

## ORIGINAL ARTICLE

# Genetic evidence for the most common risk factors for chronic axonal polyneuropathy in the general population

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## Abstract

**Background and purpose:** Chronic axonal polyneuropathy is a common disease, but the etiology remains only partially understood. Previous etiologic studies have identified clinical risk factors, but genetic evidence supporting causality between these factors and polyneuropathy are largely lacking. In this study, we investigate whether there is a genetic association of clinically established important risk factors (diabetes, body mass index [BMI], vitamin B12 levels, and alcohol intake) with chronic axonal polyneuropathy.

**Methods:** This study was performed within the population-based Rotterdam Study and included 1565 participants (median age = 73.6 years, interquartile range = 64.6–78.8, 53.5% female), of whom 215 participants (13.7%) had polyneuropathy. Polygenic scores (PGSs) for diabetes, BMI, vitamin B12 levels, and alcohol intake were calculated at multiple significance thresholds based on published genome-wide association studies.

**Results:** Higher PGSs of diabetes, BMI, and alcohol intake were associated with higher prevalence of chronic axonal polyneuropathy, whereas higher PGS of vitamin B12 levels was associated with lower prevalence of polyneuropathy. These effects were most pronounced for PGSs with lenient significance thresholds for diabetes and BMI (odds ratio [OR]<sub>diabetes, p < 1.0</sub> = 1.21, 95% confidence interval [CI] = 1.05–1.39 and OR<sub>BMI, p < 1.0</sub> = 1.21, 95% CI = 1.04–1.41) and for the strictest significance thresholds for vitamin B12 level and alcohol intake (OR<sub>vitamin B12, p < 5e-6</sub> = 0.79, 95% CI = 0.68–0.92 and OR<sub>alcohol, p < 5e-8</sub> = 1.17, 95% CI = 1.02–1.35). We did not find an association between different PGSs and sural sensory nerve action potential amplitude, nor between individual lead variants of PGS<sub>p < 5e-8</sub> and polyneuropathy.

**Conclusions:** This study provides evidence for polygenic associations of diabetes, BMI, vitamin B12 level, and alcohol intake with chronic axonal polyneuropathy. This supports the hypothesis of causal associations between well-known clinical risk factors and polyneuropathy.

## KEYWORDS

genetics, neuropathy, polygenic scores, polyneuropathy, risk factors

Noor E. Taams and Maria J. Knol share first authorship.

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## INTRODUCTION

Chronic axonal polyneuropathy is a common disease with an increasing prevalence with age [1]. Several clinical risk factors have been identified, including diabetes, vitamin deficiencies, and toxic agents like chemotherapy and alcohol [2,3]. However, in approximately 30%–50% of patients with polyneuropathy, none of these or other established risk factors is present [3,4]. Conversely, not all individuals with these risk factors develop the disease, suggesting differences in disease susceptibility due to these risk factors, or a combination of these risk factors being required to develop the disease. Although chronic axonal polyneuropathy is a common disease with multiple associated risk factors, the underlying pathophysiology has not been elucidated.

So far, evidence for aforementioned risk factors is primarily based on the associations between clinically measured variables and polyneuropathy, the so-called phenotype–phenotype associations. However, it is not proven whether this is at least partially driven by known genetic variants linked to these clinical risk factors. Polygenic scores (PGSs) are the sum of common genetic variants, each individually having a small effect size, associated with a phenotype. These scores may yield evidence for underlying genetic associations of the risk factors for polyneuropathy and could therefore provide further insight in the possible etiology of polyneuropathy.

In this study, we aimed to investigate the genetic evidence for the phenotype–phenotype associations of clinical risk factors and polyneuropathy. We developed PGSs based on the effects of associated genetic variants from published genome-wide association studies (GWASs) of common risk factors. We explored the genetic associations of PGSs of diabetes, body mass index (BMI), vitamin B12 levels, and alcohol intake with chronic axonal polyneuropathy and the sensory nerve action potential (SNAP) amplitude of the sural nerve alone.

## MATERIALS AND METHODS

### Study population

This study was part of the Rotterdam Study, an ongoing population-based cohort study in the Netherlands investigating multiple chronic diseases [5]. Since 2013 until 2017, 2069 participants were screened for chronic axonal polyneuropathy according to a predefined protocol as described below. Exclusion criteria were insufficient screening ( $n = 150$ ) or hereditary polyneuropathy ( $n = 2$ ). Of the remaining 1917 participants, genotyping was available in 1565 participants. Both genotyping and sural SNAP amplitude was available in 1153 participants.

### Standard protocol approvals, registrations, and patients consent

The Rotterdam Study has been approved by the Medical Ethics Committee of Erasmus University Medical Center (registration number MEC 02.1015) and by the Dutch Ministry of Health,

Welfare, and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR6831) and into the World Health Organization International Clinical Trials Registry Platform. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

### Assessment of polygenic scores

Genotyping was performed with the Illumina 550K, 550K duo and 610 quad arrays. Samples were removed if the call rate was  $<97.5\%$ , as well as if there was excess autosomal heterozygosity, duplicates or family relations, ethnic outliers, gender mismatches, variants with call rates of  $<95.0\%$ , failing missingness test, allele frequencies of  $<1\%$ , or Hardy–Weinberg equilibrium  $p < 10^{-6}$ . Genotypes were imputed using the reference panel of the Haplotype Reference Consortium version 1.1.

PGSs were calculated by multiplying the allele dosage by the effect size for each genetic variant divided by the number of variants (weighted PGS) using PRSice-2 software [6]. The genetic variants with corresponding effect sizes were extracted from previously performed GWASs on diabetes mellitus [7], BMI [8], alcohol intake, and vitamin B12 levels (rank-based inverse normal transformed to approximate a normal distribution; <http://www.nealelab.is/uk-biobank/>). These GWASs were chosen based on the largest sample sizes with European ancestry individuals, of which summary statistics were publicly available. Within PRSice-2, clumping was performed to identify the most significant variant in a linkage disequilibrium block, and this variant was used as a proxy for other correlated variants to reduce under- as well as overrepresentation of genomic regions. For this clumping procedure, a linkage disequilibrium  $r^2$  threshold of 0.1 and clumping window of 250 kb were used, that is, variants in linkage disequilibrium with the index variant at an  $r^2$  threshold of 0.1 and variants within 250 kb of the index variant are clumped together. The lead genetic variants, meaning the most significant variants in independent genomic regions associated with a risk factor, were used for the PGSs ( $p < 5 \times 10^{-8}$ ), and additional PGSs were calculated at multiple more lenient  $p$ -value thresholds ( $p < 5 \times 10^{-6}$ ,  $p < 5 \times 10^{-4}$ ,  $p < 5 \times 10^{-2}$ , and  $p < 1.0$ ). The additional PGSs with more lenient thresholds include more genetic variants and may therefore explain a larger proportion of the outcome [9]. Table 1 shows the GWASs used with their summary statistics. The weighted effect sizes of all genetic variants were added up into different PGSs and were standardized into z-scores. All PGSs approximated a normal distribution.

### Polyneuropathy screening

The polyneuropathy screening consisted of three components: a symptom questionnaire, neurological examination of the legs, and

**TABLE 1** Overview of the published genome-wide association studies used to calculate different polygenic scores at multiple significance thresholds

Risk factor	Author	Number of genetic variants per PGS significance threshold				
		$p < 5 \times 10^{-8}$	$p < 5 \times 10^{-6}$	$p < 5 \times 10^{-4}$	$p < 5 \times 10^{-2}$	1.0
Diabetes	Mahajan et al. [7]	496	1290	13,040	501,112	6,495,532
Body mass index	Yengo et al. [8]	1586	3185	8377	36,765	124,164
Vitamin B12 level	UK Biobank <sup>a</sup>	NA	18	1643	115,777	1,188,033
Alcohol intake	UK Biobank <sup>a</sup>	106	398	4224	133,892	1,189,039

Abbreviations: NA, not available; PGS, polygenic score.

<sup>a</sup><http://www.nealelab.is/uk-biobank/>

nerve conduction study of the sural nerves [1]. Furthermore, the participants' medical records were scrutinized for a diagnosis of chronic axonal polyneuropathy by a neurologist and the presence of risk factors or causes.

The questionnaire of the screening consisted of 12 symptoms and could be answered with never, sometimes, or (almost) continuously. Symptoms included tingling, burning sensation, cotton-wool feeling, muscle cramps, muscle pain not related to exercise, stabbing pain, weakness, numbness, tightness, and allodynia of the feet or legs. Additionally, the participants were asked if they had ever been diagnosed with polyneuropathy.

Neurological examination of the legs consisted of several sensory tests (vibration and superficial pain sensation), tendon reflexes of the knee and ankle, and dorsal flexion of the feet.

Nerve conduction study of the sural nerves was performed with a Nicolet Viking Quest (Natus Medical Incorporated), and sural SNAP amplitudes were measured from baseline to peak according to a predefined protocol. An amplitude  $< 4 \mu\text{V}$  was considered abnormal, taking limb temperature into account [1,10]. The highest of both sural SNAP amplitudes was used for analyses.

After the screening, all participants were discussed in an expert panel led by a neuromuscular specialist (P.A.v.D.) that included a neurophysiology specialist (J.D.) and two physicians trained in epidemiology with a special interest in neuromuscular diseases (N.E.T. and R.H.). Participants were categorized into "no," "possible," "probable," or "definite" polyneuropathy, based on the level of abnormality of the different components of the screening. If one component of the screening was abnormal, participants were generally categorized as possible polyneuropathy, and two components resulted in probable polyneuropathy. Three abnormal components generally resulted in definite polyneuropathy, irrespective of the cause [1]. In addition, a participant with a diagnosis from a neurologist in their medical records was also categorized as definite polyneuropathy, as a diagnosis from a neurologist was considered superior to our screening [1]. Participants newly diagnosed with chronic axonal polyneuropathy underwent blood sampling for the most common risk factors in the general population, including fasting glucose, vitamin B1 and B12, paraproteinemia, and thyroid stimulating hormone. Participants were not regularly tested for rare causes of polyneuropathy like connective tissue disease, genetic causes, and infections,

as the a priori risk in the general population is very low. However, as the medical records of participants were scrutinized, relevant additional risk factors and causes for polyneuropathy were most likely detected [1]. Definite and probable polyneuropathy were combined into "polyneuropathy cases" ( $n = 215$ ) because of their similarities in symptoms and signs by neurological examination. Participants with possible and no polyneuropathy were combined into "controls" ( $n = 1350$ ).

### Assessments of covariates

Covariates were assessed at the time of polyneuropathy screening and included age and sex. Principal components for population stratification were obtained using multidimensional scaling as implemented in PLINK [11].

### Data analysis

We investigated, as a methodological validation, whether the different PGSs were associated with their corresponding phenotypes in our study sample, for example, PGS of diabetes with glucose levels, using linear regression models. Logistic regression analysis was performed to assess the association between different PGSs and polyneuropathy, and linear regression analysis was used to assess the outcome sural SNAP amplitude. All models were adjusted for age at time of the polyneuropathy screening, sex, and the first four genetic principal components. As the different PGSs may be correlated, we used permutation testing to assess the number of independent PGSs per risk factor and of all risk factors combined. For this, we reran the logistic regression models for the different PGSs 10,000 times while each time the polyneuropathy outcome was randomly shuffled across participants. The minimum  $p$  value of all regressions was saved every time. After sorting the  $p$ -values, the  $p$ -value threshold dividing the 5% lowest and 5% highest  $p$ -values was defined. Afterward, 0.05 was divided by this threshold to calculate the number of independent tests. Based on this information, we defined the multiple testing  $p$ -value thresholds at  $p < 0.014$  for diabetes,  $p < 0.017$  for BMI,  $p < 0.013$  for alcohol

intake,  $p < 0.014$  for vitamin B12, and  $p < 0.003$  for all PGSs. The heatmap figures are based on  $t$ -values that show the calculated difference represented in units of standard error ( $t = \beta/SE$ ).  $T$ -values close to zero resemble no significant difference, and the greater the magnitude of  $t$ -values the more likely there is a significant difference.

We performed three additional analyses. First, we investigated the effect of misclassification of probable and possible polyneuropathy by excluding probable from the "polyneuropathy cases" and excluding possible polyneuropathy from the "controls." Second, we explored whether single genetic lead variants (with individual  $p < 5 \times 10^{-8}$ ) for their accompanying risk factor were also individually significantly associated with polyneuropathy and sural SNAP amplitude (significance thresholds were Bonferroni corrected using  $[0.05/\text{number of variants in the PGS}]$ ). Third, the Rotterdam Study was part of the discovery GWAS for diabetes and BMI. To exclude bias and examine whether our findings hold especially for the lenient PGS  $p$ -thresholds, we stratified by the presence of diabetes, but we were not able to do this for BMI, as this is a continuous variable.

Analyses were performed, and figures were made with IBM SPSS Statistics 25 and R software version 3.6.3.

## RESULTS

### Population characteristics

Characteristics of the population sample are shown in Table 2. In total, 1565 participants were included in this study, of whom 215 had chronic axonal polyneuropathy (13.7%) and 1350 were controls (86.3%). Median age of the participants was 73.6 years (interquartile range = 64.6–78.8), and females were slightly overrepresented (53.5%).

### Validation of the polygenic scores with their corresponding phenotypes

We confirmed the associations of PGSs of diabetes, BMI, and vitamin B12 with their corresponding clinical phenotype in our sample. The associations of diabetes (odds ratio  $[OR]_{\text{diabetes}, p < 1.0} = 2.58$ , 95% confidence interval  $[CI] = 2.19\text{--}3.06$ ), glucose levels, BMI, and vitamin B12 were in the expected direction (Figure S1). The PGS of vitamin B12 was only borderline significantly associated with small effect sizes probably due to the relatively small number of participants.

The PGS of alcohol intake was associated with a lower intake of alcohol in grams/day (Figure S1), opposite of what we expected. However, when abstainers ( $<10$  g of alcohol/day, corresponding to  $<1$  glass of alcohol/day) were excluded, this inversed association disappeared, suggesting the association is due to misclassification of this group.

**TABLE 2** Population characteristics,  $N = 1565$

Characteristic	Value
Age, years, median (IQR)	73.6 (64.6–78.8)
Female, $n$ (%)	837 (53.5)
Polyneuropathy, $n$ (%)	215 (13.7)
Definite polyneuropathy	100 (6.4)
Probable polyneuropathy	115 (7.3)
Controls, $n$ (%)	1350 (86.3)
Possible polyneuropathy	303 (19.4)
No polyneuropathy	1047 (66.9)
Sural SNAP amplitude, median (IQR) <sup>a</sup>	8.0 (5.0–11.0)

Abbreviations: IQR, interquartile range; SNAP, sensory nerve action potential.

<sup>a</sup>Available in 1153 participants.

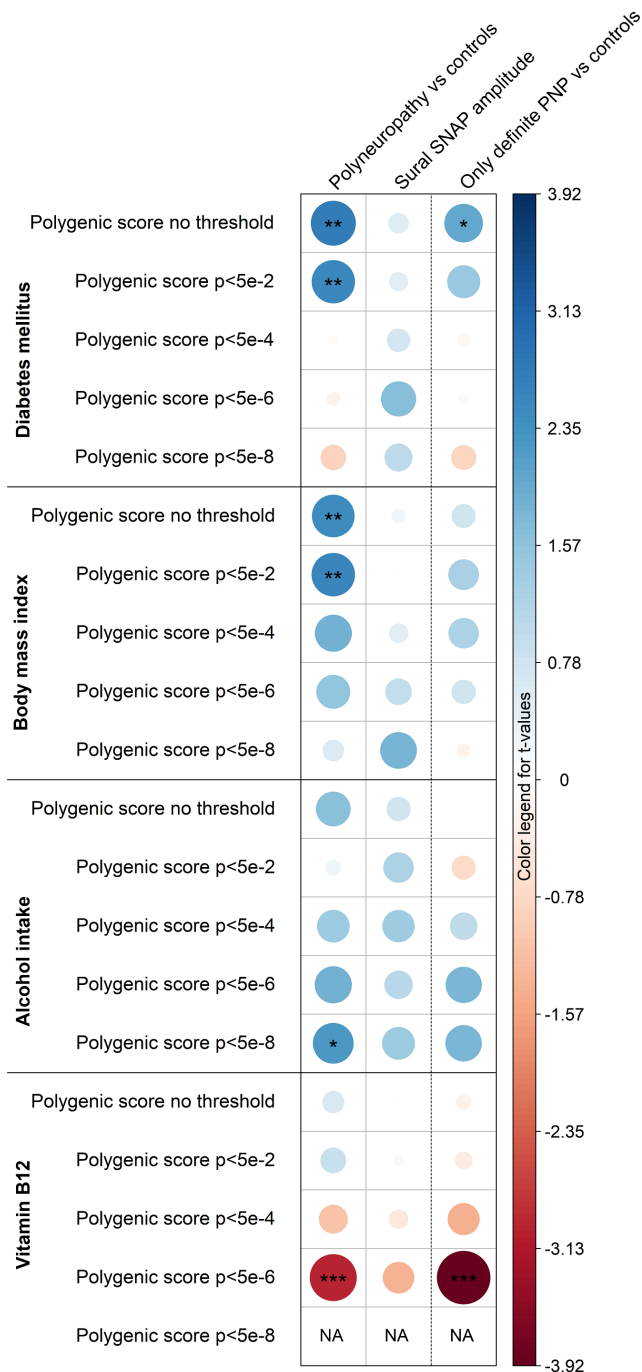
### Polygenic scores and polyneuropathy

Significant associations were observed between the PGSs of all risk factors and the outcome polyneuropathy (Figure 1 and Table 3). For the PGS of diabetes, the lenient significance thresholds, including most genetic variants, were most strongly associated with chronic axonal polyneuropathy (OR = 1.20, 95% CI = 1.04–1.39 for PGS <sub>$p < 5 \times 10^{-2}$</sub>  and OR = 1.21, 95% CI = 1.05–1.39 for PGS <sub>$p < 1.0$</sub> ). Similar effects were observed for the PGS of BMI. A higher PGS of BMI with a lenient significance threshold was strongly associated with a higher prevalence of polyneuropathy (OR = 1.22, 95% CI = 1.05–1.42 for PGS <sub>$p < 5 \times 10^{-2}$</sub>  and OR = 1.21, 95% CI = 1.04–1.41 for PGS <sub>$p < 1.0$</sub> ). A higher PGS of vitamin B12 levels was significantly associated with a lower prevalence for polyneuropathy at the strictest PGS significance threshold, including only the most significant genetic variants (OR = 0.79, 95% CI = 0.68–0.92 for PGS <sub>$p < 5 \times 10^{-6}$</sub> ; Figure 1 and Table 3). The PGS of alcohol intake was associated with polyneuropathy at the strictest level of the PGS significance threshold (OR = 1.17, 95% CI = 1.01–1.35 for PGS <sub>$p < 5 \times 10^{-8}$</sub> ), and there was no association between the more lenient PGS significance thresholds and polyneuropathy. When we adjusted the results for multiple testing based on permutation testing, all results remained significant (Figure 1).

We did not find an association between different PGSs and sural SNAP amplitude. Only a borderline significant association was found between PGS <sub>$p < 5 \times 10^{-8}$</sub>  for diabetes and sural SNAP amplitude ( $\beta = -0.25$ , 95% CI =  $-0.52$  to  $0.02$ ; Table S1).

### Additional analyses

We performed sensitivity analyses to explore misclassification of probable and possible polyneuropathy, as we combined those into "polyneuropathy" and "controls," respectively. After excluding probable polyneuropathy, the effect sizes in terms of ORs of PGSs of



**FIGURE 1** Association of polygenic scores of diabetes, body mass index, vitamin B12, and alcohol intake (at multiple significance thresholds) with chronic axonal polyneuropathy. Analyses were adjusted for age, sex, and the first four genetic principal components. Larger circles indicate higher  $t$ -values. The colors represent positive (blue) and negative (red) associations; for example, blue corresponds to a higher prevalence for polyneuropathy and lower sural sensory nerve action potential (SNAP) amplitude (results of sural SNAP amplitude are inverted) and vice versa for the color red. Statistical significant findings are indicated by asterisks: \* $p < 0.05$ ; \*\* $p$ -threshold adjusted for the number of independent polygenic scores (PGSs) per trait as calculated by 10,000 permutations:  $p < 0.014$  for diabetes,  $p < 0.017$  for body mass index,  $p < 0.014$  for vitamin B12, and  $p < 0.013$  for alcohol intake; \*\*\* $p < 0.003$  for all PGSs of the different traits. NA, not available; PNP, polyneuropathy [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

diabetes, BMI, alcohol intake, and vitamin B12 remained quite similar, but lower power resulted in less significant  $p$ -values (Figure 1 and Table 3). A similar trend was seen after excluding participants with possible polyneuropathy from the "controls" (Figure S2).

Furthermore, we explored the associations between the different individual lead genetic variants ( $p < 5 \times 10^{-8}$ ) and polyneuropathy, but none of these variants was significantly associated with polyneuropathy or sural SNAP amplitude after correction for multiple testing (data not shown).

To check whether the associations of  $PGS_{diabetes,p < 1.0}$  at the lenient PGS  $p$ -thresholds hold, as the Rotterdam Study was part of the discovery GWAS, we stratified by this risk factor (Table S2). The effect estimates in participants with diabetes remained quite similar, but were attenuated in participants without diabetes and did not remain statistically significant ( $PGS_{diabetes,p < 1.0}$ : OR = 1.21, 95% CI = 0.88–1.68;  $PGS_{no\ diabetes,p < 1.0}$ : OR = 1.09, 95% CI = 0.90–1.31).

## DISCUSSION

In this population-based study, PGSs of the clinical risk factors diabetes, BMI, alcohol intake, and vitamin B12 level were associated with chronic axonal polyneuropathy. Effects were in the clinically expected direction, and therefore support the hypothesis of causal associations. No association was found between different PGSs and sural SNAP amplitude, except for a borderline significant association between a higher PGS of BMI and lower sural SNAP amplitude. We did not find single genetic variants that were significantly associated with polyneuropathy or sural SNAP amplitude after correction for multiple testing.

In general, the clinical risk factors are used to classify chronic axonal polyneuropathy into different phenotypes, for example, diabetic or alcohol-related polyneuropathy. However, due to the clinical similarities in symptoms and signs at neurological examination, at least a partial common etiology may be expected. Furthermore, from an epidemiological perspective, not all individuals with these risk factors develop the disease, and the effects of glycemic control do not benefit all patients [12], suggesting that these risk factors are relevant but not the only component that contribute to the development of polyneuropathy [13]. From a more biological perspective, the proposed pathways between the different subtypes of polyneuropathy overlap. Diabetic polyneuropathy research has focused on pathways related to metabolic dysfunction, like hyperglycemia, dyslipidemia, and insulin resistance, and the alterations in (chronic) inflammation, oxidative stress, and mitochondrial dysfunction leading to microvascular damage, ischemia, and consequently nerve damage [14,15]. Obesity also connects to these pathways, resulting in a state of chronic inflammation with oxidative stress and microvascular changes [16]. The etiology for alcohol-related polyneuropathy or polyneuropathy due to vitamin deficiencies is not yet fully understood, but pathways related to increased oxidative stress directly or via metabolic pathways are proposed. The mechanisms suggested include protein kinase C, advanced glycation end products, and nuclear factor  $\kappa$ B [14–16].

**TABLE 3** Association of polygenic scores of diabetes, body mass index, vitamin B12 level, and alcohol intake (at multiple significance thresholds) with chronic axonal polyneuropathy

PGSs <i>p</i> -value threshold	Polyneuropathy, <i>n</i> / <i>N</i> = 215/1565 <sup>a</sup>		Definite polyneuropathy, <i>n</i> / <i>N</i> = 100/1450 <sup>b</sup>	
	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
<b>Diabetes</b>				
<i>p</i> < 5 × 10 <sup>-8</sup>	0.94 (0.81–1.09)	0.387	0.91 (0.74–1.13)	0.407
<i>p</i> < 5 × 10 <sup>-6</sup>	0.98 (0.85–1.14)	0.808	1.01 (0.82–1.25)	0.915
<i>p</i> < 5 × 10 <sup>-4</sup>	0.99 (0.86–1.15)	0.937	0.98 (0.80–1.21)	0.865
<i>p</i> < 5 × 10 <sup>-2</sup>	1.20 (1.04–1.39)	0.011 <sup>c</sup>	1.16 (0.95–1.40)	0.145
<i>p</i> < 1.0	1.21 (1.05–1.39)	0.006 <sup>c</sup>	1.21 (1.00–1.46)	0.044 <sup>c</sup>
<b>Body mass index</b>				
<i>p</i> < 5 × 10 <sup>-8</sup>	1.05 (0.90–1.22)	0.545	0.97 (0.79–1.20)	0.805
<i>p</i> < 5 × 10 <sup>-6</sup>	1.12 (0.97–1.31)	0.123	1.09 (0.88–1.33)	0.436
<i>p</i> < 5 × 10 <sup>-4</sup>	1.15 (0.99–1.34)	0.065	1.14 (0.93–1.41)	0.212
<i>p</i> < 5 × 10 <sup>-2</sup>	1.22 (1.05–1.42)	0.010 <sup>c</sup>	1.15 (0.93–1.42)	0.208
<i>p</i> < 1.0	1.21 (1.04–1.41)	0.014 <sup>c</sup>	1.08 (0.88–1.34)	0.450
<b>Vitamin B12 level</b>				
<i>p</i> < 5 × 10 <sup>-8</sup>	NA	NA	NA	NA
<i>p</i> < 5 × 10 <sup>-6</sup>	0.79 (0.68–0.92)	0.003 <sup>c</sup>	0.66 (0.53–0.81)	0.000 <sup>c</sup>
<i>p</i> < 5 × 10 <sup>-4</sup>	0.92 (0.79–1.07)	0.265	0.87 (0.72–1.07)	0.169
<i>p</i> < 5 × 10 <sup>-2</sup>	1.06 (0.91–1.18)	0.379	0.94 (0.71–1.18)	0.684
<i>p</i> < 1.0	1.05 (0.90–1.19)	0.520	0.96 (0.73–1.18)	0.760
<b>Alcohol intake</b>				
<i>p</i> < 5 × 10 <sup>-8</sup>	1.17 (1.02–1.35)	0.024 <sup>c</sup>	1.18 (0.98–1.41)	0.074
<i>p</i> < 5 × 10 <sup>-6</sup>	1.15 (0.99–1.33)	0.063	1.19 (0.98–1.44)	0.077
<i>p</i> < 5 × 10 <sup>-4</sup>	1.12 (0.96–1.29)	0.151	1.11 (0.90–1.37)	0.313
<i>p</i> < 5 × 10 <sup>-2</sup>	1.02 (0.89–1.19)	0.762	0.93 (0.77–1.13)	0.447
<i>p</i> < 1.0	1.11 (0.97–1.27)	0.105	1.00 (0.71–1.26)	0.982

Note: Adjusted *p*-values for independent PGSs per traits: *p* < 0.015 for diabetes, *p* < 0.018 for body mass index, *p* < 0.014 for vitamin B12, and *p* < 0.012 for alcohol intake. Results were adjusted for age, sex, and principal components C1–C4.

Abbreviations: CI, confidence interval; NA, not available; OR, odds ratio; PGSs, polygenic scores.

<sup>a</sup>Definite and probable polyneuropathy combined versus possible and no polyneuropathy combined.

<sup>b</sup>Definite polyneuropathy versus possible and no polyneuropathy combined.

<sup>c</sup>Significant findings based on *p* < 0.05.

As the clinical features of the disease as well as the biological hypotheses overlap, the outcome chronic axonal polyneuropathy was used independently of categorization based on clinical risk factors, as the associations between clinical risk factors may be due to bias or (unmeasured) confounding. Using PGSs, we were able to show genetic evidence for the clinical risk factors associated with polyneuropathy.

BMI was included in the study as it reflects obesity, which is after diabetes the most important driver of the association between metabolic syndrome and polyneuropathy [17,18]. We were particularly interested in this risk factor as it overlaps with the metabolic pathways that are involved in diabetes and polyneuropathy. Interestingly, we showed that BMI is also genetically associated with polyneuropathy, not only as part of the metabolic syndrome or as a confounding factor, because of its association with other

prevalent clinical risk factors like diabetes. However, further research is needed to elucidate whether BMI on its own is sufficient or whether it is a component cause in the development of polyneuropathy [13].

Strengths of the study are the population-based setting and that, to our knowledge, this is the first study that investigated whether there is genetic evidence for the clinically established risk factors of polyneuropathy identified in phenotype–phenotype associations. Although we did not find statistically significant findings with the outcome sural SNAP amplitude, probably owing to too little power, the effects were in the expected direction. This is of interest as a reduced amplitude of the sural nerve may reflect subclinical nerve damage and can therefore be used as a proxy for the disease. A limitation of this study is that we were reliant on previously performed GWASs, and unfortunately no large-scale GWAS

has been performed for vitamin B1. Vitamin B1 deficiency is of interest as it likely causes peripheral nerve damage directly but is also associated with alcohol intake [19,20]. Additionally, we should note that the Rotterdam Study was part of the discovery sample for the GWASs of diabetes and BMI [7,8]. However, these were such small proportions of the total sample size (0.97% and 1.11%, respectively) that we do not expect this influenced our findings to a large extent. The effect estimates were slightly attenuated when we stratified for the presence of the categorical factor diabetes (Table S2). Furthermore, although our discovery set consisting of previously performed GWASs was large, it may still have been underpowered at the stricter *p*-value thresholds. Moreover, the number of cases in our test set was relatively small. Consequently, both factors may have resulted in an underestimation of our results. Another limitation of PGSs is that the effects of the multiple genetic variants may be through different pathways, and it cannot be determined exactly which pathway is of interest. Furthermore, alcohol intake was measured using questionnaires, and these are known to be prone to measurement error. Misclassification of abstainers in observational studies is an issue, as this group consists of both never and former drinkers, although they systematically differ [21]. This may explain why we found an inverse association in the validation tests between the PGS and alcohol intake, especially as this association dissolved after excluding the participants who drank less than one alcoholic beverage per day.

In conclusion, we found genetic evidence for the associations between the clinical risk factors diabetes, BMI, alcohol intake, and vitamin B12 level. Higher PGSs of diabetes, BMI, and alcohol intake were associated with a higher prevalence for chronic axonal polyneuropathy, and higher PGS of vitamin B12 level with a lower prevalence for polyneuropathy. This study supports the hypothesis of causal associations between the clinical risk factors and polyneuropathy independent of their phenotypic classification. Further research on the genetic variants and their related pathways is needed to fully understand the pathophysiology of the disease.

## CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

## AUTHOR CONTRIBUTIONS

**Noor E. Taams:** Conceptualization (equal), data curation (lead), formal analysis (lead), visualization (equal), writing–original draft (lead), writing–review & editing (lead). **Maria J. Knol:** Conceptualization (equal), formal analysis (lead), visualization (equal), writing–original draft (lead), writing–review & editing (lead). **Rens Hanewinkel:** Conceptualization (supporting), data curation (supporting), writing–original draft (supporting), writing–review & editing (equal). **Judith Drenthen:** Conceptualization (supporting), data curation (equal), supervision (supporting), writing–original draft (supporting), writing–review & editing (equal). **Hieab H. H. Adams:** Conceptualization (equal), data curation (supporting), formal analysis (supporting), writing–review & editing (equal). **Pieter A. van Doorn:** Conceptualization (equal), formal analysis (supporting),

funding acquisition (equal), supervision (lead), writing–original draft (supporting), writing–review & editing (equal). **M. Arfan Ikram:** Conceptualization (equal), formal analysis (supporting), funding acquisition (equal), supervision (lead), writing–original draft (supporting), writing–review & editing (equal).

## DATA AVAILABILITY STATEMENT

Data can be obtained on request. Requests should be directed toward the management team of the Rotterdam Study ([secretariat.epi@erasmusmc.nl](mailto:secretariat.epi@erasmusmc.nl)), which has a protocol for approving data requests.

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#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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