRS is consistent with the study hypothesis [9], although interestingly the correlation was with other types of dementia rather than with AD. Given the above mentioned limitations in

our IL-33 PRS, including a lack of association with asthma, this result should be interpreted cautiouslytab:positive-control.

Generally, no significant correlation was observed between ST2 PRS and AD or with any other types of dementia, in females or males. Although for females, ST2 26IV PRS tended to display general increased odds of all types of dementia, these OR included a very broad range of CIs ranging as wide as 0.1 to 85.

The small sample size for AD cases (CA 3000) in the UK Biobank could have reduced the statistical power of our individual-level MRs. We therefore used bigger GWAS with approximately 22,000 AD cases (see methods). However, our summary-level MR analyses failed to show a statistical significance for IL-33/ST2 PRS link to AD (table 2). The IVW, Egger and Median sensitivity analysis for ST2 26IV showed no significant results and displayed disparate slopes (appendix figure 15), underscoring a lack of clear correlational trend with a few extreme outliers. Together our results fail to establish a link between genetic variation in IL-33/ST2 genes and the risk of developing AD, or other types of dementia.

Our negative results are in contrast with a number of previous clinical, experimental and genetic studies suggesting a role for IL-33/ST2 axis as AD development. To start with, our MR conditions were not similar to Jiang's study (Jiang et al. 2022), where only a single ST2 SNP was used for the MR analysis. Furthermore, their MR was only significant for females with ApoE4, an analysis which we did not perform. Moreover, our IL-33 PRS was unreliable and our ST2 PRS had only two SNPs shared with the previous studies. Although our ST2 PRS seemed robust against the serum level of sST2, their association with AD could be complicated by indirect links and pleiotropic and outlier effects.

There could be other reasons for a lack of genetic determination of AD by IL-33/ST2 despite a robust association between ST2 PRS, ST2 and AD. First, given that IL-33/ST2 is an immune regulatory pathway, it could impact the onset or the progression of the disease rather than causing it. We did not include stratification for early or late-onset AD and therefore are unable to comment on this possibility. Second, many immune inflammatory proteins can be triggered during the course of AD without being involved in the initiation of the disease. Further studies, including time-to-disease analyses and a finer subtype stratification, are granted to address these issues.

A primary limitation of our study was our instrument selection; for IL-33 we were required to lower our p-value threshold to 10-6 from 10-8 as described earlier. Also, due to high missingness in serum IL-33 in our sample, we were further unable to test the strength of our IL-33 instrument. Future studies could address this by looking at tissue expression of IL-33 at target organs[7]. Additionally, the statistical power of our study suffered from a relatively small sample size of approximately 3,000. A further limitation of our study was that the base and target populations used for the ST2 GWAS and MR were the same given a lack of GWAS availability, although we tried to partially address this by conducting a summary-level MR model using different base and target populations. Bigger, and perhaps more diverse, studies with a higher number of cases, and with better stratification and subgroup analyses can overcome several of our main limitations.

In summary, we identified IL-33/ST2 as a potential immune regulatory pathway involved in AD. Our study established that ST2 PRS can partly explain variability in serum levels of ST2,

which also associates well with AD. However, our MR models failed to establish a significant genetic link to the risk of AD. IL-33/ST2 is a drugable pathway, and the need for treatments that could prevent AD or slow the disease's progress is universal. Therefore, despite our results and given previous research underscoring the role of the IL-33/ST2 axis in AD,further studies are required to address the significance of this axis in AD.

Abbreviation

AD - Alzheimer's disease
VD - Vascular dementia
OD - Other dementia
APOE - Apolipoprotein E
IL-33 - Interleukin-33
ST2 - Suppression of tumorigenicity 2
sST2 - Soluble ST2
PRS - Polygenic risk score
MR - Mendelian randomisation
MR-IVW - Inverse variance weighted method for summary-level MR
IV - Instrument variable
GWAS - Genome Wide Association Study
SNP - Single nucleotide polymorphisms
KNN - K-Nearest Neighbors
OR - Odds ratio

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Appendix

ICD10 and ICD9 codes

- Alzheimer's Disease:
  - ICD –10: F00, F00.0, F00.1, F00.2, F00.9, G30, G30.0, G30.1, G30.8, G30.9
  - ICD –9: 331.0
- Vascular Dementia:
  - ICD -10: F01, F01.0, F01.1, F01.2, F01.3, F01.8, F01.9, I67.3
  - ICD –9: 290.4
- Other Dementia:
  - ICD -10: A81.0, F02, F02.1, F02.2, F02.3, F02.4, F02.8, F03, F05.1, F10.6, G31.1, G31.8
  - ICD –9: 290.2, 290.3,291.2, 294.1, 331.2, 331.5 (290.0, 290.1)
- Asthma:
  - ICD -10: J45
  - ICD –9: none

Ethnicity exclusion

In this study, we only included white ethnicity, which contains the following categories: 'White', 'British', 'Irish', and 'Any other white background'. Ethnicity other than these four categories was excluded.

Details of selected genetic IVs

Figure 11 presents the overview of genetic IVs for each protein and Table 5 present all details of the SNPs selected.

Protein	Population and size	Variants selection		After clumping $R^2 = 0.01$	Selected SNPs
		Threshold	*# SNPs*	*# SNPs*	
IL - 33	11,793 European *(Sweden, Denmark, U.K., Germany, Estonia, Croatia)*	p-value < 10^{-6}	10	**2**	"rs8042883", "rs10415966"
ST2	21,758 European	p-value < 5 x 10^{-8}	390	**2**	"rs1468789", "rs13029918"
		p-value < 10^{-6}	1208	**26**	"rs115540952", "rs11465602", etc.

Figure 11: Overview of the genetic IVs for each protein

Risk Factors	rsid	Chromosome	Effect size	p-value
IL-33	rs8042883	15	-0.0681	4.55E-07
	rs10415966	19	-0.1184	3.74E-07
ST2	rs115540952	2	0.656	2.79E-20
	rs11465602	2	0.3147	6.63E-21
	rs75372018	2	-0.205	1.65E-07
	rs4851574	2	-0.436	7.41E-54
	rs17776702	2	0.2052	5.02E-08
	rs951193	2	0.2469	1.54E-45
	rs111477597	2	0.3175	4.59E-73
	rs79400648	2	0.3309	1.98E-29
	rs13029918	2	-1.3873	3.05E-305
	rs148378006	2	0.3616	1.36E-31
	rs9808381	2	-0.294	2.71E-24
	rs13402123	2	-0.3234	3.88E-12
	rs1468789	2	-0.4705	1.07E-305
	rs11465740	2	0.3605	4.83E-40
	rs36120431	2	0.0829	4.40E-07
	rs77936881	2	-0.0756	7.44E-07
	rs186508685	2	0.419	3.11E-13
	rs1257221	2	-0.0772	1.20E-11
	rs35518360	4	0.1205	1.61E-08
	rs635634	9	-0.08	1.82E-10
	rs608008	11	-0.0914	1.69E-19
	rs66619583	11	-0.1372	1.06E-19
	rs11600151	11	0.1702	4.61E-27
	rs879627	16	-0.0524	7.56E-07
	rs55714927	17	0.0818	6.12E-10
	rs9914370	17	-0.0683	4.68E-07

Table 5: Genetic variants of IL-33 and ST2 proteins selected for the polygenetic risk score (PRS) construction, with chromosome position, p-value and associated effect size.

PRS distributions

Figure 12, Figure 13, Figure 14 and Figure 15 show the difference in PRS distribution between cases and controls of all types of dementia, AD, VD and OD respectively.

Summary-level MR results

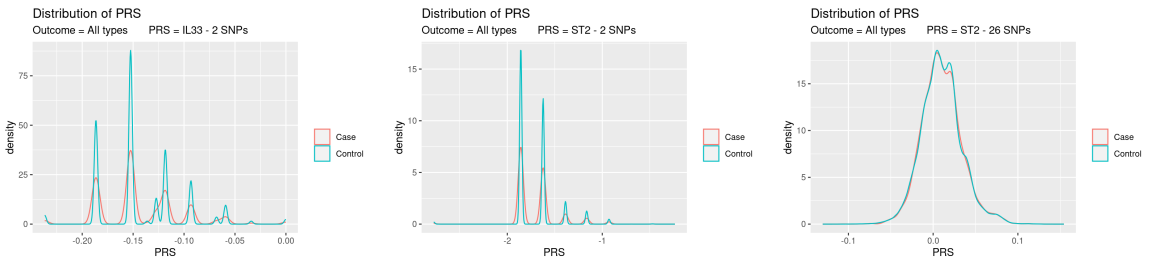

Figure 12: PRS distribution for all types of dementia

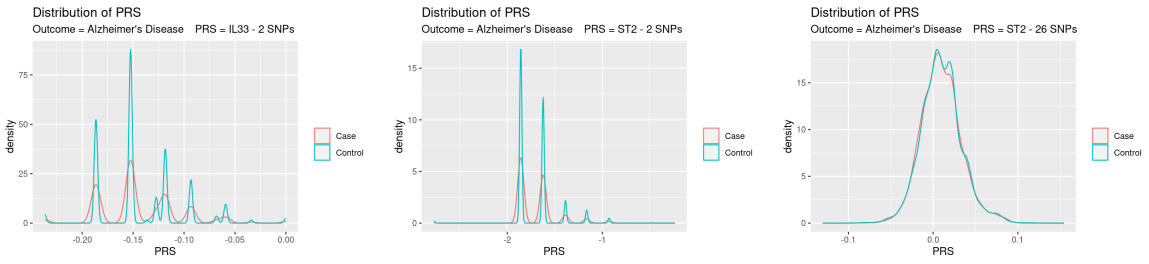

Figure 13: PRS distribution for Alzheimer's disease

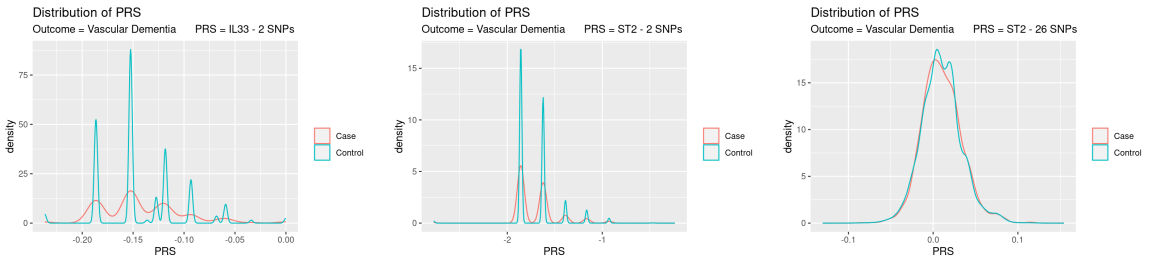

Figure 14: PRS distribution for vascular dementia

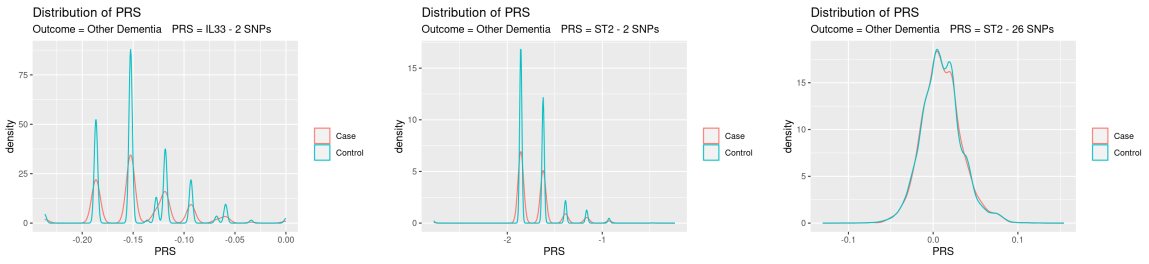

Figure 15: PRS distribution for other dementia

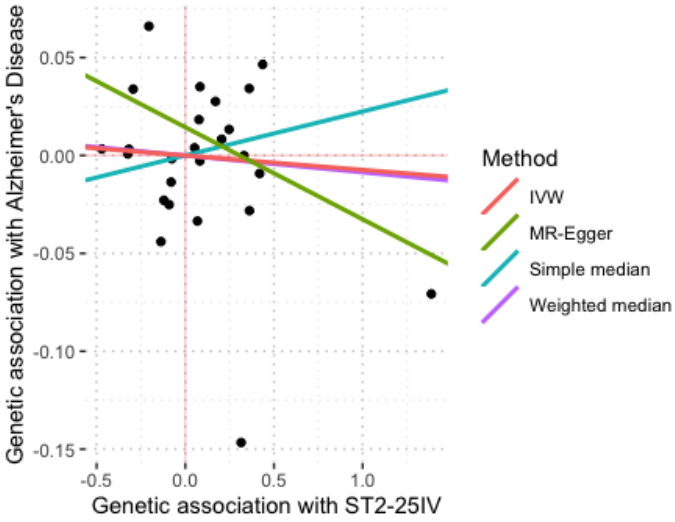

Figure 16: The plot of summary-level MR result using ST2 25IV

Variable	All participants N = 458866[1]	All types			Alzheimer's Disease			Vascular Dementia			Other Dementia		
		Case N = 7140 (1.56%)[1]	Control N = 451726 (98.4%)[1]	p-value[2]	Case N = 3027 (0.66%)[1]	Control N = 455233 (99.3%)[1]	p-value[2]	Case N = 1589 (0.35%)[1]	Control N = 455911 (99.7%)[1]	p-value[2]	Case N = 5081 (1.11%)[1]	Control N = 453674 (98.9%)[1]	p-value[2]
Current Age (Years)	72.70 (8.04)	80.31 (4.85)	72.58 (8.02)	<0.001	80.72 (4.31)	72.64 (8.03)	<0.001	81.02 (4.16)	72.65 (8.03)	<0.001	80.24 (4.97)	72.62 (8.02)	<0.001
Gender				<0.001			0.005			<0.001			<0.001
Female	249,093 (54.3%)	3,396 (47.6%)	245,697 (54.4%)		1,567 (51.8%)	247,279 (54.3%)		656 (41.3%)	247,728 (54.3%)		2,367 (46.6%)	246,680 (54.4%)	
Male	209,773 (45.7%)	3,744 (52.4%)	206,029 (45.6%)		1,460 (48.2%)	207,954 (45.7%)		933 (58.7%)	208,183 (45.7%)		2,714 (53.4%)	206,994 (45.6%)	
Education Level (if higher education)				<0.001			<0.001			<0.001			<0.001
FALSE	311,757 (68.0%)	5,712 (80.1%)	306,045 (67.8%)		2,453 (81.1%)	308,792 (67.9%)		1,327 (83.5%)	309,321 (67.9%)		4,049 (79.8%)	307,620 (67.9%)	
TRUE	146,683 (32.0%)	1,423 (19.9%)	145,260 (32.2%)		572 (18.9%)	146,017 (32.1%)		262 (16.5%)	146,165 (32.1%)		1,028 (20.2%)	145,632 (32.1%)	
Unknown	426	5	421		2	424		0	425		4	422	
Systolic Blood Presure (mmHg)	137.98 (18.64)	144.10 (19.47)	137.89 (18.61)	<0.001	144.93 (18.95)	137.93 (18.63)	<0.001	145.59 (20.26)	137.93 (18.63)	<0.001	144.06 (19.72)	137.91 (18.62)	<0.001
Unknown	26,418	438	25,980		189	26,187		116	26,212		303	26,109	
Total Cholesterol (mmol/l)	4.65 (0.94)	4.46 (1.08)	4.65 (0.94)	<0.001	4.56 (1.08)	4.65 (0.94)	<0.001	4.30 (1.12)	4.65 (0.94)	<0.001	4.44 (1.07)	4.65 (0.94)	<0.001
Unknown	200,292	3,004	197,288		1,331	198,712		639	199,059		2,124	198,123	
Body Mass Index (kg/m2)	27.40 (4.77)	27.81 (4.91)	27.39 (4.77)	<0.001	27.44 (4.75)	27.39 (4.77)	0.3	28.49 (5.05)	27.39 (4.77)	<0.001	27.84 (4.96)	27.39 (4.77)	<0.001
Unknown	1,499	53	1,446		15	1,480		7	1,485		42	1,457	
Smoking				<0.001			<0.001			<0.001			<0.001
Current	47,852 (10.4%)	750 (10.5%)	47,102 (10.4%)		264 (8.7%)	47,508 (10.4%)		196 (12.3%)	47,534 (10.4%)		562 (11.1%)	47,280 (10.4%)	
Other	248,294 (54.1%)	3,294 (46.1%)	245,000 (54.2%)		1,467 (48.5%)	246,600 (54.2%)		663 (41.7%)	246,969 (54.2%)		2,320 (45.7%)	245,917 (54.2%)	
Previous	162,720 (35.5%)	3,096 (43.4%)	159,624 (35.3%)		1,296 (42.8%)	161,125 (35.4%)		730 (45.9%)	161,408 (35.4%)		2,199 (43.3%)	160,477 (35.4%)	
If take hot drinks				0.7			0.8			0.025			0.6
FALSE	454,229 (99.0%)	7,065 (98.9%)	447,164 (99.0%)		2,998 (99.0%)	450,635 (99.0%)		1,564 (98.4%)	451,311 (99.0%)		5,033 (99.1%)	449,088 (99.0%)	
TRUE	4,637 (1.0%)	75 (1.1%)	4,562 (1.0%)		29 (1.0%)	4,598 (1.0%)		25 (1.6%)	4,600 (1.0%)		48 (0.9%)	4,586 (1.0%)	

[1] Mean (SD); n (%)

[2] Wilcoxon rank sum test; Pearson's Chi-squared test

Figure 17: Table 1 - Characteristics of the study population.