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Fava, Cristiano; Montagnana, Martina; Danese, E; Sjögren, Marketa; Almgren, Peter; Engström, Gunnar; Hedblad, Bo; Guidi, G C; Minuz, P; Melander, Olle

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221 00 Lund  
+46 46-222 00 00

**VANIN-1 T26I POLYMORPHISM, HYPERTENSION AND CARDIOVASCULAR  
EVENTS IN TWO LARGE URBAN-BASED PROSPECTIVE STUDIES IN SWEDES.**

Fava C<sup>a,b</sup>, MD, PhD; \*Montagnana M<sup>a,c</sup>, MD; \*Danese E<sup>a,c</sup>, PharmD; Sjogren M<sup>a</sup>, PhD; Almgren P<sup>a</sup>, BS; Engström G<sup>a</sup>, MD, PhD; Hedblad B<sup>a</sup>, MD; Guidi GC<sup>c</sup>, MD; Minuz P<sup>b</sup>, MD; Melander O<sup>a</sup>, MD, PhD

<sup>a</sup>Department of Clinical Sciences, Lund University, University Hospital of Malmö, Sweden.

<sup>b</sup>Department of Biomedical and Surgical Sciences and

<sup>c</sup>Department of Life and Reproduction Sciences, University Hospital of Verona, Italy.

\*These authors have contributed equally to this work.

Correspondence and requests for reprints to

Cristiano Fava, MD,

Department of Biomedical and Surgical Sciences,

Division of Internal Medicine C,

Piazzale LA Scuro 10,

37134 Verona, Italy.

Tel: +39 45 8124414; fax: +39 45 8027465;

e-mail: cristiano.fava@med.lu.se

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## **ABSTRACT**

**Background:** Vanin-1 (gene name *VNN1*) is an enzyme with pantetheinase activity generating the amino-thiol cysteamine which is implicated in the regulation of redox status through its effect on glutathione. We tested the hypothesis that the rs2294757 *VNN1* T26I polymorphism could affect blood pressure (BP) levels, hypertension prevalence, and risk of incident cardiovascular events.

**Methods:** The *VNN1* T26I polymorphism was genotyped in 5664 participants of the cardiovascular cohort of the "Malmö Diet and Cancer" (MDC-CVA) study and successively in 17874 participants of the "Malmö Preventive project"(MPP). The incidence of cardiovascular events was monitored for an average of nearly 12 years of follow-up in the MDC-CVA and for 25 years in the MPP.

**Results:** Both before and after adjustment for sex, age and BMI in the MDC-CVA the polymorphism had a mild lowering effect on diastolic BP and hypertension, especially in females. However in MPP no effect on BP phenotypes was detectable. Before and after adjustment for major cardiovascular risk factors, the hazard ratio for incident ischemic stroke and coronary events in MDC-CVA was not significantly different in carriers of different genotypes.

**Conclusions:** Our data do not support a major role for the *VNN1* T26I variant in determining BP level and incident ischemic events.

**Keywords:** Vanin 1; hypertension; association; blood pressure; oxidant stress; genetics.

## INTRODUCTION

Several strategies have been used to seek for genes implicated in the regulation of BP and in the development of hypertension but with mostly fruitless efforts. Also in the genome wide associations (GWAS) era many of the genes that can contribute to BP homeostasis remain unknown [1,2].

Zhou and colleagues have recently applied a novel approach, which uses the information on genetic architecture generated by recent admixture of historically separated populations, to map hypertension-associated genes and indicated Vanin-1 (gene name VNN1) as a possible candidate gene [3].

Vanin-1 is an enzyme with pantetheinase activity generating the amino-thiol cysteamine through the metabolism of pantothenic acid (vitamin B5), which is implicated in the regulation of redox status. Experimental works using knockout mice pointed out that Vanin-1 null (VNN<sup>-/-</sup>) mice are deficient in membrane-bound pantetheinase activity in kidney and liver with consequent absence of detectable free cysteamine [4] and are resistant to oxidative injury probably through augmented store of glutathione (GSH), the most potent cellular antioxidant [5]. The same knockout animals, challenged with non steroidal antiinflammatory drugs (NSAIDs) administration or chronic *Schistosoma* infection to test the response to inflammatory stimuli, were found to have a decreased inflammatory reaction and intestinal injury in both settings [6].

A strict link between impaired redox status and hypertension development has long been supposed but still remain controversial [7-11]: the pathophysiological basis of the link would stay in the fact that reactive oxygen species (ROS) like superoxide anion could impair nitric oxide bioavailability leading to endothelial dysfunction and hydrogen peroxide could directly stimulate vascular contraction by increasing intracellular calcium concentration.

Moreover, subjects with genetic and acquired forms of mitochondriopathy have an impaired respiratory chain with subsequent production of ROS and a tendency to higher levels of BP [12-14].

Thus, VNN-1 could have all the characteristics to be implicated in BP regulation also in humans.

A single nucleotide polymorphism in the coding region, the rs2294757 *VNN1* T26I, has demonstrated to be functional *in silico* since it is located in a splicing regulation site [15].

Thus the aim of our study was to test if the rs2294757 *VNN1* T26I polymorphism could be implicated in BP regulation and hypertension development in 2 large urban-based cohort studies: the Malmö Diet and Cancer study – cardiovascular arm (MDC-CVA) including more than 5 000 subjects and attempting replication in the Malmö Preventing Project (MPP) study which recruited more than 17 000 subjects. Since there is also a report that some *VNN1* variants could influence HDLcholesterol level, as an exploratory analysis, we evaluated also the effect of this polymorphism on Metabolic syndrome related parameters [16].

## **MATERIALS AND METHODS**

All study participants had given written informed consent. The procedures were in accordance with the institutional guidelines. The Ethics Committee of the Medical Faculty of Lund University approved the study.

### **Subjects**

#### **MDC-CVA**

Between 1991 and 1996, women aged 45 to 73 years and all men aged 46 to 73 years, with residency in Malmö (approximately 250 000 habitants), Sweden, were invited by mail and by newspaper advertisement to participate in the Malmö Diet and Cancer (MDC) Study, a population-based prospective study. In all, 28 449 participated out of an eligible population of 74 000. The participants were asked to complete a self-administered questionnaire at home, which included items on lifestyle factors, medication, previous and current diseases [17]. BP along with other cardiovascular risk factors were measured in a random subsample referred to as the MDC-CVA ( $n=6\ 103$ ). Successfully extracted genomic DNA was available from 5763 MDC-CVA participants.

#### **MPP**

In the MPP, 33 346 Swedish participants (22 444 men and 10 902 women, mean age 49 years, from Malmö participated in health screening during 1974–1992 (attendance rate 71%) [18]. All

individuals underwent a physical examination with BP measurement. Information on lifestyle factors and medical history was obtained from a questionnaire. Of individuals participating in the initial screening, 4 931 have died and 551 were lost from follow-up for other reasons. Twenty-five thousand of the eligible individuals were invited to a re-screening visit during 2002–2006, including a physical examination with measurement of BP. DNA was obtained from 18 240 individuals participating in the re-screening.

### **Blood pressure**

We performed the study of BP as a continuous variable both in MDC-CVA and in MPP before and after adjustment of measured BP values (see below) and as a dichotomized tract (hypertension vs. normotension). In both studies BP was measured by specially trained nurses on the right brachial artery using a mercury sphygmomanometer. The systolic BP was defined by ‘phase I’ and the diastolic BP defined by ‘phase V’ Korotkoff sounds.

Different modalities of BP measurements between studies are the following:

In the MDC-CVA study BP was measured after 10 minutes of rest in the supine position. In MPP, the first BP reading was taken after 1 minute of rest in the supine position. Then, the participants were asked to stand up and the second BP measurement was taken in the upright standing position after one minute. This procedure was thereafter repeated following an initial 10 minute rest in the supine position. The average BP value of all the subjects with at least 3 valid measurements was used in the present study. At reinvestigation in the MPP, BP was measured twice in the supine position and all the measurements were recorded. The average BP value of all the subjects with at least 2 valid measurements was used in the present study.

### **Definition of Hypertension and Blood Pressure adjustment**

Hypertension in both cohorts was defined as being on antihypertensive treatment or having systolic BP / diastolic BP equal or greater than 140/90 mmHg according to current diagnostic criteria [19], and normotension as having systolic BP / diastolic BP less than 140/90 mmHg.

### **Blood pressure adjustment**

To overcome the possibility that a biased selection might result from selecting only individuals who were free of antihypertensive treatments, we conducted an analysis adjusting the systolic BP and diastolic BP of hypertensive individuals that were taking antihypertensive drugs at the time of investigation by two methods recently reviewed by Stephen Harrap and colleagues (see Supplementary Methods) [20].

### **Anthropometric, behavioral and laboratory parameters**

Waist circumference (in cm) was measured with the patient standing, at the umbilicus level. The BMI was calculated as the ratio of the weight in kilograms to the square of the height in meters ( $\text{kg}/\text{m}^2$ ). Smoking habits of individuals were elicited by a self-administered questionnaire and categorized into ‘non smokers’ (including former smokers) and ‘current smokers’.

After an overnight fast, blood samples were drawn for the determination of serum lipids, whole blood glucose, C-reactive protein. Samples were analyzed by standard methods at the Department of Clinical Chemistry, Malmö University Hospital, which is attached to a recurrent standardized system (see also Supplementary Methods) [21]. Metabolic syndrome was diagnosed according to the National Cholesterol education Programme/Adult Panel treatment III (NCEP/ATP III) as previously described in the same cohort [22].

### **Follow-up, definition of end points**

All subjects were followed from the baseline examination until the first cardiovascular event, death or 31 December 2005 in MDC-CVA and 31 December 2006 in MPP. Mean follow-up time was  $11.9 \pm 2.7$  years in MDC-CVA and  $24.8 \pm 4.7$  years in MPP. A cardiovascular event was defined as fatal or non-fatal myocardial infarction (MI; ICD-9 code 410), or death due to chronic ischemic heart disease (ICD-9 code 412, 414) or fatal or non-fatal ischemic stroke (ICD-9 codes 434, 436), whichever came first. The end points were retrieved by data linkage with the national Swedish Hospital Discharge and Cause of Death Registers and local stroke and Myocardial infarction registers of Malmö [22].

## **Genotyping**

DNA was extracted from frozen granulocyte or buffy-coat samples using QIAamp-96 spin blood kits (QIAGEN, Stockholm, Sweden) at the DNA extraction facility supported by SWEGENE. The *VNN1* T26I polymorphism (dbSNP accession number rs2294757), was determined by end-point fluorescent measurements [23] using TaqMan MGB probes custom synthesized by Applied Biosystems: [wild-type/mutant], VIC/FAM- VIC- CAGCTGCA[A/G]TGAAAGT

## **Statistics**

Continuous variables are presented as the mean $\pm$ SD. All data, except for the power analysis, were analyzed with SPSS statistical software (version 14.0; SPSS Inc. Chicago, Illinois, USA). Power calculation was performed using the Power and Sample Size calculator version 2.1.31 (Vanderbilt University Medical Center, Nashville, USA).

Frequency differences and deviation from Hardy-Weinberg equilibrium were analyzed by chi-square test. Significance of differences in continuous variables was tested by analysis of variance followed by Tukey's test and *t*-test. Multiple linear and logistic regression analyses were used in the multivariate models with either BP traits or hypertension status as dependent variables and genotype, age, sex, BMI, heart rate and the interaction variables (computed by multiplying the genotype with age, sex and BMI respectively) as independent variables.

Kaplan-Meier curves and log-rank tests compared cumulative incidence of ischemic strokes and coronary events in carriers of different genotypes. Age-, sex- and risk factor adjusted Cox proportional-hazard models were used to study the relationships between the polymorphisms and time (in years) to first cardiovascular events. The fit of the proportional hazards model was confirmed by plotting the cardiovascular incidence rates over time. Hazard ratios (HR) and 95% confidence interval (CI) were calculated. For variables with skewed distributions, log-normalized values were used in the analysis. All tests were two-sided and *p*-values less than 0.05 were considered statistically significant.



## **RESULTS**

The clinical characteristics of individuals included in the study in the MDC-CVA and in the MPP (both at baseline and at follow-up) are summarized in Table 1.

The genotyping success rate was 98.3% (5 664/5 763) in MDC-CVA and 98.0% (17 874/18 240) in MPP. We found respectively in MDC-CVA/MPP 40.5%/40.8% I26I homozygotes, 46.2%/45.1% T26I heterozygotes and 13.3%/12.1 T26T homozygotes.

Genotype distributions did not deviate from Hardy–Weinberg equilibrium in both cohorts (predicted heterozygosity 0.463, observed heterozygosity 0.462;  $p=0.89$  in the MDC-CVA; predicted heterozygosity 0.457, observed heterozygosity 0.46;  $p=0.37$  in MPP).

Details about reproducibility of the genotyping process and power analysis in the populations are reported in the Supplementary Results section.

### **Blood pressure**

Crude BP data and hypertension prevalence according to genotype at MDC-CVA and MPP (both baseline and reinvestigation) are presented in table 2. A slightly lower Diastolic BP and hypertension prevalence for carriers of the 26T-allele was present ( $p<0.05$  for both) in MDC-CVA but not in MPP at baseline and reinvestigation. After adjustment for covariates and antihypertensive therapy, carriers of the “protective” 26T allele showed lower diastolic BP and lower risk of hypertension only at MDC-CVA (additive and autosomal dominant mode of inheritance respectively;  $p<0.05$  see table 3 and 5) with no detectable effect at MPP (table 4 and 5).

When excluding from the analysis in MPP 2 373 subjects (13.5%), who participated also to the MDC-CVA, there was no difference in the results; that is no association for the *VNN1* T26I polymorphism with systolic, diastolic BP and hypertension prevalence both at baseline and reinvestigation ( $p>0.05$  for all the analyses).

### **Interaction with demographic variables and stratified analysis**

#### ***MDC-CVA***

In linear regression, no interaction of the rs2294757 polymorphism with either sex, age or BMI was evident (see Supplementary Result Table S1) whereas stratifying the data by gender the protective effect of the 26T allele for hypertension prevalence was present and statistically significant in females (HR 0.857, 95% C.I. 0.737-0.996) but not in males (HR 0.935, 95% C.I. 0.776-1.126) with an autosomal dominant mode of inheritance assumption.

### ***MPP***

Neither evidence of interaction (see Supplementary table S2) nor significant results stratifying for gender were present both at baseline and at reinvestigation for all the BP related traits.

### **Coronary and cerebrovascular events**

Kaplan-Meier curves compared by log-rank test did not show any statistical difference in cardiac events (n=304 in MDC-CVA and n=1 290 in MPP) and cerebral ischemic episodes (n=261 in MDC-CVA and n=855 in MPP) in carriers of different *VNN1* T26I genotypes (figure 1).

In Cox regression analysis, adjusting for age and sex carriers of at least one 26T allele were not significantly protected in the MDC-CVA either from coronary events (HR 0.907, 95% C.I. 0.722-1.138; p=0.40) or stroke (HR 0.912, 95% C.I. 0.713-1.166; p=0.46) and in the MPP (HR for coronary events 0.992, 95% C.I. 0.888-1.108; p=0.89; HR for stroke 1.005, 95% C.I. 0.877-1.151; p=0.95). Further adjustment for cardiovascular risk factors, including smoking habit, previous cardiovascular events, hypertension, diabetes mellitus, hypercholesterolemia and metabolic syndrome prevalence did not change the results.

### **Exploratory analysis on Metabolic syndrome individual components**

No effect of the *VNN1* T26I polymorphism on the metabolic syndrome individual components was found for all the genetic model tested both in MDC-CVA and in the MPP (at baseline and at reinvestigation; see supplementary table S3).

## **DISCUSSION**

The hypothesis that the red-ox balance could be implicated in hypertension has long been proposed and in different animal models administration of antioxidant is associated with a clear BP lowering

effect [23]. In humans, the issue is much more complex and antioxidant administration, though capable to restore endothelial function, most of the time have no appreciable effect on BP. On the other hand, subjects suffering from mitochondrial disease caused by rare mutation in the enzyme involved in the ox-phos process are often hypertensive and a rare mutation in the mitochondrion genome has been implicated in a rare form of hypertension that features also hypomagnesemia and hypercholesterolemia [14].

At the population level, several polymorphisms of genes implicated in red-ox balance have been proposed as implicated in hypertension development with contrasting results [25-29].

Thus, when Zhu and colleagues proposed Vanin-1 and a potential candidate gene for hypertension, we found of interest to test the hypothesis that one polymorphism in this gene could be implicated in BP related trait. Moreover, recent studies in animal models have showed that this gene is potentially implicated in cellular red-ox balance and inflammatory state reinforcing its candidature for a role about hypertension development and CV events.

The first analysis in the well-powered sample of the MDC-CVA showed a weak but consistent association between the SNP and BP and we were prompted to seek for a confirmatory analysis in MPP. However the second test on a larger sample of Swedes failed to replicate our previous finding indicating that no association is present.

The SNP originally associated with hypertension in Zhu's study is the rs2272996 (N131S) [3]. We chose the *VNN1* T26I because in silico analysis, contrarily to the N131S, indicated it is potentially functional being located in a splicing regulation site where the change of a G to A modify a specific splicing site enhancer [15]. Thus, it is still possible that a different result could have been raised if we had used the N131S polymorphism also in our population; in that case it wouldn't have been clear how this polymorphism could affect BP phenotypes, since non functional.

Our study underlines the importance of confirmatory analysis when a SNP is first implicated in a complex trait to discover false positive results. In any case other considerations and some

particularity of our samples should be considered before ruling out this polymorphism as blood pressure related.

The population we analysed is constituted of people of Caucasian ancestry and cannot be generalized to populations with different genetic background. The two populations investigated in the present study, even if genetically very closed, are different especially regarding the mean age of the investigated subjects and the composition of gender (see table 1). A particular concern apply to the MPP where only people who survived from the first to the final examination gave DNA for analysis. Thus, people at greater risk for cardiovascular disease (i.e. carriers of deleterious polymorphisms) could have been died at a higher frequency than people not carrying deleterious polymorphism confounding the final analysis. On the other hand, in other studies involving the same population we could find polymorphisms significantly associated to BP suggesting that the large number of investigated subject render this population trustable for genetic studies [30].

BP was measured in different ways (supine vs. standing) in the studies and we cannot exclude that this could have contributed to the different results. On the other hand, we chose to average the more measurements we had to get a value closer to the “real” individual BP. In addition, an analysis restricted only to average supine BP also at MPP (2 measurements at baseline) gave similar results to those obtained averaging supine and standing BP measurements (data not shown).

Finally we cannot exclude that other SNPs in the same gene in variable grade of linkage disequilibrium with our SNP could be implicated in BP/hypertension.

Apart from BP phenotypes we analyzed the same polymorphism also for CV end-points and the results of no association is in line with the final results on BP/hypertension. We could speculate that if this polymorphism really exerts a large effect on BP this should have been reflected by an augmented incidence of CV events, as for other polymorphisms we tested in the same cohort [310,32]. Finally, since Goring and colleagues, using a genome-wide transcriptional profiles of lymphocyte samples, identified the cis-regulated *VNN1* as harbouring sequence variants that influence HDL-cholesterol concentrations, in an exploratory analysis we evaluated also a possible

effect of the T26I SNP on HDL-cholesterol and other components of the metabolic syndrome but without any evidence of association in both cohorts.

In conclusion, in a Swedish urban-based cohort including more than 5,000 subjects we found a weak association of the rs2294757 *VNN1* T26I polymorphism and lower diastolic BP and hypertension prevalence but in a larger cohort our hypothesis was not confirmed. Studies evaluating the same polymorphism in other populations or other polymorphisms in the same gene are needed before excluding *VNN1* as a possible BP candidate gene.

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#### **FINANCIAL DISCLOSURES/OTHER POTENTIAL CONFLICT OF INTEREST**

Gunnar Engström is employed as senior epidemiological scientist at AstraZeneca R&D. No other relevant conflict of interest are present for the other coauthors of the manuscript.

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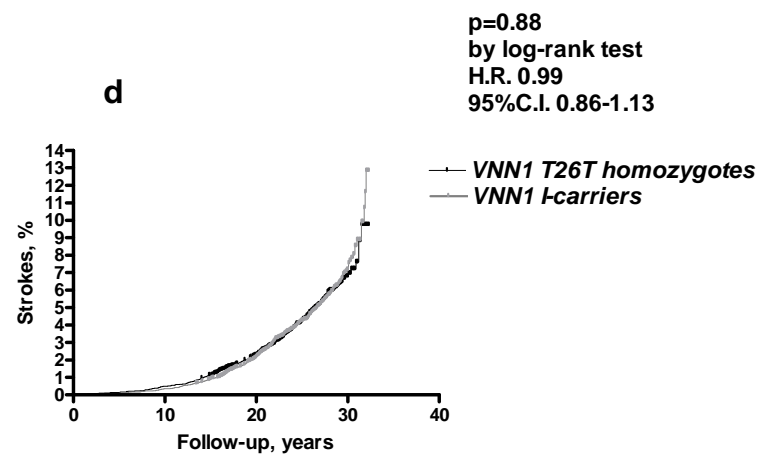
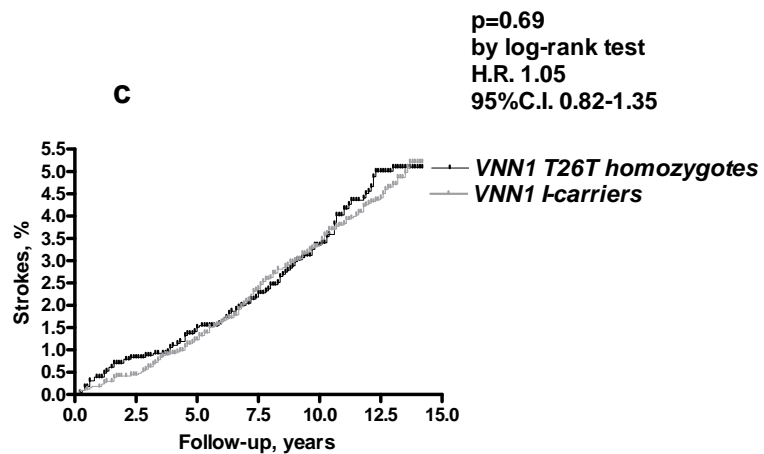
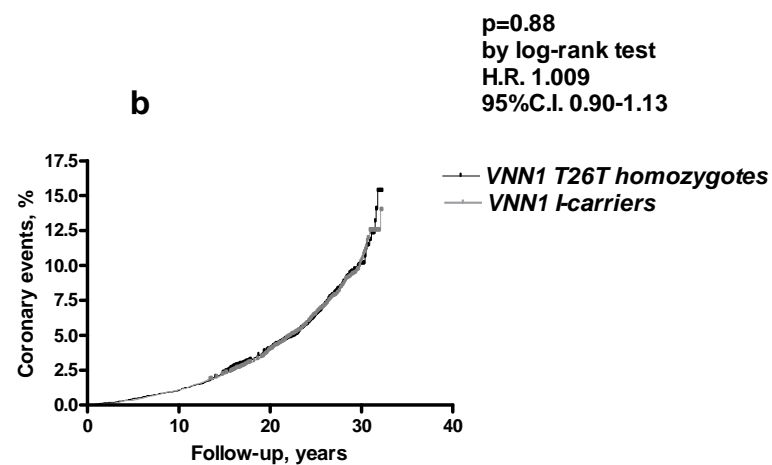
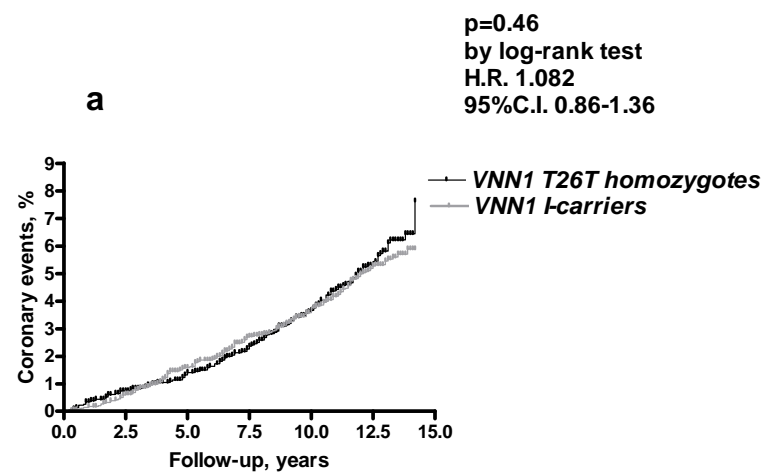
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**Figure 1:** Incident coronary events (a, b) and strokes (c, d) during follow-up at MDC-CVA (a, c) and MPP (b, d).

**Figure 1:** Incident coronary events (a, b) and strokes (c, d) during follow-up at MDC-CVA (a, c) and MPP (b, d)



**Table 1. Anthropometric and metabolic features of the investigated subjects**

<b>All subjects With at least one valid genotype</b>	<b>Data available (n)</b>	<b>MDC-CVA All subjects</b>	<b>Data available (n)</b>	<b>MPP At baseline</b>	<b>Data available (n)</b>	<b>MPP At follow-up</b>
<b>Gender, male (%)</b>	5664	41.8	17874	63.3	17874	63.3
<b>Age, years</b>	5664	57.45±5.94	17874	45.18±7.36	17874	68.25±5.77
<b>Systolic blood pressure, mmHg</b>	5664	141.14±18.95	17545	126.78±14.09	17670	144.91±20.00
<b>Diastolic blood pressure, mmHg</b>	5664	86.92±9.44	17545	85.27±8.69	17670	83.56±10.55
<b>Heart rate, bpm</b>	0	-	17867	68.30±9.55	17809	70.52±12.05
<b>Body mass index, kg/m<sup>2</sup></b>	5657	25.84±3.98	17867	24.30±3.39	17773	27.16±4.16
<b>Waist, cm</b>	5656	84.17±13.03	803	79.25	17767	94.79±12.30
<b>Glucose, mmol/L</b>	5107	5.18±1.40	17808	4.90±0.75	17852	5.84±1.43
<b>Cholesterol, mmol/L</b>	5109	6.17±1.10	17839	5.61±1.05	17866	5.59±1.09
<b>Triglycerides, mmol/L</b>	5109	1.37±0.79	17835	1.28±0.80	17864	1.27±0.80
<b>HDL, mmol/L</b>	5057	1.38±0.72	0	-	17857	1.41±0.42
<b>Obesity, %</b>	5657	13.5	17867	5.6	17773	21.7
<b>Hypertension, %</b>	5664	63.3	17559	34.2	17742	72.2
<b>Diabetes, %</b>	5115	8.7	17762	1.1	17843	13.7
<b>Smoking habit, %</b>	5341	27.6	17874	37.1	0	-
<b>Antihypertensive therapy (%)</b>	5664	16.2	17840	4.4	17871	38.3

**Table 2. Crude blood pressure according to genotypes in MDC-CVA and MPP both at baseline and follow-up.**

Type of blood pressure adjustment for AHT	VNNI T26T	VNNI T26I	VNNI I26I	VNNI T-carriers	VNNI I-carriers
<b>MDC-CVA</b>					
	n=2294	n=2617	N=753	N=4911	n=3370
<b>SBP (mmHg)</b>	141.32±18.98	141.06±18.95	140.86±18.83	141.18±18.96	141.02±18.92
<b>DBP (mmHg)</b>	87.25±9.71	86.73±9.25	86.56±9.24	86.97±9.47	86.69±9.25*
<b>Hypertension prevalence (%)</b>	64.9	62.0	62.9	63.3	62.2*
<b>MPP at baseline</b>					
	n=7321	n=8067	n=2157	N=15388	n=10224
<b>SBP (mmHg)</b>	126.89±14.06	126.66±14.02	126.86±14.50	126.77±14.03	126.70±14.12
<b>DBP (mmHg)</b>	85.34±8.68	85.23±8.60	85.15±9.04	85.28±8.64	85.22±8.70
<b>Hypertension prevalence (%)</b>	34.6	34.0	33.9	34.3	34.0
<b>MPP at follow-up</b>					
	n=7351	n=8133	n=2186	N=15484	n=10319
<b>SBP (mmHg)</b>	144.88±19.88	144.85±20.08	145.25±20.08	144.86±19.98	144.93±20.08
<b>DBP (mmHg)</b>	83.54±10.54	83.57±10.55	83.62±10.62	83.56±10.54	83.58±10.56
<b>Hypertension prevalence (%)</b>	72.4	72.1	71.9	72.3	72.1
<b>Hypertension incidence (%)</b>	63.4	62.9	64.3	63.1	63.2

\*P<0.05 by T-student test respect to I-homozygotes, SBP, systolic blood pressure; DBP, Diastolic blood pressure

**Table 3. Beta coefficient and standard error (on parenthesis) for the VNN1 T26I polymorphism tested by linear regression according to different mode of inheritance and after different kinds of BP adjustment in MDC-CVA.**

	Mode of inheritance					
Type of blood pressure adjustment for AHT	Additive	p-value	Autosomal recessive	p-value	Autosomal dominant	p-value
<b>Non adjusted</b>						
<b>SBP (mmHg)</b>	-0.309 (0.342)	0.37	-0.662 (0.686)	0.34	-0.279 (0.474)	0.56
<b>DBP (mmHg)</b>	-0.413 (0.175)	0.02	-0.550 (0.351)	0.12	-0.533 (0.243)	0.03
<b>Fixed addition</b>						
<b>SBP (mmHg)</b>	-0.252 (0.362)	0.49	-0.711 (0.725)	0.33	-0.144 (0.502)	0.77
<b>DBP (mmHg)</b>	-0.385 (0.185)	0.04	-0.575 (0.371)	0.12	-0.466 (0.256)	0.07
<b>Stepped addition</b>						
<b>SBP (mmHg)</b>	-0.276 (0.363)	0.45	-0.709 (0.728)	0.33	-0.193 (0.503)	0.70
<b>DBP (mmHg)</b>	-0.403 (0.189)	0.03	-0.594 (0.380)	0.12	-0.496 (0.263)	0.06

**Adjustment for age, sex, BMI**

**Table 4. Beta coefficient and standard error (on parenthesis) for the VNN1 T26I polymorphism tested by linear regression according to different mode of inheritance and after different kinds of BP adjustment in MPP both at baseline and at follow-up.**

	Mode of inheritance					
			MPP – baseline			
Type of blood pressure adjustment for AHT	Additive	p-value	Autosomal recessive	p-value	Autosomal dominant	p-value
<b>Non adjusted</b>						
SBP (mmHg)	-0.039 (0.142)	0.79	0.139 (0.291)	0.63	-0.134 (0.194)	0.49
DBP (mmHg)	-0.051 (0.088)	0.56	-0.088 (0.180)	0.63	-0.056 (0.120)	0.64
<b>Fixed addition</b>						
SBP (mmHg)	-0.021 (0.148)	0.89	0.162 (0.303)	0.59	-0.111 (0.202)	0.58
DBP (mmHg)	-0.040 (0.091)	0.66	-0.080 (0.187)	0.67	-0.040 (0.124)	0.75
			<b>MPP follow-up</b>			
<b>Non adjusted</b>						
SBP (mmHg)	0.199 (0.216)	0.36	0.399 (0.443)	0.37	0.194 (0.296)	0.51
DBP (mmHg)	0.073 (0.112)	0.51	0.037 (0.229)	0.87	0.121 (0.153)	0.43
<b>Fixed addition</b>						
SBP (mmHg)	0.162 (0.226)	0.47	0.247 (0.462)	0.59	0.192 (0.309)	0.53
DBP (mmHg)	0.053 (0.115)	0.65	-0.039 (0.236)	0.87	0.116 (0.158)	0.46
<b>Stepped addition</b>						
SBP (mmHg)	0.124 (0.229)	0.59	0.219 (0.469)	0.64	0.134 (0.313)	0.67
DBP (mmHg)	0.014 (0.121)	0.91	-0.085 (0.247)	0.73	0.065 (0.165)	0.70

Adjustment for age, sex, BMI, heart rate

**Table 5. Odds ratio and 95% CI for hypertension conferred by the VNN1 T26I polymorphism tested by logistic regression according to different mode of inheritance in MDC-CVA and MPP (both at baseline and at follow-up).**

	Mode of inheritance					
	Additive	p-value	Autosomal recessive	p-value	Autosomal dominant	p-value
<b>Hypertension prevalence (MVD-CVA)</b>	0.926 (0.852-1.008)	0.07	0.949 (0.802-1.123)	0.54	0.885 (0.788-0.995)	0.04
<b>Hypertension prevalence (MPP at baseline)</b>	0.985 (0.937-1.035)	0.54	0.981 (0.886-1.087)	0.72	0.980 (0.915-1.048)	0.55
<b>(Hypertension prevalence (MPP at follow-up)</b>	0.995 (0.945-1.047)	0.85	0.983 (0.886-1.091)	0.74	0.998 (0.931-1.070)	0.96
<b>(Hypertension incidence (MPP at follow-up)</b>	1.012 (0.954-1.073)	0.70	1.049 (0.93-1.184)	0.43	1.000 (0.923-1.084)	0.99

**Adjustment for age, sex, BMI, heart rate (only MPP), BMI change, years of follow-up (only for incident Hypertension)**

**Vanin-1 T26I polymorphism, hypertension and cardiovascular events in two large urban-based prospective studies in Swedes.**

**Short title:** *VNN1* T26I polymorphism and hypertension

Cristiano Fava et al.

**SUPPLEMENTARY METHODS**

**Blood pressure adjustment**

*Fixed addition*

Based on the known average treatment effects, fixed increments of 10 mmHg systolic BP and 5 mmHg diastolic BP were added to treated pressures.

*Stepped addition*

To account for the number of drugs, stepped increments of 8/4, 14/10, 20/16, 26/22 mmHg were added to the measured systolic BP/diastolic BP of treated individuals taking one, two, and three drug classes, respectively.

**Laboratory parameters**

Blood glucose was determined by a routine hexokinase method. Triglycerides and total cholesterol were determined on a DAX 48 automatic analyser with use of reagents and calibrators from the supplier of the instrument (Bayer AB, Göteborg, Sweden). High-density lipoprotein (HDL) cholesterol was determined by the same procedure as used for total cholesterol but after precipitation of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) with dextran-sulphate. LDL-cholesterol was calculated from the values for triglycerides, total cholesterol and HDL-cholesterol according to the Friedewald formula:  $LDL = total\ cholesterol - HDL - (triglycerides/2.2)$ . When serum triglyceride levels were above 4.00 mmol/l, LDL cholesterol was not determined.



## SUPPLEMENTARY RESULTS

### **Genotyping reproducibility**

We run a random subsample (190 samples) for the rs2294757 genotyping and got 100% identical call rates respect to the first genotyping.

### **Power analysis in MDC-CVA**

This study has 80% power to detect an odds ratio for hypertension greater than 1.17 according to a dominant (T26T vs. T26I&I26I) mode of inheritance, greater than 1.24 according to a recessive (T26T&T26I vs. I26I) mode of inheritance, greater than 1.12 according to an additive mode of inheritance. Regarding continuous BP variables, if an autosomal dominant/recessive/additive mode of inheritance is assumed, the study has 80% power to detect a BP difference of respectively  $>1.33 / >1.92 / >0.96$  mmHg in systolic BP and  $>0.68 / >0.98 / >0.49$  mmHg in diastolic BP between subjects carrying different genotypes.

Regarding the survival analysis this study has 80% power to detect a hazard ratio (H.R.) for ischemic stroke (n=261)/coronary events (n=304) greater than 1.18 / 1.18, according to a recessive mode of inheritance and to detect a hazard ratio greater than 1.12 / 1.12, according to a dominant mode of inheritance and greater than 1.08/1.08, according to an additive mode of inheritance.

### **Power analysis in MPP**

If an autosomal dominant mode of inheritance (T26T&T26I vs. I26I) is assumed, this study has 80% power to detect an odds ratio for hypertension greater than 1.10 / 1.10 respectively at baseline/reinvestigation, whereas under a recessive mode of inheritance (T26T vs. T26I&I26I), greater than 1.14 / 1.15 respectively at baseline/reinvestigation. If an additive mode of inheritance is assumed, this study has 80% power to detect an odds ratio for hypertension greater than 1.07 / 1.07 respectively at baseline/reinvestigation,

Regarding continuous BP variables, if an autosomal dominant mode of inheritance is assumed, the study has 80% power to detect a BP difference of  $>0.54 / >0.83$  mmHg in systolic BP and  $>0.34 / >0.43$  mmHg in diastolic BP respectively at baseline/reinvestigation. If an autosomal recessive

mode of inheritance is assumed, the study has 80% power to detect a BP difference of  $>0.82$  /  $>1.24$  mmHg in systolic BP and  $>0.51$  /  $>0.64$  mmHg in diastolic BP respectively at baseline/reinvestigation. If an additive mode of inheritance is assumed, the study has 80% power to detect a BP difference of  $>0.40$  /  $>0.60$  mmHg in systolic BP and  $>0.25$  /  $>0.31$  mmHg in diastolic BP respectively at baseline/reinvestigation.

Regarding the survival analysis this study has 80% power to detect a hazard ratio for ischemic stroke (n=855)/coronary events (n=1290) greater than 1.10 / 1.10, according to a recessive mode of inheritance, greater than 1.07 / 1.07, according to a dominant mode of inheritance and greater than 1.05 / 1.05, according to an additive mode of inheritance.

**Supplementary table S1. Linear and logistic regression analysis of systolic, diastolic blood pressure and hypertension prevalence in the MDC-CVA**

Variables	Non adjusted Systolic BP		Non adjusted Diastolic BP		Hypertension prevalence	
	Regression Coefficient (95% C.I)	P	Regression Coefficient (95% C.I)	P	O.R. (95% C.I)	P
<b>Gender †</b>	-2.60 (-3.53/-1.68)	<0.001	-3.01 (-3.48/-2.53)	<0.001	0.34 (0.63/0.80)	<0.001
<b>Age, year</b>	0.98 (0.90/1.05)	<0.001	0.14 (0.10/0.18)	<0.001	1.08 (1.07/1.09)	<0.001
<b>BMI, Kg/m<sup>2</sup></b>	0.94 (0.83/1.06)	<0.001	0.56 (0.50/0.62)	<0.001	0.12 (1.11/1.15)	<0.001
<b>VNNI T26I‡</b>	-0.28 (-1.21/0.65)	0.57	-0.53 (-1.01/-0.06)	0.03	0.88 (0.79/0.99)	0.04
<b>Model</b>		<0.001		<0.001		<0.001

BMI, body mass index; MDC-CVA, Malmö Diet and Cancer-cardiovascular arm; C.I., confidence interval.

\* N.B. Seven subjects were not included in the analysis due to missed BMI

† male sex is coded as 1 and female sex as 2.

‡ For the VNNI T26I polymorphism homozygotes for the I-allele were coded as 0 and carriers of at least one 26T allele as 1.

§ The statistical variables used for the interaction (VNNI T26I ‡x SEX§, VNNI T26I‡ x BMI, VNNI T26I‡x AGE) have been computed by multiplying the VNNI T26I genotype respectively with sex, BMI and age.

The Interaction terms VNNI T26I‡x SEX§, VNNI T26I‡ x BMI, VNNI T26I‡x AGE were discarded from the regression mode because not significant

**Supplementary table S2. Linear and logistic regression analysis of systolic, diastolic blood pressure and hypertension prevalence in the MPP.**

Variables	Non adjusted Systolic BP		Non adjusted Diastolic BP		Hypertension prevalence	
	Regression Coefficient (95% C.I)	P	Regression Coefficient (95% C.I)	P	O.R. (95% C.I)	P
<i>MPP baseline</i>						
<b>Gender †</b>	-4.39 (-4.84/-3.95)	<0.001	-4.59 (-4.86/-4.31)	<0.001	0.47 (0.43/0.51)	<0.001
<b>Age, year</b>	0.34 (0.32/0.37)	<0.001	0.19 (0.17/0.21)	<0.001	1.058 (1.05/1.06)	<0.001
<b>BMI, Kg/m<sup>2</sup></b>	1.0 (0.94/1.06)	<0.001	0.62 (0.58/0.65)	<0.001	1.16 (1.12/1.18)	<0.001
<b>Heart rate, bpm</b>	0.41 (0.39/0.43)	<0.001	0.21 (0.20/0.22)	<0.001	1.05 (1.04/1.06)	<0.001
<b>Model</b>		<0.001		<0.001		<0.001
<i>MPP reinvestigation</i>						
<b>Gender †</b>	-2.6 (-3.2/-2.0)	<0.001	-1.81 (-2.12/-1.5)	<0.001	0.70 (0.65/0.75)	<0.001
<b>Age, year</b>	0.38 (0.33/0.43)	<0.001	-0.11 (-0.13/-0.08)	<0.001	1.06 (1.06/1.07)	<0.001
<b>BMI, Kg/m<sup>2</sup></b>	0.79 (0.72/0.86)	<0.001	0.53 (0.49/0.56)	<0.001	1.15 (1.14/1.16)	<0.001
<b>Heart rate, bpm</b>	0.24 (0.22/0.27)	<0.001	0.19 (0.17/0.20)	<0.001	1.01 (1.01/1.02)	<0.001
<b>Model</b>		<0.001		<0.001		<0.001

BMI, body mass index; MPP, Malmö Preventive Project; C.I., confidence interval.

\* N.B. Seven subjects were not included in the analysis due to missed BMI at MPP baseline and 101 subjects at MPP reinvestigation.

† male sex is coded as 1 and female sex as 2.

‡ For the *VNN1* T26I polymorphism homozygotes for the I-allele were coded as 0 and carriers of at least one 26T allele as 1.

§ The statistical variables used for the interaction (*VNN1* T26I ‡x SEX§, *VNN1* T26I‡ x BMI, *VNN1* T26I‡x AGE) have been computed by multiplying the *VNN1* T26I genotype respectively with sex, BMI and age.

The *VNN1* T26I genotype and the interaction terms (*VNN1* T26I‡x SEX§, *VNN1* T26I‡ x BMI, *VNN1* T26I‡x AGE) were discarded from the regression model because not significant.

**Supplementary Table S3. Beta coefficient and standard error (on parenthesis) for the association of VNN1 T26I polymorphism and metabolic syndrome individual components, tested by linear regression according to different genetic model.**

MetS individual components	Numb.†	Additive	p-value	<i>Genetic model</i>		Autosomal dominant	p-value
				Autosomal recessive	p-value		
				<i>MDC-CVA</i>			
Waist, cm	5,029	-0.017 (0.021)	0.40	0.017 (0.042)	0.68	-0.042 (0.029)	0.15
Triglycerides, mmol/L	5,029	0.007 (0.021)	0.73	0.064 (0.042)	0.12	-0.017 (0.029)	0.55
Glucose, mmol/L	5,029	0.025 (0.021)	0.23	0.076 (0.042)	0.07	0.012 (0.029)	0.69
HDL-cholesterol, mmol/L	5,029	-0.014 (0.021)	0.51	-0.065 (0.042)	0.12	0.005 (0.029)	0.86
				<i>MPP baseline</i>			
Triglycerides, mmol/L	17,835	-0.020 (0.011)	0.07	-0.014 (0.022)	0.52	-0.029 (0.015)	0.05
Glucose	17808	-0.004 (0.010)	0.70	-0.008 (0.021)	0.71	-0.004 (0.014)	0.79
				<i>MPP reinvestigation</i>			
Waist, cm	17,767	-0.004 (0.011)	0.72	0.022 (0.023)	0.34	-0.017 (0.015)	0.26
Triglycerides, mmol/L	17,864	-0.011 (0.011)	0.34	0.016 (0.023)	0.49	-0.027 (0.015)	0.08
Glucose, mmol/L	17,857	0.011 (0.011)	0.34	0.038 (0.023)	0.10	0.003 (0.015)	0.84
HDL-cholesterol, mmol/L	17,852	-0.010 (0.011)	0.39	-0.017 (0.023)	0.46	-0.010 (0.015)	0.49

†; subjects included in the analysis

All the standardized residuals were adjusted for age and sex. No adjustment for antihypertensive, antilipidemic and antidiabetic drugs was made (see methods for detailed description).