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Rare, Damaging DNA Variants in *CORIN* and Risk of Coronary Artery Disease: Insights from Functional Genomics and Large-scale Sequencing Analyses

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Abstract

Background: Corin is a protease expressed in cardiomyocytes that plays a key role in salt handling and intravascular volume homeostasis via activation of natriuretic peptides. It is unknown if Corin loss-of-function is causally associated with risk of coronary artery disease (CAD).

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Supplemental Materials:
Supplemental Methods
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Methods: We analyzed all coding *CORIN* variants in an Italian case-control study of CAD. We functionally tested all 64 rare missense mutations in Western Blot and Mass Spectroscopy assays for pro-atrial natriuretic peptide cleavage. An expanded rare variant association analysis for Corin loss-of-function mutations was conducted in whole exome sequencing data from 37,799 CAD cases and 212,184 controls.

Results: We observed loss-of-function variants in *CORIN* in 8 of 1,803 (0.4%) CAD cases versus 0 of 1,725 controls (p-value 0.007). Of 64 rare missense variants profiled, 21 (33%) demonstrated <30% of wild-type activity and were deemed damaging in the two functional assays for Corin activity. In a rare variant association study that aggregated rare loss-of-function and functionally validated damaging missense variants from the Italian study, we observed no association with CAD – 21 of 1,803 CAD cases versus 12 of 1,725 controls with adjusted odds ratio of 1.61 (95%CI 0.79 to 3.29; p = 0.17). In the expanded sequencing dataset, there was no relationship between rare loss-of-function variants with CAD was also observed (OR:1.15; 95%CI 0.89 to 1.49; p = 0.30). Consistent with the genetic analysis, we observed no relationship between circulating Corin concentrations with incident CAD events among 4,744 participants of a prospective cohort study – sex-stratified hazard ratio per standard deviation increment of 0.96 (95%CI 0.87–1.07, p = 0.48).

Conclusions: Functional testing of missense mutations improved the accuracy of rare variant association analysis. Despite compelling pathophysiology and a preliminary observation suggesting association, we observe no relationship between rare damaging variants in *CORIN* or circulating Corin concentrations with risk of CAD.

Keywords

genetic association; functional genomics; natriuretic peptides; coronary artery disease

Subject terms:

Functional Genomics; Genetic Association Studies

Introduction:

Genetic association studies have identified multiple genetic variants associated with the risk of coronary artery disease. The majority of these associations are for common variants, but rare variant association studies (RVAS) are increasing with the reduced cost of exome sequencing. Rare variants identified in sequencing studies are defined as single nucleotide variants (SNVs) or short insertion/deletions (indels) with a minor allele frequency (MAF) of less than 5%. Many recent exome sequencing studies have sought to link genes with a burden of rare mutations to risk of coronary artery disease, congenital heart disease, dyslipidemia, and hypertension.[1–5] As the sample size of RVASs increase, there are more genes with multiple damaging rare variants to interpret for an association with common cardiovascular diseases.

One major challenge that limits the power of RVAS is knowing which rare variants can be aggregated in the association analysis. Ideally, only loss of function alleles that disrupt

gene function or gain of function alleles that confers new or enhanced activity would be considered in a testing group, and benign alleles would be ignored. For example, to enrich harmful alleles, many groups only consider null mutations (nonsense, indel frameshift, or splice-site mutations), computationally predicted deleterious missense mutations or even all non-synonymous mutations.[1] Considering only null mutations limits the power to associate genes with disease; however, the more permissive inclusion of all non-synonymous mutations is equally inaccurate since the vast majority are benign. For this reason, *in silico* algorithms exist to predict which amino acid substitutions result in disease using criteria such as evolutionary conservation, protein structure, and sequence homology.[6–9] Studies that compare the accuracy of these many predictors against a gold-standard dataset of known pathogenic variants have found weak concordance and a high rate of false positivity.[10,11] This has implications for RVAS findings, where functional missense mutations are collected using concordant scores among multiple *in silico* predictors to increase the confidence of identifying true loss of function missense mutations in a gene and then test the association of them with the risk of disease. Despite the known limitations of these computational predictions, it is not current practice to validate all predicted pathogenic variants with a biological assay.

Here we present an RVAS analysis of 1,803 cases and 1,725 controls from an early-onset coronary artery disease (CAD) cohort. Preliminary analysis identified an association between loss of function mutations in the *CORIN* gene and risk of CAD, and we set out to confirm this finding with functional analysis of all the missense mutations identified from exome sequencing. Corin is a type II transmembrane serine protease expressed in cardiomyocytes which converts pro-atrial natriuretic peptide (proANP) to biologically active ANP.[12,13] Biochemical studies have established the role of missense mutations in several *CORIN* domain structures as essential for its proteolytic function.[14,15] Mice lacking corin display salt-sensitive hypertension and cardiac hypertrophy.[16] Individual Corin variants have been linked to risk of hypertension, pre-eclampsia, and heart failure.[17,18] In larger RVAS for hypertension, however, there has been no association between Corin variants and risk of hypertension.[4] This suggests that loss-of-function (LOF) Corin variants may not be sufficient to cause cardiovascular disease. More studies that combine rare variant association analysis with functional validation are necessary to determine if Corin loss of function has a causal effect on cardiovascular disease.

To determine the accuracy of the initial association between LOF Corin variants and CAD, we identified functionally disruptive missense mutations using five computational prediction algorithms and two biochemical assays for Corin proteolytic activity. We tested the activity of wild-type Corin and 64 missense mutations in the enzyme on proANP cleavage. Our results show a high false-positive rate for *in silico* prediction algorithms compared with the functional assay. After analysis of all functional missense mutations in our study, and expanded analysis of larger rare variant sequencing studies, we do not find an association between Corin loss of function and risk of CAD/MI. Our findings demonstrate the importance of functional validation of rare variant association results and highlight the challenges of identifying the set of functionally relevant mutations within a gene using computational methods alone.

Methods:

This research was approved by the Mass General Brigham institutional review board (protocol 2013P001840). Full description of methods is provided in Supplemental Material. In order to minimize the possibility of unintentionally sharing information that can be used to re-identify private information, the human genetic data used in this study are available at the database of Genotypes and Phenotypes (dbGaP) and can be accessed through the accession number listed for each study in the Supplemental Materials.

Results

Exome sequencing of an early MI cohort identifies loss of function mutations associated with disease.

To discover new genes associated with the risk of CAD, we studied 1,803 patients who presented with first myocardial infarction(MI) at age <45 years and 1,725 controls derived from the previously described Atherosclerosis, Thrombosis, and Vascular Biology (ATVB) Italian Study Group.[19,20] The baseline characteristics of the cohort show that age and sex are matched between the cases and controls, and as expected, the prevalence of diabetes, smoking, and dyslipidemia are higher in the cases (Table 1).

Exome sequencing and burden testing identified a statistically significant association between early-onset MI and rare *LDLR* loss of function mutations ($p=2.31 \times 10^{-7}$, Table 2). Loss of function mutations for this analysis included nonsense, frameshift, and splice-site mutations with allele frequency less than 0.001. Below the threshold of significance were several genes that have not been previously associated with CAD or MI. The *CORIN* gene had 8 loss of function mutations in cases and 0 in controls (p=0.0065). The majority of the genes with sub-significant associations either had no known cellular function or their function could not be tested with in vitro methods. We prioritized the Corin association for functional testing given its possible association with CAD/MI by burden testing and known role in regulating blood pressure through cleavage of pro-atrial natriuretic peptide (proANP).

In silico identification of loss-of-function missense mutations does not demonstrate a significant association between CORIN and CAD.

We analyzed multiple sets of missense mutations to improve the genetic association between Corin loss-of-function and CAD. There is no significant association between the full list of rare missense mutations in the ATVB cohort and CAD (p=0.70, Table 3). However, it is unknown which of these non-synonymous mutations are benign, and therefore it reduces the power of this analysis to include the full set of missense mutations. To identify the pathogenic missense mutations that have a true effect on the enzymatic function of Corin, we used five *in silico* prediction algorithms (see Methods section) that prior studies have used in RVAS analysis.[1] These prediction tools (LRT, MutationTaster, Polyphen2-HDIV, Polyphen2-HDAR, SIFT) leverage conservation between protein families and between sequence homologies, protein structures, and pathogenic mutations recorded in the ClinVar and HGMD databases, to predict whether each amino acid substitution has the potential to affect protein function.[21,22] We identified 21 missense variants that are predicted as protein

damaging variants in all 5 algorithms (Table SI). When including these 5 out of 5 predicted damaging mutations there is an improvement in the association with CAD (p=0.15, Table 3) compared with using the 38 variants predicted as damaging in 4 out of 5 (p=0.61) or 3 out of 5 algorithms (p=0.63). To improve the functional assessment of CORIN mutations we designed a proANP proteolytic cleavage assay.

Design of in vitro functional assays of Corin proteolytic function.

The role of Corin in the cleavage of proANP to active ANP is well established. This suggests a connection to CAD through the regulation of blood pressure and vascular function. It also provides a target substrate in which to test the *in vitro* proteolysis of the missense Corin mutations we identified in the ATVB cohort (Table SI). We designed two complementary assays to quantify the cleavage of the 17kDa proANP protein to the biologically active ANP peptide (Figure 1). For both assays, 293T cells were transfected with either wild-type or mutant Corin. Purified proANP substrate was added to the media, and the specific activity of Corin was quantified by measuring proANP and ANP peptides by liquid chromatography mass spectroscopy (LCMS, Figure 1B). These results were confirmed with a second functional assay which also quantified Corin activity in 293T cells co-transfected with Corin and proANP. The ratio of proANP and ANP bands on a Western blot correlated with Corin proteolytic activity (Figure 1C and SI). There was a high correlation between the two functional assays (r^2=0.89, Figure 2B).

Functional testing of specific activity of Corin missense mutations

The specific activity of 64 corin missense mutations identified in the ATVB discovery cohort (Table SI) was quantified in triplicate by LCMS. Two missense mutations, S985A (in the protease domain) and D336Y (in an LDLR domain necessary for substrate identification) were included as positive controls for the assay based on published reports that they affect the enzymatic function of Corin.[15] We defined loss-of-function as specific activity of <30% normalized to wild-type Corin function.

The LCMS assay for Corin proteolytic activity identified 21 loss-of-function missense mutations (Figure 3A) with <30% normalized Corin function. As predicted, the positive control missense mutations S985A and D336Y had complete loss of Corin specific activity (Figure 3B). Other mutations with <5% specific activity were D373N, E374K, C599Y, C599R, E649K, C790Y, R801A, R809C, C970R, and G986S. These mutations are in multiple functional domains in the Corin protein, including the C-terminus active protease domain (Figure 4).

Correlation between computational predictions and functional testing of Corin

We directly compared the validity of the results from *in silico* predictions with our functional assay data. The algorithms each independently predict between 37 and 51 missense mutations out of the 64 functional tested missenses as damaging. However, among the damaging variants of each prediction tool, only 37 – 44% had less than 30% specific activity in the LCMS functional assay (Figure 5A). There was no significant difference in the accuracy of the individual computational predictors relative to the gold-standard assay (functional validation). The accuracy of the predictive algorithms improves when they are

aggregated to identify the set of missense mutations predicted as pathogenic with multiple computational tools (Figure 5B, 5C). The variants predicted as pathogenic in 4/5 or all 5 algorithms account for all the variants with a specific activity of < 30% compared with wild-type Corin in the LCMS assay (Figure 5C). However, these 4/5 and 5/5 variants also include a large number of false positives that do not show evidence that they are loss of function in the enzymatic assay (Figure 5C).

No evidence of rare variant association for Corin when including validated loss-of-function missense mutations.

We further grouped the functional validated loss-of-function missense mutations (< 30% normalized to wild-type Corin function) into the LOFTEE predicted null variants (stop gained, frameshift, and splice variants). The re-analysis was conducted in the original ATVB cohort as well as further included samples from additional three large cohorts, which then in total have 37,799 CAD/MI cases and 212,184 controls (Figure 6 and Methods section for cohort details). In this setting, we didn't observe significant association between the rare loss-of-function variants with CAD, the fixed effect meta-analysis has P value of 0.30, with OR 1.15 (95 CI 0.89 – 1.49), Figure 6. A sensitivity analysis using LOFTEE predicted null variants across all four cohorts has the same null association, meta-analysis P value of 0.12 with OR 1.35 (95 CI 0.92 – 1.98), Figure SII. Another sensitivity analysis using <5% wild-type Corin activity to select loss-of-function missense mutations together with LOFTEE null variants has null association also, meta-analysis P value of 0.24 with OR 1.19 (95 CI 0.89 – 1.58), Figure SIII. Taken together, no evidence of rare loss-of-function variant association for Corin with CAD/MI disease risk.

No association between plasma soluble Corin and first major adverse cardiovascular event.

To determine whether low plasma Corin is associated with incident risk of cardiovascular disease, we measured Corin in samples from the Malmo Diet and Cancer (MDC) study, a prospective observational study of ~30,000 residents of Malmö, Sweden enrolled between 1991 and 1996.[23] This cohort includes subjects with early onset and later onset CAD. After quality control and excluding samples with prevalent cardiovascular disease, 4,744 participants were used in the final analysis, see Methods section. The mean (SD) age at baseline was 57.4(5.9) years and 61.0% were female. The median [IQR] of Corin was 686.7 [491.7–945.3] pg/mL. Females had approximately one standard deviation lower Corin concentrations compared to males (−0.94 SD, $p < 0.001$) and 31.3% of the variance of log Corin is explained by sex. Current smoking status was also strongly associated with lower Corin concentrations (−0.43 SD, $p < 0.001$).

These participants were followed for mean (SD) 18.6 (4.8) years (total 88,250 patient follow-up years) and 543 (11.4%) sustained the primary outcome - major adverse cardiac events (MACE), see Methods. After adjusting for cardiovascular risk factors, there was no association between Corin and MACE (HR 0.96 per Corin SD, 95% CI 0.87–1.07, $p = 0.48$). Only adjusting for sex also showed no association for Corin with MACE (HR 0.99 per Corin SD, 95% CI 0.90–1.09, $p = 0.78$). There was no difference in Corin levels between incident MACE cases and controls among males ($p = 0.87$) and females ($p = 0.29$) (p interaction

= 0.63, Figure SIV). Sensitivity analyses restricting follow-up period also demonstrated no association between Corin and MACE in multivariable models: 5 years (67 events, p = 0.90), 10 years (178 events, p = 0.62), and 15 years (329 events, p = 0.54).

Analysis of secondary outcomes included congestive heart failure (CHF) and all-cause mortality in MACE. There were 416 (8.8%) coronary events, 209 (4.4%) CHF events, and 1,124 (23.7%) all-cause deaths. There was no evidence of association for incident coronary events (HR 0.93, 95% CI 0.82–1.05, p = 0.23), incident CHF (HR 0.99, 95% CI 0.83–1.18, p = 0.89), or all-cause mortality (HR 0.93, 95% CI 0.87–1.00, p = 0.06).

Discussion:

Rare variant association studies present an opportunity to identify new genes and novel mechanisms of cardiovascular disease. Since these studies are not powered to identify individual pathogenic variants, current methods look for the combined effect of all loss of function variants within a gene. Burden testing in rare variant association studies has validated previously known causal genes associated with CAD/MI, aortic dissection, and congenital heart disease.[1,2,24] To expand the power of RVAS identifying the correct loss of function mutations to include in the burden analysis remains a challenge. There are dozens of computational algorithms that seek to allow the inclusion of loss of function missense mutations, but their accuracy has not been systematically tested with functional assays.

Here we test all the missense mutations identified in a RVAS gene for early-onset CAD/MI. We initially identify null mutations in the *CORIN* gene, and found its association with early-onset MI in the ATVB cohort. Given the role of Corin in cleaving proANP to active ANP, this was a biologically plausible genetic association that warranted validation. When we expanded the analysis to include all missense mutations the association between Corin and CAD/MI weakened. This is expected since most missense mutations have no effect on Corin function. To provide a gold standard for inclusion of loss of function missense mutations in the genetic association analysis, we designed two functional assays for Corin missense mutations. The RVAS findings were then re-analyzed with quantitative knowledge of Corin enzymatic activity, and there was ultimately no significant association between Corin loss of function and CAD/MI risk. This finding was confirmed by including 246,455 more subjects from another three big sequencing cohort for CAD/MI in the RVAS analysis. Each of the five computational prediction algorithms showed a high false positive rate, and even when limited to the subset of predicted pathogenic mutations in all five algorithms, the inaccuracy of these *in silico* methods over-estimated produced a spurious association that was disapproved with our *in vitro* functional assay.

Our exhaustive analysis of an early positive signal in a RVAS serves as a cautionary tale for the interpretation of these studies. The early finding of an association between rare, damaging mutations in Corin and CAD/MI risk was invalidated by including loss-of-function missense variants tested in our functional proANP processing assays. The spurious association for Corin was also invalidated by including more sequenced subjects in the RVAS. Taken together, this suggests that small association studies that link individual Corin variants with cardiovascular disease may be specific to certain populations. For example, the

previously reported Corin variant associations such as the T555I/Q568P haplotype and the R530S missense mutation may have large effects exclusively in the African-American[25] and Han Chinese[18] populations in which they were first identified. Whether this is because of polygenic risk from other mutations in these populations is an open question that requires further study.

Functional testing of all missense mutations in a protein also provides important insight on the limited accuracy of the widely used computational prediction algorithms for variant interpretation. Each algorithm only showed less than 50% positive prediction with the gold-standard functional assay, and though aggregating all five algorithms improved the positive predictive value, there were many false positives that did not affect Corin function *in vitro*. This suggests that current *in silico* methods to include functional missense mutations in RVAS need improvement and studies should separately report true null mutations (i.e. stop gained, frameshift, and splice variants) and missense mutations associated with disease. Functional assays like the ones in this paper present one avenue for improving *in silico* prediction. Our data show that certain protein domains in Corin contain the majority of functional missense mutations (Figure 4). Many algorithms incorporate protein structure in their predictions, but functional assays provide a more concrete set of data on which to train these algorithms.

It remains a challenge to expand these variant testing methods to all genes. In this case, Corin function as a proANP convertase is well characterized, and its enzymatic function could be easily tested *in vitro*. Other genes may have complex functions that require *in vivo* testing, and therefore cannot be scaled to include more than a small fraction of missense mutations. In fact, one limitation of our study may be that Corin has other functions or even other substrates that are not captured in our assay. We expect that the mutations that reduce Corin proteolytic cleavage of proANP would similarly affect enzymatic function of other substrates, but have not tested other substrates or possible non-enzymatic functions.

Functional testing in a different cell type may also identify novel Corin substrates or biological functions relevant to CAD risk. Single cell RNA-sequencing analysis or the mouse aorta and human heart show that Corin is exclusively expressed in a subtype of ventricular cardiomyocytes.[26,27] Primary human ventricular cardiomyocytes are not amenable to high-throughput functional analysis given the technical limitations of culturing and transfecting these cells. The ideal study would test Corin function with *in-situ* genome editing of all missense mutations in ventricular cardiomyocytes, as has been done for BRCA1 in an immortalized cancer cell line.[28] These technologies have not yet available for primary human cells, but when editing efficiency in non-immortalized cells improves this will be an exciting new tool for functional genomic analysis.

It is important to note that this study does not rule out a role for Corin in the pathophysiology of cardiovascular diseases. Though we find no association between genetic loss of function and risk of disease, there are several studies that link serum Corin levels to adverse outcomes. Soluble Corin levels portend poor outcomes for patients after myocardial infarction and with congestive heart failure.[29–31] These studies do not show a relationship between Corin levels and incident risk of either disease, however there may be a larger effect

in younger cohorts. Therefore, the causal role of Corin remains unclear, while there is a pathophysiologic connection between Corin and poor clinical outcomes.

Though this study did not find a significant association for an initially promising RVAS signal in the Corin gene, the implications of this null hypothesis validation are important for future genetic association studies. We establish that functional testing of missense variants is necessary to determine pathogenicity. Aggregating predicted loss of function missense variants from *in silico* algorithms remains problematic, and will likely only improve with better gold-standard examples from systematic functional analysis. Genes with enzymatic functions, like Corin, are more easily tested with biochemical assays for the effect of a mutation on substrate cleavage. In this case the functional analysis identified an accurate set of missense mutations for association testing, and invalidated the hypothesis that Corin is causally linked to risk of CAD/MI. Many promising genetic associations will soon emerge with the expansion of RVAS to cardiovascular disease cohorts. The validation of these association findings will require some understanding of the biologic function of the gene, and variant testing in conjunction with computational analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard Abbreviations and Acronyms

ATVB	Atherosclerosis, Thrombosis, and Vascular Biology
CAD	coronary artery disease
CHF	congestive heart failure
CI	confidence interval
HR	hazard ratio
LCMS	liquid chromatography mass spectroscopy

LOF	loss-of-function
MACE	major adverse cardiac events
MI	myocardial infarction
OR	odds ratio
RVAS	rare variant association study
SD	standard deviation
proANP	pro-atrial natriuretic peptide

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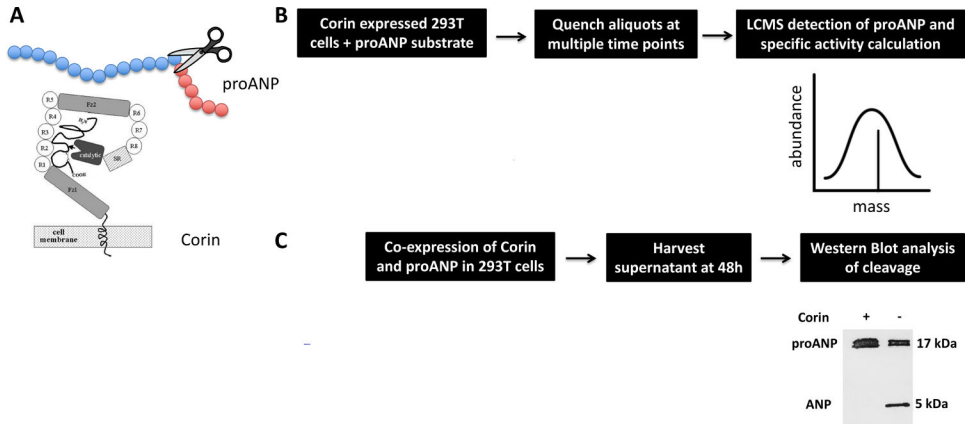

Figure 1.

Design of a functional assays to identify loss of function missense mutations in Corin. A) Functional assays to quantify Corin proteolysis of proANP to ANP. B) Liquid chromatography mass spectrometry (LCMS) assay to quantify specific activity of Corin cleavage. C) Western blot assay to detect proANP/ANP ratio in cell supernatant.

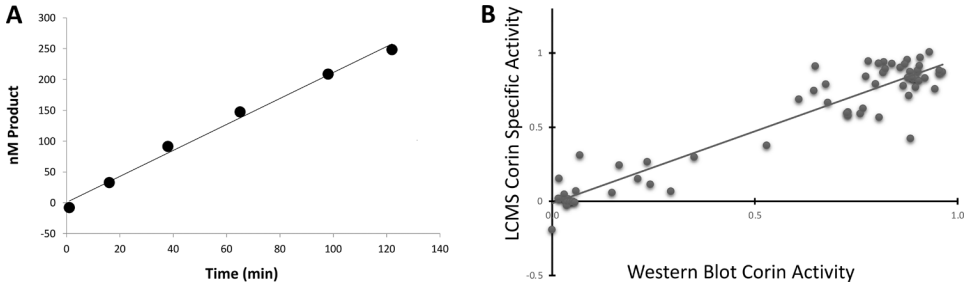

Figure 2.

A) Standardized wild-type Corin activity with liquid chromatography mass spectroscopy measurement of N-terminal proANP product. Wild-type Corin activity measured over 2 hours and normalized to ^{15}N-labeled product. Data is average of 3 technical replicates. B) Correlation between Western Blot and LCMS Assays for Corin specific activity. The two independent assays used to test all Corin missense variants for cleavage of proANP substrate show high correlation (r^2=0.89). Data is average of 3 biologic replicates.

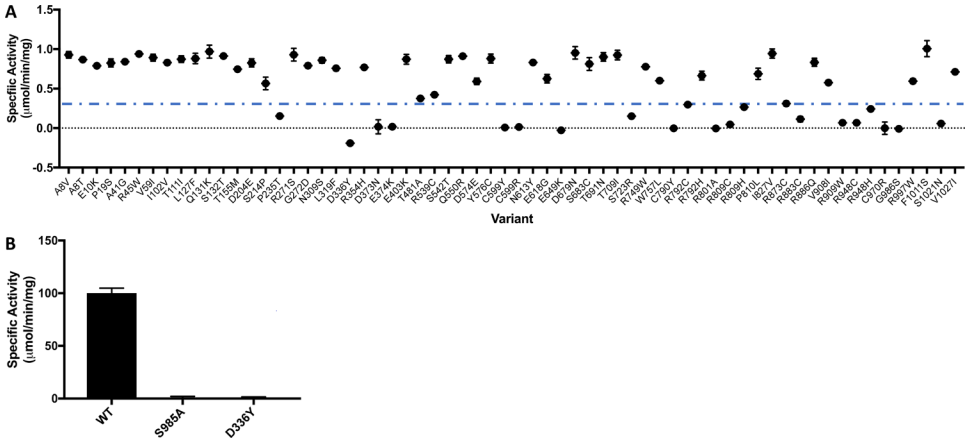

Figure 3.

A) Liquid chromatography mass spectroscopy (LCMS) specific activity for each of the 64 Corin missense mutations identified in the ATVB Discovery Cohort‡. All variants under the dotted blue line have < 30% Specific Activity (the mass of proANP substrate cleaved per minute, normalized to wild-type Corin activity), and are considered loss-of-function mutations. B) Known loss-of-function mutations S985A and D336Y show no Corin proteolytic activity on proANP substrate in the LCMS assay. All data is the average of 3 biological replicates*.

*The error bar in the figure represents standard error.

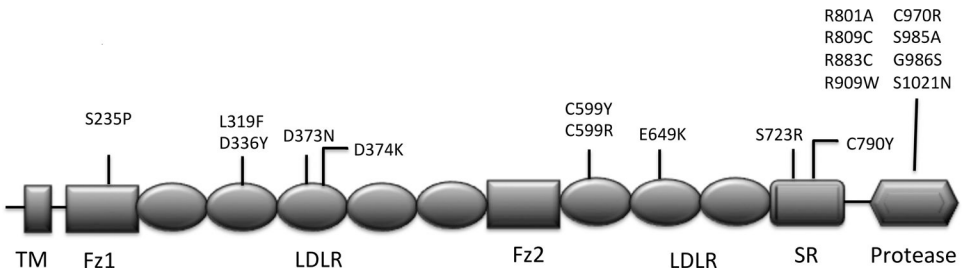

Figure 4.

Diagram of loss-of-function Corin mutations, defined by <30% normalized wild-type Corin function in the liquid chromatography mass spectroscopy functional assay. While multiple domains contain damaging mutations, the majority are in the protease domain.

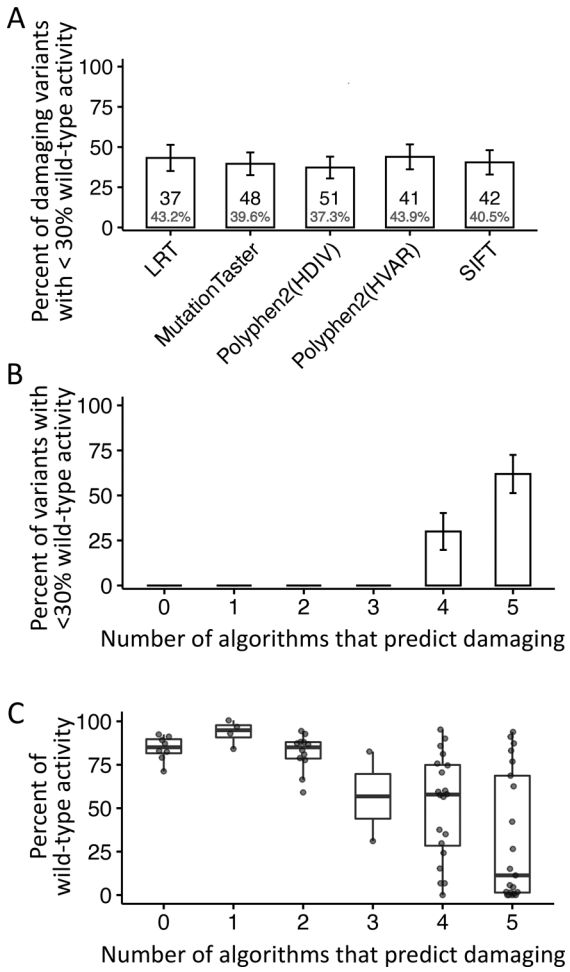

Figure 5. Correlation of functional assay with computational prediction tools.

A) The proportion of variants with < 30% normalized wild-type activity out of all the damaging variants predicted by each tool, lower percentage value in each bar. The upper values in each bar represent the number of missense variants predicted as damaging by each *in silico* prediction tools out of the 64 missense variants found in the ATVB cohort. B) The proportion of variants with < 30% normalized wild-type activity as a function of the number of algorithms that predict as damaging for each missense. C) The percentage of wild-type activity of each missense as a function of the number of algorithms that predict as damaging.

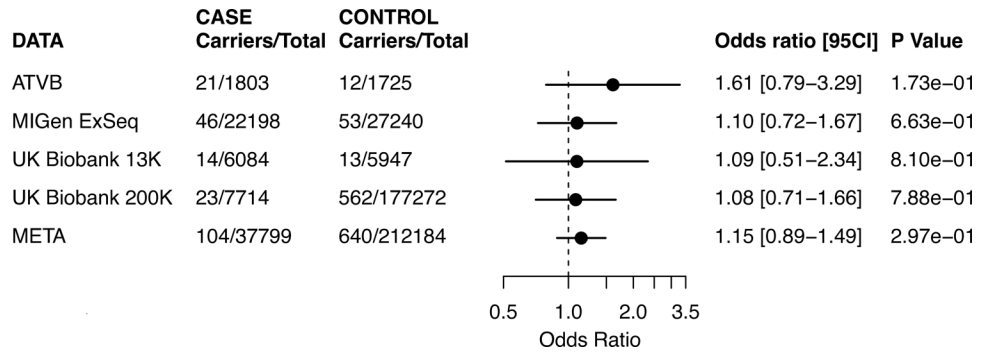

Figure 6. Forest plot for the association of the rare loss-of-function variants with coronary artery disease with functional validated data.

For each testing data set, the variant testing group includes LOFTEE predicated high confidence loss-of-functions plus missense variants with functionally validated < 30% normalized wild-type activity. The effect size odds ratio and *P* value was estimated from the firth logistic regression model. The META row is the result of the fixed effects meta-analysis of the four testing data sets.

Table 1.

Baseline characteristics for the ATVB coronary artery disease case-control study

	CASE	CONTROL	P-Value
N	1803	1725	
Baseline Age, median [Q1,Q3]	41.0 [37.0,43.0]	41.0 [37.0,43.0]	0.894
Sex (Male), n (%)	1605 (89.0)	1522 (88.5)	0.657
Type 2 diabetes, n (%)	105 (6.0)	9 (0.5)	<0.001
Current smoking, n (%)	778 (44.4)	514 (30.8)	<0.001
Total Cholesterol *, mg/dl, median [Q1,Q3]	216.0 [185.0,250.8]	197.0 [176.0,221.0]	<0.001
HDL-Cholesterol *, mg/dl, median [Q1,Q3]	40.0 [34.0,47.0]	47.0 [40.0,55.0]	<0.001
LDL-Cholesterol *, mg/dl, median [Q1,Q3]	142.0 [114.0,174.0]	120.2 [101.8,146.0]	<0.001
Triglycerides *, mg/dl, median [Q1,Q3]	152.0 [106.0,208.0]	105.0 [76.0,150.0]	<0.001

The Q1 and Q3 are the first and third quartiles of the distribution. For continuous variables, the Kruskal-Wallis test was used to test the difference. For the dichotomy variable, the Chi-squared test was used to test the difference. LDL, low-density lipoprotein. HDL, High-density lipoprotein.

* Lipid levels were measured at baseline.

Table 2:

Top 10 Genes with loss of function mutations association with CAD/MI

Gene	Chromosome	N cases	N controls	Beta*	SE (Beta)	P-value†
LDLR	19	20	0	3.77	1.47	2.31×10^{-7}
PHKB	16	9	0	2.95	1.53	0.0015
PFKP	10	9	0	2.92	1.53	0.0018
ZNF510	9	0	7	−2.81	1.57	0.0023
ZNF333	19	0	6	−2.79	1.59	0.0032
SLC12A8	3	9	0	2.76	1.52	0.0040
CNGB1	16	1	9	−1.95	0.92	0.0052
LRRC36	16	0	6	−2.64	1.59	0.0057
COL18A1	21	7	0	2.75	1.57	0.0060
CORIN	**4**	**8**	**0**	**2.70**	1.55	**0.0065**

*The effect size Beta was estimated from the firth logistic regression model.

†The P value was estimated from the SPA test, no multiple test correction shown in the table.

SE: standard error.

Table 3:

Corin rare variant association with coronary artery disease in ATVB cohort with different computational predictions

VARIANTS	*N* Case Carriers	*N* Control Carriers	Odds Ratio	Odds Ratio 95% CI	P Value
LOF	8	0	14.84	0.71–307.88	0.0065
LOF+3OF5	35	29	1.13	0.68–1.86	0.629
LOF+4OF5	34	28	1.14	0.69–1.90	0.605
LOF+5OF5	24	13	1.61	0.81–3.18	0.155
All Missense	47	47	0.92	0.61–1.39	0.698

LOF: LOFTEE algorithm predicted high confidence loss-of-function variants (stop gained, frameshift, and splice variants). For the missense variants, *n*OF5 represents *n* algorithms out of 5 algorithms predicated as damaging. The 5 algorithms are SIFT, PolyPhen2-HDIV, PolyPhen2-HVAR, LRT, and MutationTaster. “All Missense” represents all the missense variants of the CORIN gene. The effect size odds ratio was estimated from the firth logistic regression model. The P value was estimated from the SPA test, no multiple test correction shown in the table.

CI: confidence interval.