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 several 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 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 determine 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-EarlyMICheck™ 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™ 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

Clinical Validity

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. 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.

Several additional meta-analyses have been published on 9p21 genotyping. Schunkert et al. conducted a meta-analysis of 14 genome-wide association studies of coronary heart disease (CAD).\textsuperscript{[10]} 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.\textsuperscript{[11]} Authors suggested their findings may implicate new pathways for CAD susceptibility. These results compare well with Palomaki et al.\textsuperscript{[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 is small.\textsuperscript{[12]} 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.\textsuperscript{[13]} Authors suggest that the 9p21 has a stronger association with CAD compared to MI.

**Individual 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.\textsuperscript{[5,14-22]} Most results again compare well with Palomaki et al.

Scheffold et al.\textsuperscript{[5]} evaluated a population of male patients with acute MI compared to a comparable population of males without an event. Authors reported a slightly higher allele risk for several 9p21 SNPs. The estimates increased when the population was limited to those cases with a family history of MI. The authors concluded that the combination risk factor of family history plus 9p21 status was similar in value to those of traditional risk factors such as hypertension, diabetes mellitus, and current smoking.

Beckie et al. studied the allelic frequencies and haplotype structure of genetic variants on chromosome 9p21 in a cohort of black and white women with early-onset CHD.\textsuperscript{[19,20]} The authors reported interethnic diversity in the SNP risk alleles and the haplotype structure of chromosome 9p21 SNP variants, and suggested that different variants may influence CHD age of onset in whites and blacks. Shiffman et al. reported no association of the three SNP risk alleles and MI in African American men (N=228) and women (N=405) aged \( \geq \)65.\textsuperscript{[22]}

Wang et al. studied CAD in a Chinese Han cohort with and without type 2 diabetes (n=2387 patients).\textsuperscript{[23]} An adjusted (gender, hypertension, hyperlipidemia, smoking) analysis of the homozygous risk genotype for rs1333049 showed an increased risk of early-onset and severe CAD among diabetic patients.

Lee et al. identified novel susceptibility variants for CAD through genome-wide association studies (GWAS) in the Korean and Japanese populations and confirmed those previously identified in European populations.\textsuperscript{[24]} In the study, 2123 cases and 3591 controls were genotyped with 521,786 SNPs in the Korean population. In the replication, direct genotyping was performed using 3052 cases and 4976 controls from the KitaNagoya Genome study of Japan with 14 selected SNPs. CAD association was replicated for three GWAS-identified in Koreans. Authors suggested this study shows that three CAD
susceptibility loci, which were previously identified in the European populations, can be directly replicated in Koreans and also provided additional evidence implicating suggestive loci as risk variants for CAD in East Asian.

9p21 Allele Dosage and Disease Severity, Progression

Dandona et al. reported that among younger patients, 3-vessel disease has strong association with 9p21 gene dose.\(^{[16]}\) In the same study, one-vessel disease demonstrated a strong inverse association with increasing gene dosage. Patel et al. also reported greater 9p21 risk allele frequency with increasing angiographically-defined CAD severity.\(^{[17]}\) In a case-control study with a 10-year follow-up of cases (N=1,508), Ardissino et al. reported that the CAD risk allele rs1333040 was significantly associated with coronary atherosclerosis progression and the probability of coronary artery revascularization during long-term follow-up.\(^{[25]}\) There was no significant association with cardiovascular death or the recurrence of MI.

9p21 Association with Ischemic Stroke

Several 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. Anderson et al. conducted a meta-analysis of 8 studies, focusing on 2 9p21 SNPs, s1537378 and rs10757278.\(^{[26]}\) 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.\(^{[27]}\) Olsson et al. published a case-control study of the association of 9p12 and ischemic stroke in individuals aged younger than 70 years.\(^{[28]}\) 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.\(^{[21]}\) Authors reported a positive association with CAD mortality but no significant association with deaths due to stroke.\(^{[21]}\)

9p21 Association with Aneurysm

The 9p21 locus has been associated, along with 4 other genetic markers, with risk for intracranial aneurysm. However, these risk factors explain only up to 5% of the familial risk, reducing enthusiasm for genetic testing for this outcome at this time. 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.\(^{[29-32]}\) 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.\(^{[32]}\) This estimated is lower than other well-characterized risk factor estimates for AAA such as age, family history, and smoking.\(^{[33]}\)

9p21 Association with Other Conditions

A few studies have explored the association of 9p21 variants with a variety of other conditions such as peripheral arterial disease,\(^{[34]}\) coronary artery calcification,\(^{[35-37]}\) and polypoidal choroidal vasculopathy (characterized by aneurismal dilations at the border of the choroidal vascular network).\(^{[38]}\) While all studies reported positive associations, the strength of the associations was modest and none suggested clinical use.

Conclusions
The clinical validity of 9p21 with CHD/CAD outcomes is well-established and consistent in multiple independent populations, with evidence of increasing severity of outcomes with increasing risk allele dosage. The magnitude of increased risk is modest, with odds ratios for cardiovascular disease generally in the 1-2 range. The clinical validity for 9p21 and ischemic stroke or AAA is less well-studied and less certain.

Clinical Utility

Clinical utility is satisfied when the evidence shows that using a test to change medical management for at least some patients significantly improves outcomes. Palomaki et al. addressed clinical utility with a reclassification analysis, asking 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.\cite{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.\cite{39,40} Therefore based on this meta-analysis, evidence for clinical utility of 9p21 testing is insufficient.

Dutta et al. also conducted a reclassification analysis, evaluating risk first with Framingham score, then with 9p21 SNP-determined risk added to the Framingham score.\cite{21} In their cohort of community-dwelling elderly people followed for 20 years after DNA collection (N=1,095), SNP risk predictors identified an additional 6% (N=5) of the 81 CAD deaths within 10 years in the high-risk group, compared to the 21% (N=17) identified by Framingham score. However, a net reclassification index was not reported for a full evaluation of the results. In a similar analysis, Shiffman et al. found that adding a 9p21 SNP risk variant to the Framingham score did not improve the area under the curve and that the net number of individuals that were reclassified to more appropriate risk categories was 25 or fewer out of 3,651 whites.\cite{22} Adding C-reactive protein and KIF6 resulted in a larger number of correctly reclassified white men (N=93), but did not improve risk prediction for white women.

Studies have also used the odds ratio (OR) associated with an individual’s 9p21 genotype to modify a risk assessment based on traditional risk factors. For example, based on the results of Palomaki et al.,\cite{9} an individual with a 10-year CHD risk of 10% based on traditional risk factors who has two 9p21 at-risk alleles would have their risk estimate increased to about 14% compared to an individual with no at-risk alleles. Davies et al.,\cite{18} however, found that the addition of 9p21 to traditional risk factors was not significant as measured by area under the curve. Other similar attempts to add 9p21 alone as a risk factor have not demonstrated significance in addition to traditional risk factors.\cite{39-41} An improved risk calculation, if shown, would be an intermediate outcome. The expectation is that improved risk assessment might influence patient and provider decisions about preventive interventions and behavioral change. However, as Palomaki et al.\cite{9} noted, only 37% of U.S. physicians reported regular use of a heart disease risk score,\cite{42} and the evidence that such risk scores translate into net clinical benefits is minimal.\cite{43} Thus, the clinical utility of 9p21 genotyping cannot be assumed even if risk assessment is improved.

Do et al.\cite{44} tested several 9p21 SNPs in 3,820 cases and 4,294 matched controls from the multiethnic INTERHEART study of risk factors for acute non-fatal MI, and also collected dietary information. As expected, the SNPs were significantly associated with MI. An analysis of interactions found no significant effect of physical activity or smoking, but a significant interaction with the prudent diet (i.e.,
Conclusions

The clinical utility 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.

Clinical Practice Guidelines

The EGAPP Working Group 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. 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 discourages clinical use unless further evidence supports improved clinical outcomes. Based on the available evidence, the overall certainty of net health benefit is deemed “Low.”

Summary

The association of 9p21 SNP alleles with CAD/CHD outcomes (clinical validity) is well-established and consistent in multiple independent populations, with evidence of increasing severity of outcomes with increasing risk allele dosage. 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 has not been established. No studies have shown that 9p21 genotyping significantly improves risk reclassification after initial classification by traditional risk factors, nor have studies shown that addition of 9p21 genotyping to traditional risk factors improves risk assessment. 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

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