

Table 1 criteria indicate our study (Smits et al., 2017; DOI:[10.1016/j.matbio.2017.01.003](https://doi.org/10.1016/j.matbio.2017.01.003)) provides moderate to strong support that rs16881446^{G/G} is a risk factor that affects the severity of coronary artery disease. Functional significance is further strengthened by study design and results (Table 2).

Table 1. Criteria for assessing the functional significance of a genetic variant in candidate gene association studies¹

Criteria	Strong support for functional significance	Moderate support for functional significance	Evidence against functional significance
Nucleotide sequence	Variant disrupts a known functional or structural motif	Variant is a missense change or disrupts a putative functional motif; changes to protein structure might occur	Variant disrupts a non-coding region with no known functional or structural motif
Evolutionary	Consistent evidence from multiple conservation approaches for conservation across species and multigene families	Evidence for conservation across species or multigene families	Nucleotide or amino-acid residue not conserved
Population genetics	In the absence of laboratory error, strong deviations from expected population frequencies in cases and/or controls in a particular ethnicity	In the absence of laboratory error, moderate to small deviations from expected population frequencies in cases and/or controls; effects are not well characterized by ethnicity	Population genetics data indicates no deviations from expected proportions
Experimental evidence	Consistent effects from multiple lines of experimental evidence; effect in human context is established; effect in target tissue is known	Some (possibly inconsistent) evidence for function from experimental data; effect in human context or target tissue is unclear	Experimental evidence consistently indicates no functional effect
Exposures (for example, genotype–environment interaction studies)	Variant is known to affect the metabolism of the exposure in the relevant target tissue	Variant might affect metabolism of the exposure or one of its components; effect in target tissue might not be known	Variant does not affect metabolism of exposure of interest
Epidemiological evidence	Consistent and reproducible reports of moderate-to-large magnitude associations	Reports of association exist; replication studies are not available	Prior studies show no effect of variant

1. Criteria fulfilled by our study highlighted. Criteria directly taken from Table 1 of Rebbeck, T.R., Spitz, M., and Wu, X. (2004). Assessing the function of genetic variants in candidate gene association studies. *Nat Rev Genet* 5, 589-597.

Table 2. Factors that enhance significance of an initial association study¹

Criteria	Metrics for Our Study
Large sample size	n = 2144
Rigorous phenotypic assessment in patients and in controls	Coronary artery disease severity (four level scale) determined by the gold standard (coronary angiography) based on the rigorous SYNTAX score ²
Low genotyping error rate	TaqMan genotyping error rate <0.5%; association was also confirmed by genotyping a linked SNP (rs1047389)
Genomic controls or other techniques to account for population stratification	The study population (>98% Caucasian) from northern New England is highly admixed with low genetic differentiation ($F_{ST} \sim 0.05$) ³
Low P value (corrected for multiple testing)	Primary outcome adjusted P = 0.0004 (no multiple testing)
Odds ratio or attributable risk is high	Adjusted OR was 1.77 (1.29–2.43)
Gene makes biological sense	Knockout mouse studies show HS3ST1 gene controls an anti-inflammatory pathway of the blood vessel wall, inflammation is a major driving force in coronary artery disease/atherosclerosis
Functional data demonstrating a biological effect of the at-risk allele	Computational data predicts risk allele (rs16881446 ^G) occurs in transcriptional control region, ChIP analysis shows risk allele associates with impaired incorporation of transcription factor c-Myc into chromatin of the rs16881446 region
Allele affects the gene in a meaningful way	Risk allele associates with decreased HS3ST1 mRNA accumulation, which should decrease anti-inflammatory tone and thereby increase susceptibility to progression of atherosclerosis
Presence of a gene–dose response relationship	Frequency of recessive genotype progressively increased across four level scale of CAD severity: Undetectable = 3.77%; Low = 4.79%; Intermediate = 7.08%; High = 8.81%

1. Criteria directly taken from Table 2 of Dichgans, M., and Markus, H.S. (2005). Genetic association studies in stroke: methodological issues and proposed standard criteria. *Stroke* 36, 2027-2031.

2. Sianos, G., Morel, M.A., Kappetein, A.P., Morice, M.C., Colombo, A., Dawkins, K., van den Brand, M., Van Dyck, N., Russell, M.E., Mohr, F.W., et al. (2005). The SYNTAX Score: an angiographic tool grading the complexity of coronary artery disease. *EuroIntervention* 1, 219-227.

3. Sloan, C.D., Andrew, A.D., Duell, E.J., Williams, S.M., Karagas, M.R., and Moore, J.H. (2009). Genetic population structure analysis in New Hampshire reveals Eastern European ancestry. *PLoS One* 4, e6928.