

An Abbreviated ACCE¹ Review of *BMP2* Mutation Testing Among Individuals Diagnosed with Familial or Idiopathic Pulmonary Arterial Hypertension

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¹ Analytic validity, Clinical validity, Clinical utility, and Ethical, legal, and social implications

Introduction and Background

As part of a recent CDC-sponsored project (CCU119356-01), we developed a methodology (referred to as ACCE) specifically designed to facilitate the appropriate transition of genetic tests from investigational settings to clinical and public health practice. The broad objective aimed at achieving that goal is to develop and evaluate a model system to assess the availability, quality, and usefulness of existing data on DNA-based tests and testing algorithms. The ACCE process is designed to, and derives its name from, a) specify the disorder and clinical scenario, b) assess analytic validity, c) assess clinical validity, d) assess clinical utility, and e) examine ethical, social, and legal implications (ELSI).

This current project, also funded by the CDC, aims to briefly evaluate and summarize existing knowledge on testing for mutations in the bone morphogenetic protein receptor II (*BMPR2*) gene among individuals diagnosed with familial or idiopathic pulmonary arterial hypertension (PAH). The terms “familial” and “idiopathic” PAH used in this review are defined using the classification developed at the 3rd World Symposium on Pulmonary Arterial Hypertension (Venice, Italy, June 23-25, 2003). Both of these disorders are of unknown etiology (no known cause), unless a *BMPR2* mutation has been detected. Familial PAH refers to an affected individual (PAH patient) who has at least one other affected family member. Idiopathic PAH refers to an affected individual with no known affected family members. This latter group also includes an affected individual with an identified deleterious mutation in the *BMPR2* gene, but no known affected relatives. The term “mutation” used in this review is defined as a disease-causing sequence variation.

The objectives of this review are to 1) provide information to aid in developing clinical guidelines for *BMPR2* genetic testing, 2) provide information to be used in patient education materials, and 3) identify gaps in knowledge from which a research agenda can be developed. Much of the information contained in this review is also applicable to the population at risk for familial PAH (e.g. family members with two affected relatives). The American College of Chest Physicians has recommended that 1) genetic testing and counseling should be offered to relatives of patients with familial PAH and 2) that patients with idiopathic PAH should be advised about the availability of genetic testing and counseling for their relatives.¹ These two recommendations are based on expert opinion. There are few published data on *BMPR2* mutation testing in patients with either familial or idiopathic PAH; no published data on *BMPR2* mutation testing in the population at risk for PAH has been found. Wherever possible in this review, an attempt is made to apply the ACCE question to the population at risk, as well as those with diagnosed familial or idiopathic PAH.

EXECUTIVE SUMMARY

Disorder/Clinical Scenario

The main aim of this ACCE (Analytic validity, Clinical validity, Clinical utility, and Ethical, legal, and social implications) Review is to evaluate the efficacy of identifying *BMP2* mutations among all individuals with diagnosed familial or idiopathic PAH. Thus, the clinical scenario is comprised of all individuals with familial or idiopathic PAH and the disorder of interest is *BMP2*-related PAH. For every 100 cases of idiopathic PAH, there are approximately 7 familial cases. The genetic testing methodologies used to detect *BMP2* mutations are full DNA sequencing, targeted mutation analysis, Southern Blot, dye binding high resolution thermal denaturation (Hi-res Melting), and reverse transcriptase-PCR (RT-PCR).

Analytic Validity

A single laboratory, located at Columbia University, offers sequencing to detect *BMP2* mutations. This laboratory tested 16 samples with known point mutations and 10 control samples as part of the New York State certification process. The test was positive in all 16 (analytic sensitivity = 100% [95% CI 83 to 100%]). No mutations were detected in the 10 control samples (analytic specificity = 100% [95% CI 74 to 100%]).

Two laboratories (located at Columbia University and Vanderbilt University) offer targeted mutation analysis. This analysis is appropriate when a mutation has been identified in an affected family member. There has been no validation studies reported for targeted mutation analysis. RT-PCR is expected to be clinically available in October 2005 and analytic studies are underway. The laboratory at LDS Hospital has evaluated Hi-Res Melting. This laboratory tested 4 samples with known point mutations and 3 control samples. All four mutations were detected (analytic sensitivity = 100% [95% CI 40 to 100%]). No abnormalities were detected in the 3 control samples (analytic specificity = 100% [95% CI 29 to 100%]).

Because familial and idiopathic PAH are rare disorders and only two U.S. laboratories perform clinical testing, there is no external proficiency testing scheme for *BMP2* mutation testing. Both of these laboratories are CLIA-certified and the laboratory at Columbia University is also certified by the State of New York. There are plans for inter-laboratory sample exchanges to augment existing internal quality control procedures.

Clinical Validity

DNA sequencing identifies mutations in approximately 50 percent of all familial PAH cases and in slightly less than a quarter among idiopathic cases. Reverse transcriptase-PCR (RT-PCR) is a mutation detection methodology that is being used in a research setting and is likely to be available clinically in October 2005. This methodology detects *BMP2* mutations in approximately 90 percent of familial PAH cases; RT-PCR has not been used among idiopathic cases. The cost of each of these mutation detection methodologies is roughly equal. Another research methodology, Hi-Res Melting, has detected mutations in nearly 70 percent of familial PAH cases and in approximately

20 percent of idiopathic cases. This methodology is about one-tenth the cost of sequencing and RT-PCR.

The incidence of familial and idiopathic PAH is estimated to be one to two per million individuals per year, or 300 new cases per year, yielding 75 new cases of *BMPR2*-related PAH. The mutation prevalence is estimated to be between 1 per 10,000 and 1 per 100,000. These incidence and mutation prevalences are based on few data and should not be considered robust estimates.

The penetrance of *BMPR2* mutations is incomplete and varies among families, indicating possible gene/gene or gene/environment interactions. The average penetrance is approximately 20 percent. Environmental triggers for the development of PAH have been identified (HIV infection, adulterated rapeseed oil, and appetite suppressant drugs). A number of potential genetic modifiers are currently under investigation to determine their role in the development of familial and idiopathic PAH.

Clinical Utility

At present, treatment for individuals with familial or idiopathic PAH does not differ according to whether they are a *BMPR2* mutation carrier or not. Thus, results of the DNA test will not affect patient care. DNA test results may, however, have implications for family members. Familial and idiopathic PAH are rare diseases and clinical genetic testing has only recently become available. As such, there are few data on the utilization of this testing. Anecdotally, few family members of known mutation carriers have undergone genetic testing. The Pulmonary Hypertension Association maintains a web site (www.phassociation.org) that contains many resources for patients, patient families, and providers.

Ethical, Legal, and Social Implications

Cases of genetic discrimination have been documented, although none involved *BMPR2* mutation carriers. There is currently a bill in the legislative process that would prevent this type of discrimination by health insurers. However, there is no such protection from discrimination by other sources (e.g. life insurance, adoption, etc.). The Health Insurance Portability and Accountability Act protects the privacy of individually identifiable health information, including genetic information. Absent a national standard, many states have enacted legislation to specifically define how genetic information can be used.

In the case of familial and idiopathic PAH, there are currently no medical benefits to the patient by *BMPR2* mutation testing. There may be benefits and risks to other family members or future family members. Ongoing and future research may lead to medical benefits for mutation carriers. Because PAH is a disorder that also occurs in individuals less than 18 years of age, social and ethical issues about genetic testing in children and adolescents must be considered.

Informed consent is strongly recommended for patients considering genetic testing, and laboratories complying with New York State certification are required to seek evidence

of informed consent. Because familial and idiopathic PAH are rare disorders, clinical genetic testing is not likely to become widespread, thus minimizing any legal issues regarding patents, licensing, and proprietary testing. The two laboratories that offer genetic testing for *BMPR2* mutations are located at the two institutions that hold patents for the *BMPR2* gene (Columbia University and Vanderbilt University).

Gaps in Knowledge – Research Agenda

What proportion of variants of current unknown clinical significance are disease-causing mutations?

What are the analytic sensitivity and specificity of the RT-PCR methodology?

What proportion of idiopathic PAH cases have *BMPR2* mutations that are identified by the RT-PCR and exon counting methodology?

Are there racial and/or ethnic differences in the occurrence of familial and idiopathic PAH or the frequency of *BMPR2* mutations?

What is the role of genetic and environmental modifiers in the development of PAH?

What is the utilization of *BMPR2* mutation testing by patients and their family members?

There is a lack of existing educational materials written at an appropriate reading level for the average U.S. adult and that satisfy the Suitability Assessment of Materials (SAM) evaluation criteria.

Are there long-term monitoring schemes that can be established?

What guidelines should be developed for evaluating program performance of *BMPR2* mutation testing?

DISORDER/TEST/CLINICAL SCENARIO

1. What is the specific clinical disorder to be studied?

The focus of this review is a subset of familial and idiopathic pulmonary artery hypertension (PAH) cases caused by a mutation in the *BMPR2* gene. About 90% of the PAH cases that are diagnosed annually in the United States can be explained by some precipitating clinical circumstance. The remaining 300 cases can be grouped into two classifications: 1) familial and 2) idiopathic. It is within this former category that the majority of PAH cases caused by *BMPR2* mutations is believed to reside. Some patients with idiopathic PAH may actually be familial cases, since the penetrance of *BMPR2* mutations is incomplete (e.g. an affected individual with few family members). For every 100 cases of idiopathic PAH, there are approximately 7 familial cases.² Idiopathic PAH is most common in women between the ages of 21 and 40; however, it can affect anyone at any age.

2. What are the clinical findings defining this disorder?

BMPR2 mutation-related PAH is characterized by vascular obstruction and the variable presence of vasoconstriction leading to increased pulmonary vascular resistance and right-sided heart failure, shortness of breath on exertion, loss of energy, fatigue, dizziness, fainting, and chest pain. Physical findings include abnormal heart sounds, low blood pressure, diminished pulse pressure, peripheral edema, cool extremities, and cyanosis. The clinical findings have recently been reviewed.¹ They are indistinguishable from the larger group of idiopathic PAH cases.

3. What is the clinical setting in which the test is to be performed?

The present review examines the implications of offering *BMPR2* mutation testing to all individuals with a diagnosis of familial or idiopathic PAH. This testing scenario goes beyond that currently recommended by the American College of Chest Physicians Clinical Practice Guidelines, which recommend that genetic counseling and *BMPR2* mutation testing be offered only when at least one other family member is affected. This approach does not take into account that perhaps 11 to 40 percent of idiopathic PAH cases with a negative family history are also associated with *BMPR2* mutations³⁻⁵, although it is not known what proportion of these mutations may, in fact, be polymorphisms.

4. What DNA test(s) are associated with this disorder?

This review includes several methods of DNA testing (DNA sequencing, Southern blot, reverse transcriptase-PCR [RT-PCR], and melting curve analysis). These types of testing are performed in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory, as well as in non-CLIA certified laboratories. Both types of laboratories provide test results that are used for patient management. Congress passed the CLIA in 1988 establishing quality standards for all laboratory testing to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed. Ideally, all clinical testing should be performed in a CLIA certified laboratory. However, this may not be feasible for testing associated with rare disorders such as idi-

opathic or familial PAH due to the time and expense required to comply with CLIA regulations.

The *BMPR2* gene is located on chromosome 2q31-32 and was initially mapped using linkage analysis.^{6,7} The *BMPR2* gene is large (13 exons), and many deleterious mutations have been identified, few of them common to more than one family. Gene sequencing, the current choice for detecting point mutations, identifies only a proportion of the *BMPR2*-related cases of PAH. In addition, sequencing identifies variants of unknown clinical significance (many of which may be benign) in 10 to 15 percent of familial and idiopathic PAH cases tested (C.G. Elliott, University of Utah School of Medicine, Salt Lake City, UT, personal communication), leading to complicated interpretations. Most of the remaining mutations are detectable using a Southern blot technique. Currently, the most likely testing scheme is to perform gene sequencing, followed by a Southern blot if no deleterious mutation is identified. Southern blot analysis for *BMPR2* mutations is only available from a non-CLIA certified laboratory. The Southern blot detects large gene rearrangements but does not identify specific mutations. Another methodology used in a non-CLIA certified laboratory, RT-PCR, is being validated for use in a CLIA certified laboratory, with a target date for implementation in October 2005. The RT-PCR methodology sequences amplified mRNA products (cDNA) to detect large gene rearrangements (i.e. exon deletions, duplications), as well as the mutations that are detectable by conventional DNA sequencing and Southern blot, and most exon duplications and deletions. RT-PCR cannot detect exon 1 or exon 13 deletions. Hi-Res Melting is also available from a non-CLIA certified laboratory. This analysis identifies exons with a sequence variation. These exons are then sequenced to determine the specific mutation.

Gap in Knowledge: Variants of unknown clinical significance are sequence variations that may or may not be disease-causing. Because familial and idiopathic PAH are rare disorders and the penetrance of *BMPR2* mutations is usually low, it is difficult to identify which of these variants segregate with disease. What proportion of these variants of unknown clinical significance are disease-causing mutations?

Note: Targeted mutation analysis (cost between \$250 and \$350) can be used for testing other family members after a *BMPR2* mutation has been identified in an index case (cascade testing). The clinical scenario of this review does not extend to cascade testing, however.

Note: Other methodologies that are, or might be, used in a research setting for detecting *BMPR2* mutations are exon counting (detects rare exon deletions), quantitative PCR (not being used), and linkage analysis (does not identify specific mutations). Exon counting identifies complete exon deletions that cannot be identified by RT-PCR (exons 1 and 13).

5. Are preliminary screening questions employed?

No. In this review, all patients diagnosed with familial or idiopathic PAH are considered to be candidates for *BMPR2* mutation testing.

6. **Is it a stand-alone test or is it one of a series of tests?**
7. **If it is part of a series of tests, are all tests performed in all instances (parallel) or are some tests performed only on the basis of other results (series)?**

No current molecular testing technology will detect all deleterious *BMPR2* mutations. For clinical purposes, initial testing would involve sequencing, followed by Southern blot if a mutation were not identified. The RT-PCR methodology, when introduced for clinical purposes, is expected to detect most *BMPR2* mutations, and could replace the two-step protocol. Its performance in a clinical setting has not yet been demonstrated. Hi-Res Melting is a relatively inexpensive screening technique that can limit costs by identifying exons that show potential for containing a mutation for sequencing.

ANALYTIC VALIDITY

8. Is the test qualitative or quantitative?

Testing for *BMP2* mutations by current methodologies is qualitative and yields three categories of results:

- Positive for deleterious mutation
- Negative for deleterious mutation
- Genetic variant of uncertain clinical significance

9. How often is a test positive when a mutation is present (analytic sensitivity)?

10. How often is the test negative when a mutation is not present (analytic specificity)?

Sequencing - The single laboratory at Columbia University offering sequencing for *BMP2* mutations tested 16 samples with known point mutations and 10 control samples as part of the New York State certification process. The test was positive in all 16 (analytic sensitivity = 100% [95% CI 83 to 100%]). No mutations were detected in the 10 control samples (analytic specificity = 100% [95% CI 74 to 100%]). (M Mansukhani, Columbia University Medical Center, New York, NY, personal communication).

Southern Blot - There are no analytic validation data available for this methodology.

RT-PCR – Analytic validation studies will be performed prior to clinical implementation of this methodology for *BMP2* mutation testing.

Hi-Res Melting – The laboratory at LDS Hospital tested 4 samples with known point mutations and 3 control samples. All four mutations were detected (analytic sensitivity = 100% [95% CI 40 to 100%]). No abnormalities were detected in the 3 control samples (analytic specificity = 100% [95% CI 29 to 100%]).⁸ In other diseases, the reported sensitivity and specificity are 96 percent and 99 percent, respectively.⁹

Note: Analytic sensitivity is defined as the proportion of positive test results when a mutation that is detectable by the test is present (true positives divided by the sum of true positives and false negatives). False negative results can be due to technical errors in the analytic phase (e.g. contamination, expired reagents, or non-specific reactions) or to administrative or clerical errors in the pre-analytic or post-analytic phases. A laboratory that performs *BMP2* mutation testing for specified mutations would have 100% analytic sensitivity if it was able to detect one of those mutations every time that the mutation was present in a sample. Therefore, if a given laboratory claimed that it could test for only 50% of the mutations in the *BMP2* gene, but was able to identify one of those mutations every time it occurred, the analytic sensitivity would be 100%.

Note: Analytic specificity is defined as the proportion of negative test results when no detectable mutation is present (true negatives divided by the sum of the true negatives and false positives). False positive results can be due to technical errors in the analytic phase (e.g. contamination, misinterpretation of a polymorphism as a deleterious mutation, expired reagents, or non-specific reactions) or to administrative or clerical errors in

the pre-analytic or post-analytic phases. A laboratory that sequences the *BMPR2* gene would have 100% analytic specificity if it reported finding no mutation in this gene every time that no mutation was actually present.

Note: External proficiency testing is one good source of information to determine analytic sensitivity and specificity, but there currently is no external proficiency testing program for *BMPR2* mutations. Idiopathic PAH is a rare disorder, and only two laboratories in the United States provide clinical testing for *BMPR2* mutations (Columbia University, New York City, NY and Vanderbilt University, Nashville, TN).

Gap in Knowledge: What are the analytic sensitivity and specificity of the RT-PCR methodology?

11. Is an internal quality control program defined and externally monitored?

Both of the laboratories in the United States that offer *BMPR2* mutation testing report having an internal quality control/quality assessment program consisting of the blind insertion of samples of known abnormal and normal sequences. These data are usually not available for review outside the laboratory. In addition, these two laboratories are planning to exchange samples as part of an inter-laboratory comparison program. The expectation is that all laboratories offering *BMPR2* DNA testing for clinical purposes will be CLIA certified. Although this is a minimum qualification, it does ensure that the laboratory meets specifications for a high-complexity test and is externally reviewed. A more comprehensive review is required for New York State certification (although for rare tests, an exemption can be obtained). For sequence analysis, it is recommended by the Clinical and Laboratory Standards Institute (CLSI) that a quality score be determined for each sequence run (MM9-A). These scores could be used to evaluate quality control.

12. Have repeated measurements been made on specimens?

Repeated DNA measurements in multiple assay runs offer the opportunity for the laboratory to assess consistency of performance and the impact on testing of external factors (e.g., quality of the sample, exposure to high temperatures). Data are not available on repeated measurements from the two U.S. laboratories.

13. What is the within- and between-laboratory precision?

This question is not applicable to the use of DNA testing for *BMPR2*-related PAH, since such testing is qualitative. However, comparisons between/among quality scores in sequencing runs (Question 11) could provide a measure of precision.

14. If appropriate, how is confirmatory testing performed to resolve false positives in a timely manner?

When a *BMPR2* mutation is found, both laboratories in the United States routinely perform confirmatory analyses. This consists of sequencing the same PCR product in the reverse direction or performing a Southern blot to confirm deletions.

15. What range of patient specimens has been tested?

Whole blood is used to obtain DNA from patients for clinical testing. The Southern blot and RT-PCR methodologies cannot use archived or paraffin-embedded tissue samples.

16. How often does the test fail to give a useable result?

The failure rate for laboratory testing of satisfactory specimens is very low (less than 0.1%). While none have been documented by the two laboratories that offer clinical *BMPR2* mutation testing, poor sample quality (e.g. obvious contamination or hemolysis, exposure of sample to extreme temperature, or delay in transit) or insufficient sample quantity (for RT-PCR) can result in test failure. It is relatively common for a PCR reaction to fail. However, another DNA sample can easily be obtained from the original specimen.

17. How similar are results obtained in multiple laboratories using the same, or different, technology?

No data are available from laboratories that perform *BMPR2* mutation testing to make this comparison, but the expectation is that laboratory results will have a high degree of concordance. There are plans for an inter-laboratory exchange of samples to compare results. It is important for laboratories to report mutations using the same system of nomenclature. Recommendations have been made for standardizing human sequence variations, and these should be adhered to (www.hgvs.org).

CLINICAL VALIDITY

18. How often is the test positive when the disorder is present?

Familial PAH

Clinical sensitivity is defined as the proportion of individuals with a *BMPR2* mutation that can be identified among cases of familial PAH. Sequencing will identify approximately 50 percent of the mutations, and Southern blotting and exon counting collectively will identify another 16 percent, among familial cases. RT-PCR has been reported to identify approximately 90 percent of *BMPR2* mutations among familial cases, with exon counting identifying another 5 percent. Table 1 displays the estimated clinical sensitivity for mutation detection methodologies currently being utilized in both the research and clinical setting.

Table 1. *BMPR2* mutation detection methodologies used by laboratories in the United States along with the associated clinical sensitivity among individuals diagnosed with familial pulmonary arterial hypertension (PAH)

Detection Methodology	Clinical Sensitivity (95% confidence interval)
Sequencing	47 (37 – 59) ^{a,b}
RT-PCR ¹	78 (61 – 90) ^c
RT-PCR ¹	89 (74 – 97) ^a
Southern Blot	11 (3 - 26) ^c
Hi-Res Melting	68 (43 – 87) ^a
Exon counting	~ 5 ^a

^a unpublished data; ^b Machado *et al.*, 2001; ^c Cogan *et al.*, 2005

¹ Anticipated to be available in October 2005;

Note: One important consideration is that some small proportion of idiopathic PAH patients may have been misdiagnosed. For example, a cause for the pulmonary arterial hypertension may have gone undetected (e.g. thromboembolic disease, lung disease, exposure to appetite suppressants). This will result in an “artificial” elevation of the denominator for clinical sensitivity (total number of idiopathic PAH cases) and a corresponding underestimate of clinical sensitivity for each of the mutation detection methodologies.

Sequencing

A single published study has reported the proportion of *BMPR2* mutations detected among familial PAH patients.¹⁰ Among probands from 47 unrelated families, 23 (49%) were found to be mutation carriers. Cogan *et al.*¹¹ state that *BMPR2* mutations were found by full sequencing in 24 of 53 (45%) unrelated kindreds with familial PAH. However, these data have not been published. These two clinical sensitivity estimates have

been combined using the der Simonian and Laird methodology (random effects model) in Table 1.¹²

Reverse transcriptase PCR (RT-PCR) and Southern blot

In a study of 12 individuals with familial PAH who were previously found to be negative for *BMP2* mutations by DNA sequencing, 4 (33%) had large gene rearrangements that were identified by Southern blot.¹¹ Two of these four were further analyzed by RT-PCR to determine the effects of the altered patterns seen in the Southern blots. Ongoing studies have shown that eight of these 12 individuals (67%) have *BMP2* mutations that were identified using RT-PCR (J. Cogan, Vanderbilt University School of Medicine, Nashville, TN, personal communication).

The clinical sensitivity estimate given for Southern blot in Table 1 is 11 percent. This estimate is obtained by using 36 as the denominator (24 kindreds with mutations identified by sequencing + 12 kindreds with no mutations identified by sequencing) and 4 as the numerator. In Table 1, two clinical sensitivities are given for RT-PCR. The first is based on published data by Cogan *et al.*¹¹; this estimate of 78 percent is obtained by using 36 as the denominator and 28 as the numerator (24 mutations identified by sequencing and 4 identified by Southern blot, all of which are assumed to be identified by RT-PCR). The second estimate of 89 percent is based on unpublished data; the same denominator is used (36) and the numerator is 32 (24 mutations identified by sequencing and 8 mutations identified by RT-PCR). Studies of RT-PCR among idiopathic PAH cases are underway.

Research Assays

Hi-Res Melting identified *BMP2* mutations in 13 of 19 individuals with familial PAH. (CG Elliott, University of Utah School of Medicine, Salt Lake City, UT, personal communication). Thus, the clinical sensitivity is 68 percent.

Exon counting has identified two *BMP2* mutations in four samples from kindreds in which no mutations had been detected by either sequencing or RT-PCR (J. Cogan, Vanderbilt University School of Medicine, Nashville, TN, personal communication). The clinical sensitivity of exon counting is calculated as 2 out of 36 (5%), where the denominator includes all 36 samples that were studied.

Idiopathic PAH

Among idiopathic PAH cases, sequencing will identify the presence of a deleterious *BMP2* mutation in between 20 and 25 percent, and it is not known what remaining proportion will be detected by Southern blot, RT-PCR, and exon counting. These proportions are expected to be less than those found among familial cases. However, the actual number of cases with an identified mutation may be equal to or greater than mutation carriers among familial PAH cases.

Sequencing

A study of 50 unrelated individuals without a family history of PAH found that 13 (26%) carried a *BMP2* mutation.⁵ A similar study that was conducted in Germany found 11

BMPR2 mutation carriers (11%) among 99 individuals diagnosed with idiopathic PAH.³ These mutations were confirmed using denaturing high performance liquid chromatography (dHPLC). In contrast, a Japanese study of 30 patients with idiopathic PAH found 12 (40%) mutation carriers.⁴ When these three clinical sensitivity estimates are combined using the der Simonian and Laird methodology (random effects model)¹², the pooled estimate is 22 percent.

Research Assays

Hi-Res Melting has identified *BMPR2* mutations in 11 of 52 (21%) idiopathic PAH patients (CG Elliott, University of Utah School of Medicine, Salt Lake City, UT, personal communication).

Gap in Knowledge: What proportion of idiopathic PAH cases have *BMPR2* mutations that are identified by the RT-PCR and exon counting methodology?

19. How often is the test negative when the disorder is not present?

When *BMPR2* mutation testing is offered to all individuals diagnosed with familial or idiopathic PAH, the estimate of clinical specificity is primarily dependent on the test performance. In this highly selected population, it is unlikely that a *BMPR2* mutation might be identified coincidentally that is not actually causing the PAH. While the *BMPR2* mutation prevalence in the general population is not known, it has been estimated to be between 1 per 10,000 and 1 per 100,000 (0.001 and 0.01%).¹³ Given this mutation prevalence, a false positive based on such a coincidental mutation occurrence might occur only once in every 50 years or more.

20. Are there methods to resolve clinical false positive results in a timely manner?

Clinical false positive results can result when there is an analytic error (pre-analytic, analytic, or post-analytic – see Questions 9 and 10) or if a suspected deleterious mutation is actually a polymorphism that is linked to an undetectable deleterious mutation. Either of these occurrences should be extremely rare. Under the scenario of this review, no adverse clinical consequences will occur to the patient as a result of the mutation being reported, because all patients have been diagnosed with PAH. There are, however, implications to an unaffected relative. For example, if a polymorphism is considered to be a mutation in an affected individual and an unaffected relative is also found to carry this polymorphism, this unaffected relative is now, falsely, considered “at risk”.

21. What is the prevalence of the disorder in this setting?

The prevalence of *BMPR2*-related PAH among individuals with familial or idiopathic PAH is approximately 1 in 4. The incidence of familial and idiopathic PAH is estimated to be one to two per million individuals per year, or 300 new cases per year,¹⁴ yielding 75 new cases of *BMPR2*-related PAH. The prevalence of familial and idiopathic PAH in the general population has not been reported, but given the prognosis (Question 26), it must be at least several times higher. These prevalence and incidence estimates are based on very few data and should not be considered robust.

22. Has the test been adequately validated on all populations to which it may be offered?

Insufficient information is available to determine whether specific populations have different frequencies of familial or idiopathic PAH or its associated mutations. Based on other DNA tests, variations in mutation and polymorphism frequencies by race/ethnicity are possible. Mutation detection methodologies are designed to identify mutations in the *BMP2* gene, regardless of the characteristics of the individual being tested (e.g., race or ethnicity).

Gap in Knowledge: Are there racial and/or ethnic differences in the occurrence of familial or idiopathic PAH or the frequency of *BMP2* mutations?

23. What are the positive and negative predictive values?

In the present scenario, the positive predictive value (PPV) is the proportion of individuals with a positive test that has *BMP2*-related PAH. Given the nature of the population being tested, the PPV approaches 100%, assuming an analytic specificity of 100% (Question 10). Positive predictive value is not dependent on the mutation detection methodology.

Note: In the clinical scenario of counseling and testing relatives for targeted *BMP2* mutation testing, the PPV is essentially equivalent to the penetrance (earlier reported to be about 20%).

The negative predictive value (NPV) is the proportion of individuals with a negative test that does not have *BMP2*-related PAH. NPV varies depending on the mutation detection methodology and the proportion of misdiagnosed idiopathic PAH cases. Expert opinion is that between 5 and 8% of individuals with idiopathic PAH are misdiagnosed. Using the estimated annual incidence of familial and idiopathic PAH of 300 cases (Question 21), between 15 and 24 of these cases do not have idiopathic PAH. The association between *BMP2* mutations and PAH secondary to other known causes has not been well described. While the mutation prevalence in the general population is not known, it has been estimated to be between 0.001 and 0.01%.¹³ An example for calculating NPV is found in Appendix A. For sequencing, the NPV ranges between 9 and 14% if there are no mutation carriers among the misdiagnosed cases and ranges between 6 and 10% if there are two mutation carriers. These calculations assume that the clinical sensitivity for full DNA sequencing is 47% among familial PAH cases and 26% among idiopathic PAH cases. For RT-PCR, the NPV ranges between 10 and 15% if there are no mutation carriers among the misdiagnosed cases and ranges between 9 and 14% if there are two mutation carriers. These calculations assume that the clinical sensitivity for RT-PCR is 89% among familial PAH cases and 50% among idiopathic PAH cases. The latter clinical sensitivity is a best-guess estimate; there are no data that support this.

24. What are the genotype/phenotype relationships?

In the current scenario, all candidates for testing are diagnosed with familial or idiopathic PAH. Penetrance, therefore, is not an issue for those found to have *BMP2* mutations. It is unknown whether certain types of mutations (e.g. missense, rearrange-

ments) or mutations in specific regions of the gene are associated with distinct phenotypes.

Note: There is no evidence to suggest that the penetrance of *BMPR2* mutations differs between familial and idiopathic PAH. However, there may be yet undiscovered genotype/phenotype relationships that result in such differences. The penetrance of *BMPR2* mutations is variable among families^{15, 16} indicating possible gene/gene or gene/environment interactions. The average penetrance is approximately 20%.

25. What are the genetic, environmental or other modifiers?

The following is a partial list of genetic modifiers that may be involved in the development of familial and idiopathic PAH.¹⁶ The impact of these modifiers is unknown.

- a. nitric oxide synthases (1 and 3)
- b. vasoactive intestinal polypeptide
- c. urea cycle enzymes
- d. prostacyclin receptor
- e. beta-adrenergic receptors
- f. coagulation cascade polymorphisms
- g. immunogenetic background related to HLA and immune processing
- h. somatic mutations in *BMPR1*
- i. potassium channel disorders.

A number of potential environmental triggers for the development of familial and idiopathic PAH has been identified, including appetite suppressant drugs (e.g. dexfenfluramine and fenfluramine), HIV infection, and consumption of adulterated rapeseed oil.^{15, 17-19}

Gap in Knowledge: What is the role of genetic and environmental modifiers in the development of PAH?

CLINICAL UTILITY

26. What is the natural history of the disorder?

The natural history of familial and idiopathic pulmonary arterial hypertension (PAH) is partially described in Questions 1 and 2 and has been summarized by others.^{1, 20, 21} Prior to the advent of specific medical therapy, the mean survival after diagnosis was 2.8 years.² Since that study, which from a therapeutic standpoint only included medical therapy considered “conventional,” e.g. anticoagulation with warfarin, digitalis and diuretics, calcium channel blockers if clinically indicated, and supplemental oxygen, significant advancements have been made in the understanding of the pathobiology of idiopathic PAH, providing the rationale for the clinical development of various specific medical therapies targeting the PAH directly. These therapeutic agents and their timeline of development are as follows:

- chronic intravenous epoprostenol (prostacyclin analogue)- the first FDA approved drug for the treatment of PAH in 1995
- bosentan (endothelin receptor antagonist) - the first oral FDA approved drug in 2001
- continuous subcutaneous infusion treprostinil (prostacyclin analogue) – FDA approved in 2002
- continuous intravenous treprostinil – FDA approved in 2004
- iloprost administered by inhalation (prostacyclin analogue) - FDA approved in 2004
- sildenafil (oral phosphodiesterase inhibitor) – FDA approved in 2005

Additional therapeutic modalities are in clinical development. These include alternative oral endothelin receptor antagonists such as sitaxsentan and ambrisentan, as well as the alternative oral phosphodiesterase inhibitor tadalafil. The prostacyclin analogue treprostinil by inhalation is also being evaluated. In addition, due to scientific rationale for increased benefit by combining drugs from the three classes of medications, (i.e. prostacyclins, endothelin receptor antagonists, and phosphodiesterase inhibitors), combination therapy is also being studied.

Due to these therapeutic advancements, exercise capacity overall has increased approximately 30 to 50 meters (10-15% improvement), functional capacity has improved in approximately 20 to 40 percent of patients, there has been a variable reduction in time to clinical worsening, a small decrease in pulmonary artery pressure and a modest improvement in cardiac output with marginal improvements in quality of life, and an approximately 20 to 30 percent improvement in survival.²¹⁻²³ However, although the achievements to date are clinically significant, exercise capacity in approximately 50 percent of patients remains less than 400 to 450 meters, approximately 50 percent of patients remain in functional class III or IV, patients continue to have frequent hospitalizations for worsening PAH, and overall pulmonary hemodynamics remain at least moderately severe. Quality of life remains suboptimal and survival remains reduced. Experts have set targets for treatment of PAH patients to improve exercise capacity to within 10 percent of normal, improve functional class to class I, improve hemodynamics to mild PAH, normalize quality of life, indefinitely delay time to clinical worsening, and ultimately normalize survival. In attempts to achieve these goals, future strategies in-

clude combination therapy, transition therapy, early therapy and new pathophysiologic targets.

For PAH patients in whom medical therapy isn't effective, there are several interventional procedures available as additional treatment: atrial septostomy, single lung transplant, bilateral lung transplant, and heart-lung transplant. The efficacy of these interventions has been reviewed elsewhere.^{24, 25}

Atrial septostomy (AS) – There have been no randomized controlled trials that have evaluated the efficacy of this procedure. Most of the case series that have been reported included patients with severe disease. In most reports, syncope does not recur, and signs and symptoms of right heart failure improve following AS. Exercise capacity (6-minute walk) and survival also appear to improve after AS. Although this is a high risk procedure, it can be effective in patients with recurrent syncope and/or significant right heart failure despite maximal medical therapy. It can also be used as a palliative bridge to transplantation. Due to its high risk (e.g. procedural mortality), it has been recommended that AS be performed only at institutions with significant procedural and clinical experience.

Lung and Heart-Lung Transplantation – Selection of transplant recipients and the timing of this procedure are complex. A pre-transplant assessment is performed to confirm the familial or idiopathic PAH diagnosis, assess the severity of disease, and to optimize medical management. The prognosis of the disease compared to that of the transplant must be evaluated and a decision on the type of transplant must be made. Usually, a heart-lung transplant is not required for familial or idiopathic PAH patients, unless there is a significant cardiac problem. Both single and bilateral lung transplants have been successful in the treatment of PAH. There have been no randomized controlled studies to assess the efficacy of each transplant procedure. Studies that assessed post-operative hemodynamic data show that most patients had a significant reduction in mean pulmonary arterial pressure and pulmonary vascular resistance. There are no functional outcomes data available that are specific to patients with PAH. There appears to be similar survival for single lung, bilateral lung, and heart-lung transplants at all time points, up to 10 years (Table 2). It has been recommended that transplantation procedures be performed only at institutions with significant procedural and clinical experience.

Table 2. Survival among familial and idiopathic pulmonary arterial hypertension patients after lung and heart-lung transplants.²⁴

Survival	Single Lung	Bilateral Lung	Heart-Lung
1 year	65%	70%	70%
3 years	50%	55%	50%
5 years	40%	45%	40%
10 years	23%	20%	25%

Note: The natural history needs to take into account the adverse effects of drug administration and surgical procedures. Epoprostenol and Treprostinil require continuous infusion. Complications from indwelling catheters include clogging or dislodging of catheter, line-related infections, catheter associated venous thrombosis, thrombocytopenia, ascites, and pain and erythema at the infusion site with subcutaneous infusion. Abrupt interruption of epoprostenol infusion may cause death. Common side effects of the prostacyclin analogues include headache, flushing, jaw pain, diarrhea, nausea, rash, and musculoskeletal aches and pains. Abnormal hepatic function has occurred with use of the endothelin receptor antagonists. Health risks associated with surgical procedures include hemorrhagic complications, graft dysfunction, and graft rejection. Death while on the waiting list for transplant organs is a problem, particularly among patients with idiopathic PAH and poor New York Heart Association functional status.

27. What is the impact of a positive (or negative) test on patient care?

At present, treatment for individuals with familial or idiopathic PAH does not differ according to whether they are a *BMP2* mutation carrier or not. Thus, results of the DNA test will not affect patient care. DNA test results may, however, have implications for family members.

28. If applicable, are diagnostic tests available?

Under the clinical scenario for this review, the diagnostic test for familial and idiopathic PAH, right heart catheterization, has been performed prior to DNA testing. The diagnosis is confirmed by excluding other known causes of pulmonary hypertension.

29. Is there an effective remedy or acceptable action, or other measurable benefit?

At present, treatment for individuals with familial or idiopathic PAH does not differ according to whether they are a *BMP2* mutation carrier or not. Thus, results of the DNA test will not affect patient care.

30. Is there general access to that remedy or action?

The majority of the remedies discussed in Question 26 are expensive. In addition, both the disorder and its treatments require frequent physician visits, some of which may require lengthy travel to a facility with specialized procedural and/or clinical experience. Thus, there may be financial and/or logistical barriers for individuals with no or insufficient health insurance. Financial assistance for drug therapy is available from some of the individual pharmaceutical distributors. Other limiting factors include the time necessary for, and availability of, services related to the remedies. Even if services are available, individuals may not be able to take time away from work to attend. Similar barriers exist for the actual surgical procedures. Transplants are only performed in tertiary care hospitals and require recovery periods that necessitate time off from work, for patients and their family caregivers. Additional counseling and/or mental health care may be necessary following these surgeries. If affected individuals are employed, time away from work for provider visits and/or treatment could be substantial.

31. Is the test being offered to a socially vulnerable population?

In the present scenario, those being offered testing already have the clinical disorder. The population is, therefore, undergoing testing only to learn about a possible cause, and would not be considered socially vulnerable.

32. What quality assurance measures are in place?

For the laboratory component, see Questions 11 to 17. Regarding the counseling component, DNA testing generally involves an informed consent process and genetic counseling.

33. What are the results of pilot trials?

Because familial and idiopathic PAH are rare diseases and clinical genetic testing has only recently become available, there has been only one published pilot trial.²⁶ That study of 47 individuals showed that one-third was offered a genetic test; of these, 70 percent accepted. Of those who had not been offered testing, two-thirds said they probably or definitely would be tested if it were available. The most frequently cited reasons for avoiding testing were fears about impact on the family, ability to handle the results, and insurance. Anecdotally, few family members of known mutation carriers have undergone genetic testing.

Gap in Knowledge: What is the utilization of *BMP2* mutation testing by patients and their family members?

34. What health risks can be identified for follow-up testing and/or intervention?

There are no special health risks associated with identification of *BMP2* mutations in individuals with familial or idiopathic PAH; however, anxiety related to test results is often an issue for individuals undergoing genetic testing. There may be implications for family members.

35. What are the financial costs associated with testing?

The financial costs associated with genetic testing include the costs of the genetic test, genetic counseling, and the ancillary physician visit(s). For an individual with familial or idiopathic PAH who does not have an affected relative with a known mutation, full DNA sequencing, Reverse Transcriptase-PCR, or Hi-Res Melting can be offered. The costs for the former two tests are between \$700 and \$900. The Hi-Res Melting screening technique can be done prior to sequencing for a cost of less than \$100. If an individual has an affected family member with a known mutation, single site analysis can be performed at a cost of \$250 to \$350. Individuals who choose to undergo genetic testing are also recommended to have pre- and post-test genetic counseling. The cost for two 1-hour counseling sessions may be \$300 or more. The ancillary physician visit(s) cost depends on the level of the encounter.

36. What are the economic benefits associated with actions resulting from testing?

At present, there are no different actions to be taken as a result of genetic testing. This may change as a result of ongoing and future research.

37. What facilities/personnel are available or easily put in place?

There are two clinical genetic testing facilities (Columbia University and Vanderbilt University) for *BMPR2* mutations. Columbia University provides single site mutation testing when a mutation has been identified in a family and DNA sequencing. Vanderbilt University currently offers testing for 2 specific mutations, and soon will offer Reverse Transcriptase-PCR testing. The Utah Pulmonary Hypertension Genetics Project (at LDS Hospital) currently performs genetic testing only as part of a research program. The development of a clinical program is underway at LDS Hospital. This program will include screening for *BMPR2* mutations for individuals without a known mutation as well as confirmation of suspected mutations by gene sequencing.

The Pulmonary Hypertension Association (www.phassociation.org) maintains a medical resources page that includes information, by state, on physicians who treat familial and idiopathic PAH, and pulmonary hypertension clinics. These providers should have access to genetic counselors. Additional information on locating genetic counselors can be found at www.nsgc.org.

38. What educational materials have been developed and validated, and which of these are available?

Patient educational materials have been developed and made available by the clinical genetic testing programs (Columbia and Vanderbilt Universities). These materials have not yet been validated. They have been evaluated by an adult education consultant and have been found to require a literacy level greater than that of the average adult reading level in the United States. Other factors that were evaluated using the Suitability Assessment of Materials (SAM) criteria²⁷ and found to have some deficiencies include: whether the purpose of the material is readily understandable, whether the scope is limited to the purpose, whether there is a summary of the message at the end of the material, whether the material uses the active voice for the most part, whether common, explicit words are used, good use of graphics, layout and typography factors, and cultural appropriateness. The Pulmonary Hypertension Association is in the process of developing genetic counseling and DNA testing informational materials (www.phassociation.org).

Gap in Knowledge: There is a lack of existing educational materials written at an appropriate reading level for the average U.S. adult and that satisfy the Suitability Assessment of Materials (SAM) evaluation criteria.

39. Are there informed consent requirements?

The American Society of Clinical Oncology has developed a list of 11 elements of informed consent for cancer genetic testing.²⁸ It is recommended that all genetic testing, including *BMP2* mutation testing, utilize a comprehensive informed consent document. These elements are also applicable to genetic testing for PAH and include:

- information about the specific test being performed,
- implications of a positive and negative result
- the possibility that the test will not be informative
- options for risk estimation without genetic testing
- the risk of passing a mutation to children
- technical accuracy of the test
- fees involved in testing and counseling
- risks of psychological distress
- risks of insurer or employment discrimination
- confidentiality issues
- options and limitations of medical surveillance and screening following testing

For research studies, institutional review boards serve as patient advocates and try to ensure that patients' rights are respected through full disclosure and voluntary participation and insist on measures to insure confidentiality. Increasingly informed consent documents have also included statements about disposition of samples. Certificates of confidentiality can be obtained by investigators to provide a guarantee that records cannot be subpoenaed. However, these certificates have not been tested in court.

40. What methods exist for long term monitoring?

A patient registry had been established by the National Institutes of Health (NIH) to collect data on individuals with primary pulmonary hypertension from 32 centers. This registry was active from 1981 to 1985. The NIH is funding a Grant (through 2008) for a five-year study that includes establishing a registry of families with familial PAH at Vanderbilt University. The registry was initiated in 1994. The purpose of the registry is to find and enroll as many families with hereditary PAH as possible. The major goals of the study are to find the gene(s) that causes PAH, identify possible modifier genes and/or environmental influences on disease incidence and severity, understand the function of these genes in causing disease, work toward understanding the effects of clinical genetic counseling and testing, and be a resource of information for patients and physicians.

Gap in Knowledge: Are there long-term monitoring schemes that can be established?

41. What guidelines have been developed for evaluating program performance?

No guidelines have been located for evaluating program performance of *BMP2* mutation testing.

Gap in Knowledge: What guidelines should be developed for evaluating program performance of *BMP2* mutation testing?

ETHICAL, LEGAL AND SOCIAL IMPLICATIONS

42. What is known about stigmatization, discrimination, privacy / confidentiality and personal/family social issues?

The Genetic Information Nondiscrimination Act of 2005 was passed by the Senate in February 2005 and is supported by the President. This bill will need to complete the legislative process before it can be signed into law. This act prohibits a group health plan or health insurance issuer from adjusting premiums on the basis of genetic information or requesting or requiring an individual or a family member of such individual to undergo a genetic test. This act further prohibits employment discrimination on the basis of genetic information. There have been no known reported cases of discrimination against individuals with *BMP2* mutations. There are, however, documented cases of genetic discrimination against individuals with other mutations.²⁹ This bill does not address discrimination by life insurers against mutation carriers. While there have been no reported cases of life insurance discrimination, this remains a concern. Absent a national standard, many states have enacted legislation to specifically define how genetic information can be used.

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) (hhs.gov) required the Health and Human Services to develop standards for protecting the privacy of individually identifiable health information from inappropriate use and disclosure. The resulting Privacy Rule came into effect on April 14, 2003. Within the Privacy Rule, genetic information is treated as all other "Protected Health Information." The Privacy Rule does not preempt more stringent state law; therefore, there are many state laws that prevail over the Privacy Rule.

Genetic information not only affects the individual considering testing; family members are also affected. Individuals who are found to carry a mutation may experience a variety of emotions, including anxiety, guilt, shame, fear of what will happen, worry about other family members, and depression. An individual who has a negative genetic test may feel guilty about being "spared". These emotions may lead to a perception of being stigmatized. Additionally, some negative tests are not informative. For example, an individual who carries a mutation may receive a negative test result because the genetic test does not detect the type of mutation they have.

Because PAH is a disorder that also occurs in individuals less than 18 years of age, social and ethical issues about genetic testing in children and adolescents are a concern. This topic has been addressed in a joint policy statement from the American College of Medical Genetics and the American Society of Human Genetics.³⁰ One of the "points to consider" states that "*Timely medical benefit to the child should be the primary justification for genetic testing in children and adolescents. Under this condition, genetic testing is similar to other medical diagnostic evaluations. Medical benefits include preventive measures and therapies, as well as diagnostic information about symptomatic children. If the medical benefits are uncertain or will be deferred to a later time, this justification for testing is less compelling.*" In the case of idiopathic PAH, there are currently no

medical benefits to *BMP2* mutation testing. There may be benefits to other family members or future family members. In addition, ongoing and future research may lead to medical benefits for the patient.

43. Are there legal issues regarding consent, ownership of data and/or samples, patents, licensing, proprietary testing, obligation to disclose, or reporting requirements?

Informed consent is strongly recommended for patients considering genetic testing (Question 39), and laboratories certified under New York State are required to seek evidence of informed consent. Ownership of data and/or samples has not, as of yet, presented any cause for litigation. Patents have been obtained for a method of diagnosing pulmonary hypertension and the role of *PPH1* gene in pulmonary hypertension. Because familial and idiopathic PAH are rare disorders, clinical genetic testing is not likely to become widespread, thus minimizing any legal issues regarding patents, licensing, and proprietary testing. The two laboratories that offer genetic testing for *BMP2* mutations are located at the two institutions that hold the previously mentioned patents (Columbia University and Vanderbilt University). The Privacy Rule (Question 42) prohibits inappropriate disclosure of genetic information.

44. What safeguards have been described and are these safeguards in place and effective?

Current safeguards include the informed consent (Question 39), appropriate genetic counseling, internal quality control, and regulatory oversight (e.g. CLIA). The effectiveness of these safeguards has not been formally evaluated.

Appendix A.

Table 3 shows the framework for calculating the negative predictive value (NPV) of *BMPR2* mutation testing via DNA sequencing among individuals with diagnosed familial or idiopathic pulmonary arterial hypertension (PAH). The following assumptions are incorporated into this table:

- The annual incidence of familial and idiopathic PAH in the United States is 300 cases
- Five percent of all cases is misdiagnosed ($300 * 0.05 = 15$)
- One of the 15 misdiagnosed cases is a *BMPR2* mutation carrier
- The clinical sensitivity for full DNA sequencing among familial PAH cases is 47% and is 26% among idiopathic cases. ($300 * 0.06 = 18$ familial cases and $285 [300-15] - 18 = 267$ idiopathic cases)

The calculation for NPV is the number of individuals without the disease that has a negative test (14) divided by the total number of individuals with a negative test (222). Thus, the negative predictive value is 6.3 percent, using the values in Table 2.

Table 3. A two-by-two contingency table for deriving negative predictive values of *BMPR2* mutation testing among individuals with familial or idiopathic pulmonary arterial hypertension

<i>BMPR2</i> mutation	Idiopathic Pulmonary Arterial Hypertension		Totals
	Yes	No	
Yes	77	1	78
No	208	14	222
Totals	285	15	300

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