rs629301 *CELSR2* polymorphism confers a ten years equivalent risk of critical stenosis assessed by coronary angiography.

Davide Noto[a]* MD PhD, Angelo B Cefalù[a]* MD PhD, Nicola Martinelli[b]* MD, Antonina Giammanco [a]* MD PhD, Rossella Spina[a]* BS PhD, Carlo M Barbagallo MD[a], Marco Caruso[c] MD, Salvatore Novo [c] MD, Filippo Sarullo[d] MD, Vincenzo Pernice[e] MD, Federica Brucato[a] BS, Valeria Ingrassia[a] BS PhD, Francesca Fayer[a] BS PhD, Grazia Ida Altieri[a] BS PhD, Chiara Scrimali[a] BS, Gabriella Misiano[a] BS, Oliviero Olivieri[b] MD, Domenico Girelli[b] MD, Maurizio R Averna[a] MD.

* These authors equally contributed to the manuscript.

[a] Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo.

[b] Department of Medicine, Unit of Internal Medicine, University of Verona, Italy.

[c] Intensive Care Unit, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo.

[d] Intensive Care Unit, "Buccheri La Ferla" Hospital, Palermo.

[e] Intensive Care Unit, "Villa Maria Eleonora" Hospital, Palermo.

Running Title:

rs629301 in coronary angiography patients.

Corresponding Author:

Dr. Davide Noto

Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical

Specialties, University of Palermo. Via del Vespro 141 I90127 PALERMO, ITALY

E-Mail: davide.noto@policlinico.pa.it

Telefax no.: + 390916554332

Words Count: (abstract & text) : 2662, references: 18, Tables: 2, Figures: 2

Abstract

Background and Aims: Novel genetic determinants associated with coronary artery disease (CAD) have been discovered by genome wide association studies. Variants encompassing the *CELSR2-PSRC1-SORT1* gene cluster have been associated with CAD. This study is aimed to investigate the rs629301 polymorphism association with the extent of CAD evaluated by coronary angiography (CAG), and to evaluate its associations on an extensive panel of lipid and lipoprotein measurements in a large Italian cohort of 2429 patients.

Methods and Results: The patients were collected by four Intensive Care Units located in Palermo and Verona (Italy). Clinical Records were filed, blood samples were collected, lipids and apolipoproteins (apo) were measured in separate laboratories. CAD was defined by the presence of stenotic arteries (>50% lumen diameter) by CAG.

The presence of CAD was associated with the rs629301 genotype. Patients with CAD were 78% and 73% (p = 0.007) of the T/T vs. T/G+G/G genotype carriers respectively. T/T genotype was also correlated with the number of stenotic arteries, with a 1.29 (1.04 - 1.61) risk to have a three-arteries disease. T/T genotype correlated with higher levels of LDL-, non-HDL cholesterol, apoB, apoE and apoCIII, and lower HDL-cholesterol. Logistic Regression confirmed that rs629301was associated with CAD independently from the common risk factors, with a risk similar to that conferred by ten years of age [odds ratios were 1.43 (1.04-1.96) and 1.39 (1.22-1.58) respectively].

Conclusions: rs629301 risk allele was independently associated with the extension and severity of CAD and positively with apoE and apoB containing lipoproteins.

Introduction

High throughput DNA sequencing technologies have fueled the discovery of novel causative genes in monogenic diseases and of novel genetic and epigenetic determinants in complex multifactorial diseases as coronary artery disease (CAD). High density single nucleotide polymorphisms (SNPs) genotyping in large cohorts identified old and novel genetic loci associated with CAD and atherosclerosis (1,2). A novel locus related to lipid traits (3) and cardiovascular disease (CVD) has been identified in the 1p13 chromosomal region (4). This locus includes a cluster of SNPs in three genes, *CELSR2*, *PSRC1* and *SORT1* in strong linkage disequilibrium (5). The contribution to lipid traits of these genes has been explored in knock-out /overexpression mouse models and *SORT1* gene showed to be the most relevant in affecting circulating cholesterol levels (5). Sortilin, encoded by *SORT1* gene, has been demonstrated to regulate VLDL and LDL production and secretion in the liver, but other functions in peripheral tissues have been investigated in order to explain the correlation with CAD and glucose metabolism (6).

The association of the 1p13 locus with CAD has been replicated in different studies, but very few studies have analyzed the association of this locus in cohorts of patients subjected to coronary angiography (CAG) (7). More, the relationships between CAD and the modifications of an expanded lipid and apolipoprotein panel in carriers of SNPs within the 1p13 haplotype have not been investigated.

In the present study we studied the rs629301 SNP from the 1p13 locus, since it has been considered relevant in a panel of six SNPs able to measure the polygenic burden in hypercholesterolemia (8), and we evaluated its association with CAD and with an extensive lipid and apolipoprotein panel in a large cohort of patients who underwent CAG into four Italian Intensive Care Units (ICU).

Methods

Study patients

Patients were enrolled in the study among those admitted to four ICUs of Verona and Palermo, Italy.

The Verona cohort is part of the "Verona Heart Study" project from the "Azienda Ospedaliera Universitaria Integrata" ICU, Verona, Italy and it has been described elsewhere (16). The three cohorts from Palermo have been enrolled in the "Azienda Ospedaliera Universitaria Policlinico Paolo Giaccone " ICU at the University Hospital of Palermo , in the "Buccheri La Ferla" Hospital ICU and in the "Villa Maria Eleonora" Hospital ICU of Palermo. The relative cohorts have been described elsewhere (17,18), and their contributions to the study and data availability are presented in Supplemental Table 1. All patients were enrolled among those acceding consecutively to study ICUs to undergo a CAG procedure and willing to participate to the study. Years of enrollment are also indicated in Supplemental Table 1.

All patients were investigated to obtain clinical history with careful ascertainment of CV risk factors as smoking status, diabetes mellitus, hypertension, dyslipidemia by careful revision of the patients documented clinical history. A physical examination was performed and a blood sample was withdrawn for lipids and apolipoprotein determinations the day before the CAG procedure.

CAD was assessed by CAG and coded according to the number of epicardial coronary arteries presenting a critical stenosis (> 50% of the vessel lumen), as no critical stenosis = 0, or 1,2,3 critical arteries.

Lipids and apolipoprotein determination.

Non anticoagulated blood was separated by centrifugation and serum was stored at -80°C after mixing with a commercial antiprotease and beta-hydroxy-toluene cocktail. Total cholesterol (TC), triglycerides (TG), HDL-C were measured using standard enzymatic-colorimetric assays, while LDL-C and non-HDL-cholesterol (nonHDL-C) were calculated by Friedewald formula and TC – HDL-C difference respectively. Apoliproteins AI, B, CIII, E were measured by immuno-turbidimetry as described (16).

Real-time-PCR rs629301 genotyping assay.

Genomic DNA from all subjects were extracted from EDTA treated whole blood samples using standard commercial systems and stored at 4°C degrees.

Genotyping of rs629301 in CELSR2 gene was carried out by a validated commercial TaqMan assay (Thermo Fisher) in a ViiA7 Real Time PCR System (Thermo Fisher). Each assay reaction

was composed of 5 μL TaqMan Universal Master Mix (2X) (Thermo Fisher), 0.25 μL assay mix (40X), 1-2 μL DNA (total input of 1-10 ng template), 3.75-2.75 μL H2O. Thermal cycling was performed at the following conditions: 95°C, 10 minutes; (95°C, 15 seconds; 60°C, 1 minute) for 40 cycles. The genotyping call was done with SDS software v.1.3.0 (ViiA7 software RUO, Life Technologies).

Ethical consideration

All study patients signed a written consent to participate to the relative studies and to have their DNA sample stored. All the study protocols were approved by the respective local Ethical Committees and all the procedures adopted followed the Helsinki Declaration of 1973, as revised in 1983. All data were handled anonymously and no personal data able to identify patients were supplied to the researchers.

Statistics

Differences in prevalence rates between groups were assessed by Chi-square test, after adjustment for age and gender with Mantel-Haenszel test whenever appropriate. Age was used as predictor after conversion to decades (10 years intervals). Difference in means of numeric variables were assessed by ANOVA test after adjustment for age and gender as covariates. Homogeneity of variances were assessed by Levene's test and, in case of non-homogeneous variances, post-hoc one-to-one comparisons were performed by Games-Howell's test. Multiple Regression Analysis was performed with simultaneous evaluation of variables, without step-wise exclusion procedure. Multiple coefficients with relative standard errors are presented in tables. Alpha value was set to 0.05, multiple hypothesis comparisons were performed by Bonferroni test for ANOVA and by setting confidence intervals to 97.5 % for multiple regression. The power test for non-equality of proportions showed that the difference of CAD rates for the overall cohort (All), has a power of sample size of 0.834 with an alpha value of 0.05. The independent proportion power analysis was used to check the power of the sample to detect the difference in proportions. Statistics were performed using the SYSTAT 12 statistical package (Systat corporation, CA U.S.A.) or R statistical package (www.r-project.org/) when appropriate.

Results

A total of 2429 patients were genotyped for rs629301, and 2013 of them had CAG data available. The relative contribution of each ICU involved in the study and the clinical and laboratory data available are shown in Supplemental Table 1.

Table 1 shows the clinical and laboratory characteristics of the study cohort according to gender. Males presented significantly higher prevalence of smokers but lower prevalence of hypertension and dyslipidemia. Significantly more males accessed to ICU for AMI. Males showed higher levels of TG and lower TC and HDL-C, and nearly significantly (p=0.061) higher LDL-C levels, while in subset of patients Lipoprotein (a) levels was higher in males. Apolipoprotein AI and E were significantly lower in a subgroup of male patients (n = 1260).

The rs629301 genotype frequencies in the whole cohort were: T/T = 0.69, G/T =0.28, G/G =0.03 and agreed to Hardy Weinberg equilibrium (p = 0.209) as shown in Supplemental Figure 1.

Figure 1 shows the association of the rs629301 T/T genotype with CAD by gender revealed by CAG. The T/T genotype was associated with a higher proportion of subjects with one or more coronary arteries with critical stenosis, with an increased risk of having stenotic coronary arteries for T/T carriers. Age and gender adjusted odds ratios were 1.10 (0.83 - 1.46), 1.33 (1.01- 1.74) and 1.54 (1.17- 2.02) for 1 ,2 or 3 stenotic arteries vs. no critical stenosis, respectively.

The rs629301 T allele exerted a gene-dose association on the number of subjects with triple-vessel disease (TVD), and conversely a decreasing effect on the number of subjects without CAD detected by CAG (Supplemental Figure 2).

Overall rate of CAD was 0.786 in T/T risk genotype carriers vs. 0.732 in non-carriers (cumulated G/T and G/G genotypes) with an age and gender adjusted odds ratio of 1.34 (1.07- 1.67) as shown in Supplemental Figure 3. Power of the sample size was 0.834 with an alpha level of 0.05. Similar results were found according to gender, though results were not significant for females due to the low numerosity of the sample (Supplemental Figure 3).

Table 2 shows the clinical and laboratory parameters of the study cohort according to rs629301 genotype. Carriers of the T/T genotype presented higher prevalence of dyslipidemia, with significantly higher levels of LDL-C, nonHDL-C, apoB, apoE, apoCIII, and lower levels of HDL-C. A

higher number of subjects with CAD characterized by CAG were found among carriers of T/T genotype in comparison with non-carriers.

The association of rs629301 T/T risk genotype with lipid and lipoprotein levels were also confirmed after excluding from the analysis the patients that either were on lipid lowering therapy or without any information about lipid lowering therapy (Supplemental Table 2).

The rs629301 T allele showed a clear additive gene-dose effect (for 0, 1 or 2 alleles) for most of the significant variables analyzed, though the small numerosity of the G/G genotypes limited the power of the statistical test (Supplemental Table 3).

Figure 2 shows the independent risk of CAD for the main CV risk factors considered in the study. Age, male gender, diabetes, hypertension, smoking habit, all significantly contributed to risk of CAD. The rs629301 T/T genotype remained independently associated with CAD with an odds ratio of 1.43 (1.04-1.96), after adjustment for all the risk factors.

Contribution of the rs629301 T allele was investigated in Supplemental Table 4, confirming the positive associations of rs629301 with most of the lipid measurements. The presence of a single copy of the T allele was associated with an significant increase of TC, LDL-C and non HDL-C levels of 0.09, 0.11 and 0.13 mmol/L respectively, and with a decrease of HDL-C levels of 0.04 mmol/L. Also, apoCIII levels were increased by 0.05 g/L and Diabetes prevalence was incremented by 7%.

Discussion

The 1p13 locus was identified by several studies as associated with CAD and CVD (9,10). The analysis of the locus identified many SNPs in strong linkage disequilibrium corresponding to a relatively common haplotype conferring an increased risk of CAD and CVD (5). The risk haplotype in the chromosomal region 1p13 was expressed "in vitro" and displayed an increased expression of the three nearest genes, *CELSR2*, *PSRC1* and *SORT1*. The rs12740374 was identified as the main determinant of the haplotype effect by selective knock-out of the haplotype SNPs in a luciferase expression model (5). *SORT1* was also demonstrated to be associated with cholesterol levels in mice models of knockdown/overexpression genetic models (5). The analysis of the effects

of different SNPs in linkage disequilibrium on lipid values and CVD showed that the risk is conferred by the common homozygous haplotype (5,7). Our data in Supplemental Table2 and Supplemental Table4 suggest that the risk of CAD is proportional to the number of T alleles. The increase was more evident for non-HDL cholesterol, increased by 0.13 mmol/L by a single T allele. Since also Diabetes prevalence was increased by 7%, with an estimated 14% increase in T/T homozygotes.

The sortilin protein, the product of SORT1, is expressed in many tissues and organs, and it is involved in protein-trafficking among cell organelles (11). Sortilin works also as a cell membrane signaling protein and can be secreted in the plasma after cleavage (6). The mechanism behind the association of SORT1 and CVD resides in the role of sortilin in regulating lipid and glucose metabolisms, though the studies that evaluated the alteration of SORT1 expression did not bring consistent results (6). It has been suggested that the lack of sortilin might decrease the liver output of apoB containing lipoproteins, but other factors, as insulin resistance or free fatty acids availability, seem able to modulate this effect (6). SORT1 expression is reduced by insulin resistance (IR) in the liver (6,10), while fibrates and polyunsaturated fatty acids antagonizes IR and seem to rescue sortilin production, reducing the hepatic lipoprotein output (12). Sortilin has been linked also to a direct effect on plaque inflammation and calcification (10).

The main aim of this paper is to investigate the association of the rs629301 SNP with the extension of CAD evaluated by CAG. This SNP has been correlated with lipid levels in large GWAS settings (13) and it has been included in a list of SNPs able to predict the polygenic burden in hypercholesterolemia (8), resulting among the most powerful SNPs affecting LDL-C levels (8). Our study shows that the rs629301 T allele, which is the allele included in the 1p13 risk haplotype (5), is associated with the presence of CAD and with its extension, expressed as the number of coronary arteries with critical stenoses. The T/T genotype is associated with CAD independently from the conventional CV risk factors, increasing by roughly 43% the risk to detect a critical stenosis by CAG, but it is also associated to the extension of CAD, as shown in Figure 1.

Three SNP within the 1p13 locus, rs599839, rs646776, and rs4970834 were associated with CAD documented by CAG in a smaller German cohort independently from common risk factors (7). The

same cohort showed association of SNP risk alleles with lipid and lipoprotein levels similar to the present study (7). In a New Zealand cohort, the rs599839 homozygous common genotype was associated with a prospective risk for non-ST elevation myocardial infarction (NSTEMI) of 1.33 in the 8 years of follow-up (14). Results comparable to the present Italian cohort were found in a Chinese cohort of patients subjected to CAG (15), demonstrating that the effect of the 1.p13 locus is shared among different ethnic groups.

A secondary aim of this study was to understand how the 1p13 haplotype affects lipoprotein characteristics using an extensive panel of lipids and apolipoproteins, including apoAI, apoB, apoE, apoCIII and Lp(a), that were measured in a large subset of the present cohort. The rs629301 T/T genotype was associated with an increase of all apoB-containing lipoproteins, as suggested by the increase of LDL-C, nonHDL-C, apoB and apoE. Since apoE represents a measure of the amount of circulating triglyceride rich lipoproteins (TRL), it is plausible that VLDL and IDL contributed to the observed increase in apoB and TC levels. Lp(a) was measured in about 25% of the study subjects, and its levels seemed unaffected by rs629301, but the low number of assayed samples limits the strength of this result.

HDL-C cholesterol levels have been shown to be reduced by the 1p13 risk haplotype in most of the published cohorts (7,14–15). It is interesting that HDL-C but not apoA1 levels were reduced in the T/T genotype carriers of the present study. These data suggest that rs629301 T/T genotype might be associated with the presence of smaller circulating HDL particles.

We found that ApoCIII levels were increased in the rs629301 T/T genotype carriers. The increase of apoCIII, the main inhibitor of lipoprotein lipase (LPL), might contribute to the observed increase of TRL and to the reduction of HDL-C levels with an apparent reduction of HDL size. These findings might be explained by the inhibition of LPL activity, that could play a role in the increase of TRL and the impairment of HDL particles lipidation.

Study limitations:

The main limit of this study is that it is an association study. The relationships of rs629301 with various lipids and lipoproteins are only associative and do not elucidate causative mechanisms. More, the cohort is not completely homogeneous in terms of clinical history, because patients

undergo CAG for different reasons and the admission to different ICUs might follow different rules. On the other hand, this heterogeneity depicts the real-life scenario of the subjects admitted to ICU.

Conclusions:

In conclusion, the study of a large cohort of more than two thousand patients admitted to ICU showed that the rs629301, belonging to the 1p13 risk haplotype, is associated with an increased risk of CAD and CAD extension, and that this risk is independent from the other common CV risk factors. The analysis of the lipid and apolipoprotein patterns indicated that some of the observed effects on CAD might be explained by the increase of all apoB-containing lipoproteins, including TRLs. An impairment of lipoprotein lipolysis, related to increased apoCIII levels in risk genotype carriers, might worsen the effect of the increased liver lipoproteins output, but further studies are required to unravel the mechanisms behind the data presented in this paper. The strong independent association of the rs629301 with the CAG outcome makes rs629301 a valuable tool that could be used to refine the CV risk of patients admitted to ICU.

Acknowledgments:

References

1) Rao AS, Knowles JW. Polygenic risk scores in coronary artery disease. Curr Opin Cardiol 2019; 34(4):435-440. doi: 10.1097/HCO.0000000000000629.

2) McPherson R, Tybjaerg-Hansen A. Genetics of Coronary Artery Disease. Circ Res. 2016;118(4):564-78. doi: 10.1161/CIRCRESAHA.115.306566.

3) Kathiresan S, Melander O, Guiducci C, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 2008; 40(2):189-97. doi: 10.1038/ng.75.

4) Myocardial Infarction Genetics Consortium, Kathiresan S, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ, Schunkert H, Erdmann J, Reilly MP, Rader DJ, Morgan T, Spertus JA, Stoll M, Girelli D, McKeown PP, Patterson CC, Siscovick DS, O'Donnell CJ, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Melander O, Altshuler D, Ardissino D, Merlini PA, Berzuini C, Bernardinelli L, Peyvandi F, Tubaro M, Celli P, Ferrario M, Fetiveau R, Marziliano N, Casari G, Galli M, Ribichini F, Rossi M, Bernardi F, Zonzin P, Piazza A, Mannucci PM, Schwartz SM, Siscovick DS, Yee J, Friedlander Y, Elosua R, Marrugat J, Lucas G, Subirana I, Sala J, Ramos R, Kathiresan S, Meigs JB, Williams G, Nathan DM, MacRae CA, O'Donnell CJ, Salomaa V, Havulinna AS, Peltonen L, Melander O, Berglund G, Voight BF, Kathiresan S, Hirschhorn JN, Asselta R, Duga S, Spreafico M, Musunuru K, Daly MJ, Purcell S, Voight BF, Purcell S, Nemesh J, Korn JM, McCarroll SA, Schwartz SM, Yee J, Kathiresan S, Lucas G, Subirana I, Elosua R, Surti A, Guiducci C, Gianniny L, Mirel D, Parkin M, Burtt N, Gabriel SB, Samani NJ,

Thompson JR, Braund PS, Wright BJ, Balmforth AJ, Ball SG, Hall A; Wellcome Trust Case Control Consortium, Schunkert H, Erdmann J, Linsel-Nitschke P, Lieb W, Ziegler A, König I, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Schunkert H, Samani NJ, Erdmann J, Ouwehand W, Hengstenberg C, Deloukas P, Scholz M, Cambien F, Reilly MP, Li M, Chen Z, Wilensky R, Matthai W, Qasim A, Hakonarson HH, Devaney J, Burnett MS, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Knouff CW, Waterworth DM, Walker MC, Mooser V, Epstein SE, Rader DJ, Scheffold T, Berger K, Stoll M, Huge A, Girelli D, Martinelli N, Olivieri O, Corrocher R, Morgan T, Spertus JA, McKeown P, Patterson CC, Schunkert H, Erdmann E, Linsel-Nitschke P, Lieb W, Ziegler A, König IR, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Hólm H, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Engert JC, Do R, Xie C, Anand S, Kathiresan S, Ardissino D, Mannucci PM, Siscovick D, O'Donnell CJ, Samani NJ, Melander O, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Altshuler D. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009;41(3):334-41. doi: 10.1038/ng.327.

5) Musunuru K, Strong A, Frank-Kamenetsky M, Lee NE, Ahfeldt T, Sachs KV, Li X, Li H, Kuperwasser N, Ruda VM, Pirruccello JP, Muchmore B, Prokunina-Olsson L, Hall JL, Schadt EE, Morales CR, Lund-Katz S, Phillips MC, Wong J, Cantley W, Racie T, Ejebe KG, Orho-Melander M, Melander O, Koteliansky V, Fitzgerald K, Krauss RM, Cowan CA, Kathiresan S, Rader DJ. From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. Nature 2010; 466(7307):714-9. doi: 10.1038/nature09266.

6) Conlon DM. Role of sortilin in lipid metabolism. Curr Opin Lipidol 2019;30(3):198-204. doi: 10.1097/MOL.0000000000000598.

7) Muendlein A, Geller-Rhomberg S, Saely CH, Winder T, Sonderegger G, Rein P, Beer S, Vonbank A, Drexel H. Significant impact of chromosomal locus 1p13.3 on serum LDL cholesterol and on angiographically characterized coronary atherosclerosis. Atherosclerosis. 2009;206(2):494-9. doi: 10.1016/j.atherosclerosis.2009.02.040.

8) Futema M, Shah S, Cooper JA, Li K, Whittall RA, Sharifi M, Goldberg O, Drogari E, Mollaki V, Wiegman A, Defesche J, D'Agostino MN, D'Angelo A, Rubba P, Fortunato G, Waluś-Miarka M, Hegele RA, Aderayo Bamimore M, Durst R, Leitersdorf E, Mulder MT, Roeters van Lennep JE, Sijbrands EJ, Whittaker JC, Talmud PJ, Humphries SE. Refinement of variant selection for the LDL cholesterol genetic risk score in the diagnosis of the polygenic form of clinical familial hypercholesterolemia and replication in samples from 6 countries. Clin Chem. 2015 Jan;61(1):231-8. doi: 10.1373/clinchem.2014.231365.

9) Hsu J, Smith JD. Genome-wide studies of gene expression relevant to coronary artery disease. Curr Opin Cardiol. 2012; 27(3) : 210-213. doi: 10.1097/HCO.0b013e3283522198.

10) Goettsch C, Kjolby M, Aikawa E. Sortilin and Its Multiple Roles in Cardiovascular and Metabolic Diseases. Arterioscler Thromb Vasc Biol. 2018; 38(1):19-25. doi: 10.1161/ATVBAHA.117.310292.

11) Willnow TE, Petersen CM, Nykjaer A. VPS10P-domain receptors – regulators of neuronal viability and function. Nat Rev Neurosci 2008; 9:899–909.

12) Li J, Bi L, Hulke M, Li T. Fish oil and fenofibrate prevented phosphorylation-dependent hepatic sortilin 1 degradation in Western diet-fed mice. J Biol Chem. 2014; 289(32):22437-49. doi: 10.1074/jbc.M114.548933.

13) Feng Q, Wei WQ, Levinson RT, Mosley JD, Stein CM. Replication and fine-mapping of genetic predictors of lipid traits in African-Americans. J Hum Genet. 2017;62(10):895-901. doi: 10.1038/jhg.2017.55. Epub 2017 May 25.

14) Ellis KL, Frampton CM, Pilbrow AP, Troughton RW, Doughty RN, Whalley GA, Ellis CJ, Skelton L, Thomson J, Yandle TG, Richards AM, Cameron VA. Genomic risk variants at 1p13.3, 1q41, and 3q22.3 are associated with subsequent cardiovascular outcomes in healthy controls and in established coronary artery disease. Circ Cardiovasc Genet. 2011;4(6):636-46. doi: 10.1161/CIRCGENETICS.111.960336.

15) Zhou L, Ding H, Zhang X, He M, Huang S, Xu Y, Shi Y, Cui G, Cheng L, Wang QK, Hu FB, Wang D, Wu T. Genetic variants at newly identified lipid loci are associated with coronary

heart disease in a Chinese Han population. PLoS One. 2011;6(11):e27481. doi: 10.1371/journal.pone.0027481.

16) Martinelli N, Baroni M, Castagna A, Lunghi B, Stefanoni F, Tosi F, Croce J, Udali S, Woodhams B, Girelli D, Bernardi F, Olivieri O. Apolipoprotein C-III Strongly Correlates with Activated Factor VII-Anti-Thrombin Complex: An Additional Link between Plasma Lipids and Coagulation. Thromb Haemost. 2019;119(2):192-202.

17) Barbagallo CM, Rizzo M, Cefalu' AB, Noto D, Scimeca A, Castello A, Sarullo F, Notarbartolo A, Averna MR. Changes in plasma lipids and low-density lipoprotein peak particle size during and after acute myocardial infarction. Am J Cardiol. 2002; 89:460-462. doi: 10.1016/s0002-9149(01)02270-6.

18) Rizzo M, Barbagallo CM, Noto D, Pace A, Cefalú AB, Pernice V, Pinto V, Rubino A, Pieri D, Traina M, Frasheri A, Notarbartolo A, Averna MR. Family history, diabetes and extension of coronary atherosclerosis are strong predictors of adverse events after PTCA: A one-year follow-up study. Nutr Metab Cardiovasc Dis. 2005;15:361-7. doi: 10.1016/j.numecd.2005.02.004.

Table 1: Clinical and Biochemical features of the study cohort.

Clinical Parameters n= 2429	M/F	Males	Females	p value[1]
Age (years)	1824/605	60.5 ± 0.3	65.6 ± 0.5	<0.0001
Former/Current Smokers (%)	1824/605	0.238	0.691	<0.0001
Hypertension (%)	1824/605	0.598	0.698	<0.0001
Diabetes (%)	1824/605	0.224	0.315	<0.0001
Dyslipidemia (%)	1824/605	0.534	0.568	0.108
Lipid lowering therapy (%), n=2050	954/1093	0.532	0.467	0.827
Admitted for acute MI (%) n=2281	1208/1073	0.585	0.479	<0.0001
Lipids n= 2429				
Total Cholesterol (mmol/L)	1824/605	4.98 ± 0.03	5.25 ± 0.05	<0.0001
Triglycerides (mmol/L)	1824/605	1.64 ± 0.02	1.56 ± 0.04	0.057
HDL Cholesterol (mmol/L)	1824/605	1.05 ± 0.01	1.25 ± 0.02	<0.0001
LDL Cholesterol (mmol/L)	1824/605	3.24 ± 0.02	3.33 ± 0.04	0.069
Non HDL cholesterol (mmol/L)	1824/605	3.93 ± 0.03	4.0 ± 0.05	0.209
Lipoprotein(a) (g/L) n=457	114/343	259(1524)	214 (1700)	0.025[2]
Apolipoproteins n= 1260				
Apolipoprotein B (g/L)	362/898	1.10 ± 0.01	1.08 ± 0.02	0.129
Apolipoprotein AI (g/L)	362/898	1.29 ± 0.01	1.41 ± 0.02	<0.0001
Apolipoprotein E (g/L)	362/898	0.04 ± 0.002	0.05 ± 0.001	0.001
Apolipoprotein CIII (g/L)	362/898	0.11 ± 0.001	0.12 ± 0.002	0.158

Data are expressed as mean ± standard error or proportions for dichotomous variables. The number of subjects with available data is also presented in column 1.

[1] p value = age and gender adjusted multiple logistic analysis for dichotomous variables, age and gender adjusted ANOVA for numerical variables where ANOVA post-hoc pairwise comparisons p-values were corrected by Bonferroni adjustment.

[2] ANOVA p value after log transform of Lp(a) values. Lp(a) cells show median values and ranges in brackets.

Table 2: Clinical and Biochemical features of the study cohort according to rs629301 genotype

Clinical Parameters n= 2429	T/T	G/T + G/G	p value[a]	Odds ratio (c.int)[b]
Age (years)	61.76 ± 0.29	61.63 ± 0.44	0.817	0.025 (0.053)[c]
Male gender (%)	0.746	0.753	0.700	1.04 (0.85-1.27)
Former/Current Smokers (%)	0.581	0.578	0.290	1.03 (0.85-1.25)
Hypertension (%)	0.616	0.636	0.038	0.9 (0.75-1.08)
Diabetes (%)	0.258	0.219	0.005	1.24 (1.01-1.53)
Dyslipidemia (%)	0.566	0.498	0.069	1.32 (1.08-1.61)
Lipid lowering therapy (%), n=2050	0.249	0.212	0.318	1.23 (0.98-1.54)
Admitted for acute MI (%) n=2281	0.566	0.543	0.290	1.03 (0.85-1.25)
Lipids n= 2429				**Multiple Coeff (std error)[c]**
Total Cholesterol (mmol/L)	5.08 ± 0.03	4.99 ± 0.04	0.052	0.099 (0.052)
Triglycerides (mmol/L)	1.64 ± 0.02	1.59 ± 0.03	0.227	0.048 (0.226)
HDL Cholesterol (mmol/L)	1.08 ± 0.01	1.13 ± 0.01	0.001	-0.050 (0.001)
LDL Cholesterol (mmol/L)	3.30 ± 0.03	3.19 ± 0.04	0.052	-0.947 (0.208)
Non HDL cholesterol (mmol/L)	4.00 ± 0.03	3.85 ± 0.04	0.002	0.149 (0.040)
Lipoprotein(a) (g/L) n=457	250 (1699)	200 (1513)	0.403 [d]	15.07 (32.86)
Apolipoproteins n= 1260				
Apolipoprotein B (g/L)	1.12 ± 0.01	1.05 ± 0.02	<0.0001	0.065 (0.001)
Apolipoprotein A1 (g/L)	1.32 ± 0.01	1.33 ± 0.01	0.230	-0.020 (0.229)
Apolipoprotein E (g/L)	4.41 ± 0.13	4.04 ± 0.18	0.105	0.364 (0.225)
Apolipoprotein CIII (g/L)	1.19 ± 0.02	1.13 ± 0.02	0.036	0.053 (0.025)

Data are expressed as mean ± standard error or proportions for dichotomous variables. The number of subjects with available data is also presented in column 1.

[a] p value = age and gender adjusted multiple logistic analysis for dichotomous variables, age and gender adjusted ANOVA for numerical variables where ANOVA post-hoc pairwise comparisons p-values were corrected by Bonferroni adjustment.

[b] Logistic analysis odds ratios with 97.5% confidence intervals in brackets. The odds ratios represent the risk conferred by the rs629301 T/T risk genotype.

[c] Multiple regression analysis coefficients, representing the increase conferred by the T/T genotype, with coefficients standard errors in brackets

[d] Lp(a) cells show median values and ranges in brackets. p-value after log transform of Lp(a) values.

Figure 1: Proportion of patients with angiography documented coronary artery disease according to rs629301 genotype.
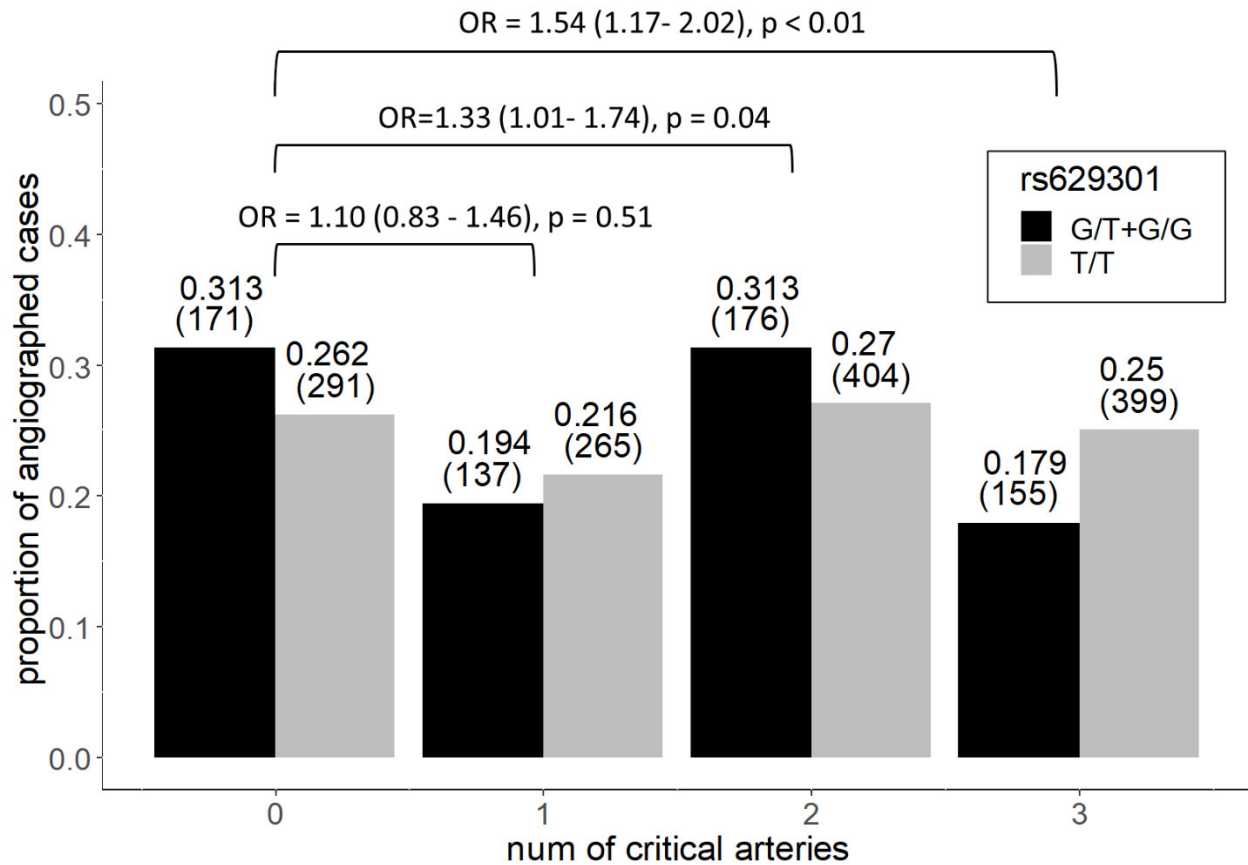
The figure shows the proportion of patients with 0, 1 ,2 or 3 (x-axis) coronary arteries presenting critical stenosis according to the rs629301 genotype. Cases are presented as T/T (homozygous risk allele, black bars) vs. pooled T/G and G/G genotypes (gray bars). Proportions are shown as bar labels with absolute cases in brackets. The upper panel shows the age and gender adjusted (by multiple logistic regression) odds ratios, with confidence intervals in brackets, of non CAD patients vs. patients with 1 2 or 3 stenotic arteries respectively.

Figure 2: Odds ratios for coronary artery disease of the main cardiovascular risk factors including the rs629301 T/T risk genotype.
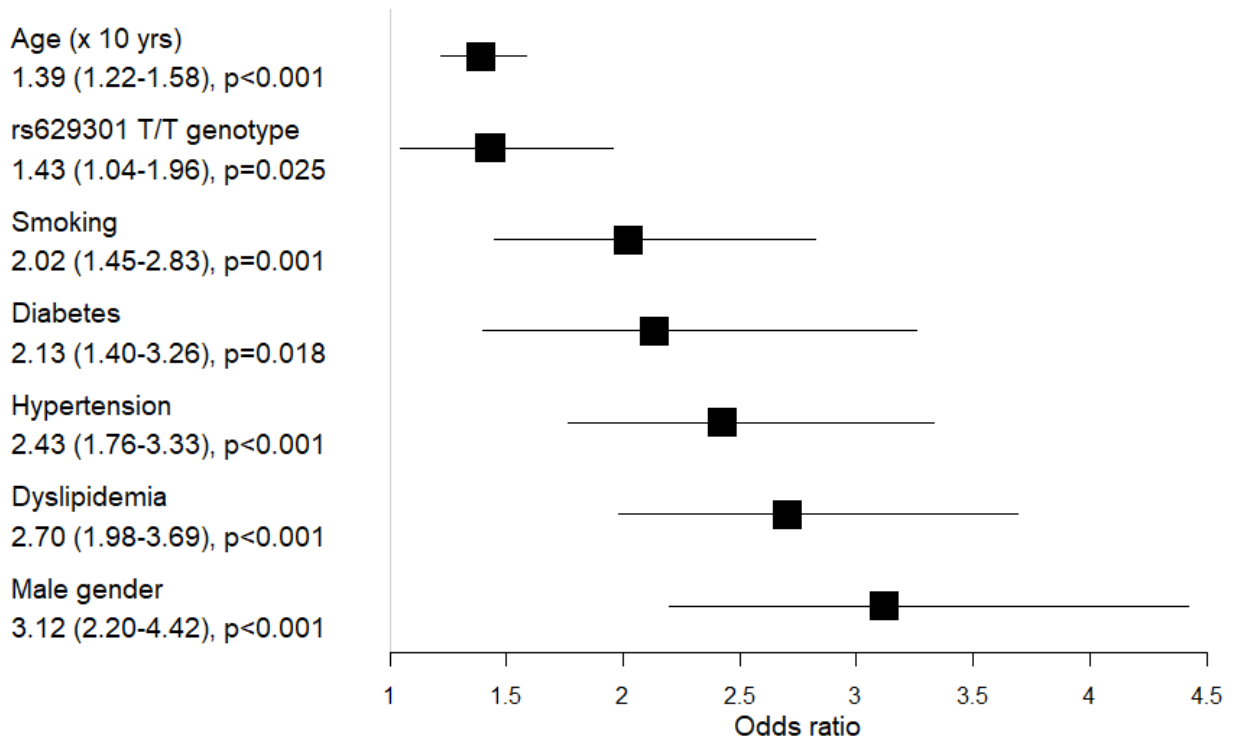
The figure shows the independent odds ratios and confidence intervals of the CV risk factors calculated by multiple logistic regression analysis. Odds ratios are presented in the labels and 97.5% confidence intervals in brackets.