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**Medical Policy Manual**

**Topic:** Genotyping for 9p21 Single Nucleotide Polymorphisms to Predict Risk of Cardiovascular Disease or Aneurysm

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## IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

## DESCRIPTION

A number of highly correlated single-nucleotide polymorphisms (SNPs) found in the chromosome 9 region p21 locus (9p21) have been significantly associated with myocardial infarction (MI), particularly early onset MI, and other manifestations of cardiovascular disease (CVD). Associations with abdominal aortic aneurysm and with intracranial aneurysm have also been reported. Genotyping for 9p21 SNPs may be offered as an approach to identify patients who may be at increased risk of some of these outcomes.

SNPs occur normally throughout a person's DNA. They occur once in every 300 nucleotides on average, which means there are roughly 10 million SNPs in the human genome. Most commonly, these variations are found in the DNA between genes. They can act as biological markers, helping scientists locate genes that are associated with disease. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene's function.

SNPs are not absolute indicators of disease development. Most SNPs have no effect on health or development. SNPs do not cause disease, but they can help determine the likelihood that someone will develop a particular illness. Some of these genetic differences, however, have proven to be very important in the study of human health. Researchers have found SNPs that may help predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins, and risk of

developing particular diseases. SNPs can also be used to track the inheritance of disease genes within families. Future studies will work to identify SNPs associated with complex diseases such as heart disease, diabetes, and cancer.

## Background

In 2007, genome-wide association studies using single nucleotide polymorphism (SNP) arrays resulted in the near simultaneous reporting of the first common genetic variant that affects the risk of coronary heart disease (CHD) in Caucasians at chromosome 9p21.3 (also known as 9p21). CHD is defined as inadequate circulation to cardiac muscle and surrounding tissue resulting in myocardial infarction (MI), unstable angina pectoris, coronary revascularization, or death.[1-4] Estimates of CHD risk were confirmed in case-control replication studies in a variety of study populations, showing that the identified SNPs were associated with CHD and even more specifically with MI.[5] In all studies, the association of any identified SNP with CHD risk was shown to be independent of traditional risk factors.[5]

Several studies have extended the 9p21 association to other vascular diseases including ischemic stroke; thus 9p21 may be reported as associated with cardiovascular disease (CVD) outcomes, defined as including CHD outcomes plus ischemic stroke. Associations have also been reported with abdominal aortic aneurysm and with intracranial arterial aneurysm.[6]

Several genes are found at the 9p21 locus, including ANRIL, which encodes a large noncoding RNA that may have regulatory functions, and CDKN2A and CDKN2B, which encode cyclin-dependent kinase inhibitors.[6] The mechanisms by which the SNPs lead to increased CHD risk have been largely unknown. Recently, Harismendy et al. identified several potential enhancer regulatory DNA sequences in the 9p21 region.[7] They reported that the SNP rs10747278, consistently associated with increased risk of CHD, occurs in one of these enhancer sequences and that the risk allele disrupts a transcription factor binding site involved in the inflammatory response (STAT1). The interaction of STAT1 with part of the inflammatory signaling pathway, interferon-gamma, is impaired in 9p21 risk carriers. Congrains et al. genotyped 18 SNPs across the CVD-associated region and determined the impact of 9p21 variants on gene expression.[8] The authors reported that, “several SNPs in 9p21 locus affect the expression of ANRIL, which is further in control of the regulation of CDKN2A/B and cell growth. Cell proliferation mediates the progression of atherosclerosis and is also directly or indirectly involved in the pathogenesis of diseases associated with this locus.”

## Availability

The Berkeley HeartLab offers the 9p21 Genotype Test, which detects the rs10757278 A>G and rs1333049 G>C SNPs within the 9p21 locus of chromosome. The information on the website (available online at: http://www.bhlinc.com/clinicians/test-descriptions/9p21) indicates that the SNPs have been shown to predict increased risk for early onset MI, for abdominal aortic aneurysm, and for myocardial infarction / coronary heart disease in general. It is suggested that the test may help identify patients at increased risk for these conditions, alerting providers to characterize and reduce other contributing risk factors.

Cardiac risk genotyping panels offered by other laboratories may include and individually report 9p21 SNP results. For example, the deCODE MI™ (deCODE Genetics) test genotypes 9p21.3 rs10757278 in addition to 7 other SNPs from other chromosomal loci to estimate the risk of coronary heart disease and MI.

**Regulatory Status**

There is no manufactured test kit for 9p21 genotyping that has been reviewed by the U.S. Food and Drug Administration (FDA). Clinical laboratories may develop and validate tests in-house for 9p21 ("home-brew") and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

**MEDICAL POLICY CRITERIA**

The use of genotyping for 9p21 single nucleotide polymorphisms is considered **investigational** for all indications, including but not limited to identification of:

A. Patients who may be at increased risk of cardiovascular disease or its manifestations (e.g., MI, ischemic stroke, peripheral arterial disease, coronary artery calcification), or
B. Patients who may be at increased risk for aneurysmal disease (abdominal aortic aneurysms, intracranial aneurysms, polypoidal choroidal vasculopathy).

**SCIENTIFIC EVIDENCE**

Validation of the clinical use of any genetic test focuses on 3 main principles:

1. Analytic validity, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. Clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. Clinical utility, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of this review is on evidence from well-designed, studies related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

**Literature Appraisal**

<u>9p21 Polymorphisms and Coronary Heart Disease (CHD)</u>

A number of studies address analytical validity, clinical validity, and clinical utility as described below. Clinical utility is satisfied when the evidence shows that using a test to change medical management for at least some patients significantly improves outcomes. Most of the evidence regarding the clinical utility of 9p21 testing is related to its role in risk-stratifying patients for coronary heart disease; a smaller body of evidence exists for its utility in other conditions. Direct evidence for how patient management is changed with 9p21 testing is not addressed in the literature.

*Systematic Reviews/Meta-analyses*

- Palomaki et al. conducted the first formal systematic review of the 9p21 literature to estimate the strength of the association between established 9p21 SNP variants and coronary heart disease and to examine clinical utility.[9] Authors reviewed the published literature for effect size, heterogeneity, publication bias, strength of evidence, and evidence of clinical utility of the test. Authors analyzed 47 data sets from 22 articles were analyzed including 35, 872 cases and 95, 837 controls. In summary, the authors concluded the association between 9p21 SNPs and heart disease that varied by age at disease onset was statistically significant; however, the magnitude of the association was small.

- In a systematic review and meta-analysis, Patel and others compared the association between variants at the chromosome 9p21 locus (Ch9p21) and risk of first versus subsequent coronary heart disease (CHD) events.[10] The authors identified 31 cohorts reporting on 193,372 individuals. Among the 16 cohorts of individuals without prior CHD (n=168,209), there were 15,664 first CHD events. Ch9p21 was associated with a pooled hazards ratio (HR) of a first event of 1.19 (95% CI: 1.17, 1.22) per risk allele. In individuals with established CHD (n=25,163) there were 4,436 subsequent events providing >99% and 91% power to detect a per-allele HR of 1.19 or 1.10, respectively. The pooled HR for subsequent events was 1.01 (0.97, 1.06) per risk allele. There was strong evidence of heterogeneity between the effect estimates for first and subsequent events (P-value for heterogeneity= $5.6x10^{-11}$). The authors concluded that 9p21 shows differential association with risk of first versus subsequent CHD events.

- Schunkert et al. conducted a meta-analysis of 14 genome-wide association studies of coronary artery disease (CAD).[11] Authors concluded that their large-scale meta-analysis identified the association of CAD with 13 novel chromosomal loci. Authors suggested these newly identified loci affect CAD risk carriers and may improve treatment of this common disease.

- The Coronary Artery Disease Genetics Consortium meta-analyzed 4 large genome-wide association studies of CAD and identified 5 loci newly associated with CAD.[12] Authors suggested their findings may implicate new pathways for CAD susceptibility. These results compare well with Palomaki et al.[9]

- In 2012, Zhou et al. conducted a meta-analysis of 7 case-control studies (n=7123 total). Authors suggest the genetic variation on the 9p21 chromosome may contribute to early-onset CAD however the effect size was small.[13]

- In a meta-analysis of 21 studies that included patients with information on CAD, MI status and 9p21 genotype (n=33,673), Chan et al. also found associations with CAD and the 9p21 locus.[14]Authors suggest that the 9p21 has a stronger association with CAD compared to MI.

- In a meta-analysis of 21 case-control studies evaluating the association between 9p21 SNPs and CHD in an East Asian population, including 25,945 cases and 31,777 controls, Dong et al. found a significant association between the allele rs1333049 and CHD (OR 1.30, 95% CI 1.25-1.35, P<0.001).[15]

- Palomaki et al. addressed clinical utility with a reclassification analysis, evaluating whether or not genotyping helped reclassify individuals more accurately than traditional risk factors according to

their known outcomes, which was measured by calculating the net reclassification index (NRI) with data from 3 studies/4 data sets.[9] None of the NRIs were statistically significant. In addition, the study showing the largest NRI achieved most of the risk reclassification because of reduced risk in individuals without events, which would have less chance of improving outcomes. Moreover, in 2 individual studies the NRI actually worsened when 9p21 risk alleles were added to algorithms that also included family history as a CAD risk factor.[16,17] Therefore based on this meta-analysis, evidence for clinical utility of 9p21 testing is insufficient.

*Nonrandomized Studies*

Several studies analyzing individual patient cohorts or case-control populations for association of 9p21 and CHD/CAD have been published since the Palomaki et al. review.[5,18-29] Most results again compare well with Palomaki et al.

Evidence for the clinical utility of 9p21 mutation testing is not addressed in the literature. Risk assessment may influence patient and provider decisions about preventive interventions and behavioral change. However, as Palomaki et al.[9] noted, only 37% of U.S. physicians reported regular use of a heart disease risk score,[30] and the evidence that such risk scores translate into net clinical benefits is minimal.[31] Thus, the clinical utility of 9p21 genotyping cannot be assumed even if risk assessment is improved. As noted, the evidence related to the clinical utility of 9p21 testing is related to its role in risk-stratifying patients for coronary heart disease; a smaller body of evidence exists for its utility in other conditions.[16,17,22,25,26,32,33]

*Conclusions*

The clinical utility, or how patient management changes, as a result of 9p21 mutation testing has not been established. The contribution of 9p21 to overall cardiovascular risk, above that of traditional risk factors, is small and not likely to be clinically important. Studies of risk reclassification do not report that 9p21 testing results in substantial numbers of patients being reclassified to clinically relevant categories.

<u>9p21 Association with Ischemic Stroke</u>

Several analytical and clinical validity studies have reported, with mixed results, on the association of 9p21 with ischemic stroke, an outcome not included in the studies discussed in the prior text. There are no clinical utility studies which address how 9p21 test results are used to improved health outcomes in patients at risk for ischemic stroke.

*Meta-analyses*

- Anderson et al. conducted a meta-analysis of 8 studies, focusing on 2 9p21 SNPs, s1537378 and rs10757278.[34] Authors concluded that the variants on 9p21 were associated with ischemic stroke.

- In a meta-analysis by Traylor et al. of 15 studies that included 12,389 individuals with ischemic stroke and 62,004 controls, the 9p21 locus was only associated with large-vessel stroke.[35]

*Nonrandomized Studies*

- Olsson et al. published a case-control study of the association of 9p12 and ischemic stroke in

individuals aged younger than 70 years.[36] In this study, the low-risk allele of 9p21 SNP rs7857345 showed significant association with decreased risk of large vessel disease after adjusting for traditional risk factors.

- Dutta et al. studied CAD mortality at older ages (71-to 80-year olds) in association with 9p21 variants. [25] Authors reported a positive association with CAD mortality but no significant association with deaths due to stroke.[25]

- Chou et al. conducted a retrospective analysis of neuropathology and genotyping on 755 deceased participants from two longitudinal cohort studies on memory and aging.[37] The authors evaluated the association between macro- and microscopic infarcts on neuropathology and 74 SNPs associated with well-established ischemic stroke risk factors and those previously found to be associated with clinical stroke in GWAS. A 9p21 SNP at the CDKN2A/B locus (rs2383207) was significantly associated with the presence of macroscopic infarct on pathology (OR 1.26; 95% CI 1.02-1.55, P=0.0314).

- Dichgans et al. analyzed data from the CARDIOGRAM/C4D consortium study described above [11,38] in conjunction with data from the METASTROKE consortium[35] to evaluate whether CAD and ischemic stroke share genetic risk in respect to common genetic variants.[39] The authors found that the 9p21 locus was significantly associated with both CAD and the phenotype of large artery stroke (PLAS =3.85 x 10-6; Spearman's rho coefficient for large artery stroke/CAS = 0.85, P=2.9E$^{-35}$).

*Conclusions*

Studies on the clinical and analytical validity of 9p21 association with ischemic stroke reported mixed results. Further, the clinical utility of 9p21 mutation testing for ischemic stroke has not been established.

<u>9p21 Association with Aneurysm</u>

The 9p21 locus has been associated with risk of both intracranial and abdominal aortic aneurysms. There are no clinical utility studies on the association of 9p21 with aneurysm.

*Systematic Review/Meta-analysis*

In 2013, Alg et al. reported results from a systematic review and meta-analysis of all genetic association studies of sporadic intracranial aneurysm to identify genetic risk factors for intracranial aneurysm[40]. The authors included 66 cohort or case-control studies of intracranial aneurysms that examined a total of 41 SNPs, not limited to the 9p21 locus, in 29 genes. Among polymorphisms with the strongest associations with intracranial aneurysm were the 9p21 SNPs rs10757278 (odds ratio [OR] 1.29, 95% confidence interval [CI] 1.21-1.38) and rs1333040 (OR 1.24; 95% CI 1.20–1.29).

*Nonrandomized Studies*

There has been a greater focus on the association of 9p21 with abdominal aortic aneurysm (AAA). Several studies reported 9p21 allele-specific estimates of risk in the range of 1.2-1.8.[41-44] Biros et al. combined the results of their study with the results of previous studies and reported a combined estimate of about 1.3 for both 9p21 SNPs rs10757278 and rs1333049.[44] This estimated is lower than other well-characterized risk factor estimates for AAA such as age, family history, and smoking.[45]

*Conclusions*

The evidence that addresses the clinical validity of 9p21 for both intracranial and abdominal aortic aneurysms is limited. Further, the clinical utility of 9p21 mutation testing for aneurysm has not been established.

9p21 Association with Other Conditions

Analytical and clinical validity studies have been reported, with mixed results, on the association of 9p21 with other conditions. There are no clinical utility studies on the association of 9p21 with other conditions described below.

A few studies have explored the association of 9p21 variants with a variety of other conditions such as peripheral arterial disease,[46] coronary artery calcification,[47-49] aortic calcification,[48] polypoidal choroidal vasculopathy (characterized by aneurismal dilations at the border of the choroidal vascular network)[50], and arterial stiffness in hypertensive individuals.[51] The strength of the associations was modest and none suggested clinical use.

In contrast, Folsom et al. found no association between SNPs at the 9p21 locus with arterial elasticity and retinal microvascular diameter.[52]

Downing et al. evaluated the impact of adding 9p21 polymorphism (rs10757269) in a risk-factor-based model predicting peripheral artery disease.[53] Among 393 subjects in the prospective Genetic Determinants of Peripheral Artery Disease study who met study inclusion criteria, the rs10757269 allele was associated with the presence of peripheral artery disease (defined as ankle–brachial index < 0.9) after controlling for traditional cardiovascular risk factors and other biomarkers (OR 1.92; 95% CI, 1.29– 2.85). The addition of 9p21 genotype to a previously-validated peripheral artery disease risk model (including age, sex, race, smoking history, body mass index, hypertension stage, diabetes status, and history of cardiovascular disease, congestion heart failure, and CAD) led to improved risk classification (net reclassification index 33.5%, P=0.001).

*Conclusions*

Evidence that addresses the analytical and clinical validity, as well as the clinical utility of 9p21 association with other conditions, including but not limited to peripheral artery disease, coronary artery calcification, aortic calcification, polypoidal choroidal vasculopathy, and arterial stiffness is insufficient.

**Clinical Practice Guidelines**

EGAPP Working Group (EWG)

The EGAPP Working Group (EWG) published a recommendation on “genomic profiling to assess cardiovascular risk to improve cardiovascular health” which included a recommendation on 9p21 profiling alone based on Palomaki et al.[9] In general, the EWG found “… insufficient evidence to recommend testing for the 9p21 genetic variant or 57 other variants in 28 genes . . . to assess risk for cardiovascular disease (CVD) in the general population, specifically heart disease and stroke.” The EWG found that the magnitude of net health benefit from use of any of these tests alone or in combination is negligible. The EWG discouraged clinical use unless further evidence supports improved

clinical outcomes. Based on the available evidence, the overall certainty of net health benefit was deemed "Low."[54]

**Summary**

The association of 9p21 SNP alleles with coronary artery disease/coronary heart disease (CAD/CHD) outcomes (clinical validity) is well-established and consistent in multiple independent populations, with evidence demonstrating an increased severity of outcomes with increased 9p21 allele copies. The clinical validity for 9p21 and ischemic stroke or abdominal aortic aneurysm is less well-studied and less certain. Despite the clinical validity evidence for CAD/CHD outcomes, clinical utility, or how patient management changes as a result of 9p21 genotyping, has not been established. No studies have shown that 9p21 genotyping significantly improves risk reclassification after initial classification by traditional risk factors. Thus, 9p21 genotyping for all applications is considered investigational.

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## CROSS REFERENCES

Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

| CODES | NUMBER | DESCRIPTION |
|---|---|---|
| CPT | 81479 | Unlisted molecular pathology procedure |
| HCPCS | None | |