Building a Pulmonary Hypertension Genetics Program at Stanford: Challenges and Opportunities

Vinicio A. de Jesus Perez, MD
Assistant Professor of Medicine
Stanford University Medical Center
PH Grand Rounds 2017
Case #1

- 45 y/o male with history of progressive SOB and leg swelling.
- Family history of 3 deaths due to respiratory and heart failure. One brother has “similar issues”.
- Echo shows severe RV dilation and RVSP of >100mmHg. RHC confirms PAH.
- Diagnostic workup is non-revelatory.
- Patient has a daughter and is worried she could “catch PAH”.

1. Is this familial or sporadic PAH?
2. Should a genetic test be offered?
3. Does the presence of pathogenic variants in candidate genes influence clinical course and prognosis of PAH?
4. Does knowing the carrier status make a difference in management of PAH?
Case #2

- 35 y/o female with history of progressive SOB and leg swelling.
- Patient is adopted and does not know her family history.
- Echo shows severe RV dilation and RVSP of >100mmHg. RHC confirms PAH.
- Diagnostic workup is non-revelatory.
- Patient has a daughter and is worried she could “catch PAH”.

1. Is this familial or sporadic PAH?
2. Should a genetic test be offered?
3. Does the presence of pathogenic variants in candidate genes influence clinical course and prognosis of PAH?
4. Does knowing the carrier status make a difference in management of PAH?
Updated Classification of Pulmonary Hypertension (2015)

1. Pulmonary arterial hypertension
   1.1 Idiopathic
   1.2 Heritable
      1.2.1 BMPR2 mutation
      1.2.2 Other mutations
   1.3 Drugs and toxins induced
   1.4 Associated with:
      1.4.1 Connective tissue disease
      1.4.2 Human immunodeficiency virus (HIV) infection
      1.4.3 Portal hypertension
      1.4.4 Congenital heart disease (Table 6)
      1.4.5 Schistosomiasis

1'. Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis
   1'.1 Idiopathic
   1'.2 Heritable
      1'.2.1 EIF2AK4 mutation
      1'.2.2 Other mutations
   1'.3 Drugs, toxins and radiation induced
   1'.4 Associated with:
      1'.4.1 Connective tissue disease
      1'.4.2 HIV infection

1''. Persistent pulmonary hypertension of the newborn

2. Pulmonary hypertension due to left heart disease
   2.1 Left ventricular systolic dysfunction
   2.2 Left ventricular diastolic dysfunction
   2.3 Valvular disease
   2.4 Congenital / acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
   2.5 Congenital / acquired pulmonary veins stenosis

3. Pulmonary hypertension due to lung diseases and/or hypoxia
   3.1 Chronic obstructive pulmonary disease
   3.2 Interstitial lung disease
   3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern
   3.4 Sleep-disordered breathing
   3.5 Alveolar hypoventilation disorders
   3.6 Chronic exposure to high altitude
   3.7 Developmental lung diseases (Web Table III)

4. Chronic thromboembolic pulmonary hypertension and other pulmonary artery obstructions
   4.1 Chronic thromboembolic pulmonary hypertension
   4.2 Other pulmonary artery obstructions
      4.2.1 Angiosarcoma
      4.2.2 Other intravascular tumors
      4.2.3 Arteritis
      4.2.4 Congenital pulmonary arteries stenoses
      4.2.5 Parasites (hydatidosis)

5. Pulmonary hypertension with unclear and/or multifactorial mechanisms
   5.1 Haematological disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy
   5.2 Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis, neurofibromatosis
   5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
   5.4 Others: pulmonary tumoral thrombotic microangiopathy, fibrosing mediastinms, chronic renal failure (with/without dialysis), segmental pulmonary hypertension

BMPR2 = bone morphogenetic protein receptor, type 2; EIF2AK4 = eukaryotic translation initiation factor 2 alpha kinase 4; HIV = human immunodeficiency virus.

Galie N et al. EHJ 2016
5th World Symposium on Pulmonary Hypertension, Nice
Knowledge of PAH Pathogenesis has Grown Over the Past Two Decades

Gene Mutations

- BMPR2
- ALK-1
- SMAD9
- Caveolin-1
- KCNK3
- EIF2AK4
- TBX4
- Endoglin

Environment

- Drugs and toxins
- Hypoxia
- Viruses

Fibrosis and Matrix

- Fibroblast
- Proliferation
- Collagen Production
- Elastase

Epigenetics

- miRNAs
- DNA methylation

Angiogenesis

- Small vessel loss
- Impaired angiogenesis

Altered Metabolism

- Warburg Effect
- ER stress
- Channelopathies
- Altered Estrogen Metabolism
- Autophagy
- Increased HIF-1α
- Unfolded Protein Response

Inflammation

- Reduced Tregs
- Increased macrophage
- B Lymphocytes
- NK cells
- Tertiary Lymphoid Follicles
- Mast Cells
- Dendritic Cells
- Neutrophils
- Autoantibodies
- Cytokines

De Jesus Perez, Heart Failure Rev, 2016
Abbreviated Pedigree of a Large Kindred Comprising Five Subfamilies over Seven Generations

BMPR2 Mutations are Associated with Most Cases of Familial PAH

• Autosomal Dominant transmission
  – ‘Reduced penetrance’

• BMPR2 gene
  – Chromosome 2q
  – Germline mutation: one WT; one mutated allele
  – Mutated in >75% known families w/ PAH
  – Variable severity within and across families
• USA - VU ~ 150 families 62 BMPR2+
• USA - Columbia ~ similar to VU cohort
• France - 65 families / 44 BMPR2 (68%)
• UK - 95 families or mutation positive
• Germany - 22 families (85% BMPR2)
• Italy - 18 families/12 BMPR2 (67%)
• China – Beijing and Shanghai

Information courtesy of Drs. Eric Austin, Marc Humbert and Greg Eliot; circa 2012
Clinical Outcomes of Pulmonary Arterial Hypertension in Carriers of BMPR2 Mutation

Benjamin Sztrymf, Florence Coulet, Barbara Girerd, Azzedine Yaici, Xavier Jais, Olivier Sitbon, David Montani, Rogério Souza, Gerald Simonneau, Florent Soubrier, and Marc Humbert

1Université Paris-Sud 11, UPRES EA 2705, Centre National de Référence de l’Hypertension Artérielle Pulmonaire, Service de Pneumologie et Réanimation Respiratoire, Institut Paris-Sud Cytokines, Hôpital Antoine-Bécère, Assistance Publique des Hôpitaux de Paris, Clamart, France; and 2Université Pierre et Marie Curie–Paris 6, Laboratoire d’Oncogénétique et Angiogénétique Moléculaire, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

(AJRCCM, 2008)
BMPr2 mutation carriers have less vasoreactivity and severe disease compared to noncarriers.
**BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis**

Transplant-Free Survival

![Graph showing transplant-free survival with and without BMPR2 mutation]

Survival

![Graph showing survival with and without BMPR2 mutation]

BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis

The main reason to test for the presence or absence of a BMPR2 mutation in a patient with PAH is to guide predictive genetic testing in unaffected relatives. Although our findings show that BMPR2 mutations are associated with worse survival, the usefulness of this result for prognostic purposes might be restricted in the clinic, since the majority of this risk appears to be accounted for by the known haemodynamic predictors of mortality measured during the diagnostic assessment during right heart catheterisation. Despite this, in younger patients, in which the increased risk appears to persist after adjustment for these factors, albeit only in subgroup analyses, screening for mutations might add value, and this warrants further investigation.
BMPR2 mutation are Not Limited to Familial PAH: Implications to Outcome and Therapeutic Response?

<table>
<thead>
<tr>
<th>Type of PAH</th>
<th>Reported Mutation Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial PAH</td>
<td>&gt; 75%</td>
</tr>
<tr>
<td>Idiopathic PAH</td>
<td>20%</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>6%</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>Not detected</td>
</tr>
<tr>
<td>HIV</td>
<td>Not detected</td>
</tr>
<tr>
<td>Hemolytic disease</td>
<td>Not reported</td>
</tr>
</tbody>
</table>
Not All BMPR2 Mutation Carriers Develop Pulmonary Arterial Hypertension

Penetrance of BMPR2 Mutations is ~20%
Hereditary Hemorrhagic Telangiectasia (HHT) and PAH

• Autosomal dominant vascular disease
  – Mucocutaneous telangiectasias
  – Multiorgan AVMs
  – PAH rare, may precede HHT dx

• ALK1: TGFβ type I receptor ($\leq 10\%$ PAH)
• Endoglin: TGFβ co-receptor ($< 1\%$ PAH)
• Smad 4: TGFβ signaling mediator ($< 1\%$ PAH)
• GDF2: TGFβ Ligand

Johnson *Nat Gen* 1994  Trembath *NEJM* 2001
Patients with Alk-1 Mutations Without HHT Have Worse Prognosis

- 1.8% of IPAH
- Shorter survival
- Vasodilator unresponsive
- Younger Dx. & Death
  - $< \text{BMPR2}$ mutation
- Less severe hemodynamics

Table:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Survival Time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMPR2/ACVRL1-</td>
<td>277 225 193 166 134 114 99 84 73 56 40 33 26</td>
</tr>
<tr>
<td>BMPR2+</td>
<td>91 82 70 63 50 44 37 30 25 23 18 13 9</td>
</tr>
<tr>
<td>ACVRL1+</td>
<td>9 5 4 4 3 1 1 0 0</td>
</tr>
</tbody>
</table>

Girerd AJRCCM 2010
# Beyond BMPR2: Recently Discovered Mutations

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Specialized Information</th>
<th>Cases</th>
</tr>
</thead>
</table>
| **Caveolin-1 (CAV1)** | • Caveolin-1  
• Caveolar structure  
• Caveolae:TGFβ rich  (Nickel AJRCCM 2015) | 1 Family & 1 IPAH |
| **KCNK3**       | • TASK-1  
• pH sensitive potassium channel | 3 Families & 3 IPAH |
| **EIF2AK4 (GCN2)** | • Eukaryotic Translation Initiation Factor Superfamily member  
• Association with recessive and sporadic forms of PVOD and PCH  
• PVOD ↔ PCH | |
| **TOPBP1**      | • DNA damage  
• Vascular cell stress | IPAH cases w/o BMPR2 |

Exact estimates of frequency among PAH patients TBD

References:
- Austin, Ma *Circ: Card Res*, 2012
- Ma, Roman-Campos *NEJM*, 2013
- Best *CHEST*, 2014
- Eyries *ERJ*, 2014
- de Jesus Perez *AJRCCM*, 2014
Gene Mutations are Present in Subsets of PAH Patients: Impact on Familial vs. Sporadic PAH

- **PAH patients without family history of PAH (n=423)**: 17% mutation carriers, 83% mutation noncarriers
  - BMPR2 mutation: 62
  - ACVRL1 (ALK1) mutation: 9
  - ENG mutation: 1
  - KCNK3 mutation: 0
  - EIF2AK4 mutations: 0
  - Total mutations: 72

- **PAH patients with family history of PAH (n=106)**: 93% mutation noncarriers
  - BMPR2 mutation: 89 (65 families)
  - ACVRL1 (ALK1) mutation: 3 (2 families)
  - ENG mutation: 0
  - KCNK3 mutation: 0
  - EIF2AK4 mutations: 0
  - Total mutations: 94

- **PVOD patients without family history of PVOD (n=81)**: 100% mutation noncarriers
  - BMPR2 mutation: 0
  - ACVRL1 (ALK1) mutation: –
  - ENG mutation: 0
  - KCNK3 mutation: –
  - EIF2AK4 mutations: 7
  - Total mutations: 7

- **PVOD patients with family history of PVOD (n=19)**: 100% mutation noncarriers
  - BMPR2 mutation: 0
  - ACVRL1 (ALK1) mutation: –
  - ENG mutation: 0
  - KCNK3 mutation: –
  - EIF2AK4 mutations: 19
  - Total mutations: 19

- **Relatives (n=272)**: 36% mutation carriers
  - BMPR2 mutation: 96
  - ACVRL1 (ALK1) mutation: 1
  - ENG mutation: –
  - KCNK3 mutation: 2
  - EIF2AK4 mutations: –
  - Total mutations: 99

Girerd B et al, ERJ 2016
Structure of a PH Genetic Program

```
PH Center
- Diagnostic tools
- Access to therapy
- Patient Registries
- Clinical Trials

Clinician/Geneticist
- MD expert in PH
- Diagnosis and Management
- Follow-up

Genetic Counselor
- Master in Science
- Explains Genetic testing
- Works with team
- Drafts pedigree
- Provides education and support
- Risk assessment and reduction
- Family planning
- Regular Follow-up.

Genetic Testing
- CLIA Certificate
- Full sequencing capability
- Communication with care team

Barbara Girerd
Nick Morrell
Greg Eliot
Eric Austin
```
OS-Seq™ Technology: The Stanford PH Gene Screen Strategy

DNA Source: Saliva or PBMC
Turnaround: 21 days

**SEQUENCING LIBRARY**

**RANDOM SHEARING**
Capture of genomic targets in situ
Flow cell
Fully integrated target enrichment and sequencing

**SEQUENCING LIBRARY**

**FLOW CELL:**

Sequencing on Illumina system

**Oligonucleotide library**

**SEQUENCING LIBRARY**

**TARGET ENRICHMENT & SEQUENCING**

Sequencing library

**Flow Cell:**
All 9 PAH genes

**Oligonucleotide library**

**PREPARATION**

**PROCESSING**

**SEQUENCING**

**Bioinformatic Analysis**

Final Report
OS-Seq Helps Identify Mutations Across the Entire Gene

55kb deletion deleting most of the **BMPR2** gene: 203325001 - 203380000
After Genetic Testing:
Surveillance and Treatment

Genetic Counseling for the PAH Patients and Family

- PAH cases: Consider PH-specific mutation testing

PAH Patient Test: Positive or Negative

- Positive → consider test family members
- Negative → clinical & ECHO q1-3 yrs

Family Members’ Care Based Upon Their Testing Results

- Positive → clinical & ECHO q1-3 yrs
- Negative → no specialized monitoring

Barbara Girerd
Eric Austin

DELPHI (NCT01600898)
Screening of Pulmonary Arterial Hypertension in BMPR2 Mutation Carriers (DELPHI-2)

This study is ongoing, but not recruiting participants.

Sponsor:
Assistance Publique - Hôpitaux de Paris

Information provided by (Responsible Party):
Assistance Publique - Hôpitaux de Paris

ClinicalTrials.gov Identifier:
NCT01600898
First received: May 15, 2012
Last updated: September 28, 2016
Last verified: September 2016

Purpose

In this prospective study, the investigators will implement a systematic screening program and 3-year follow-up in a cohort of asymptomatic BMPR2 mutation carriers. This study is designed to:

- determine predictive factors (biological, functional, radiological and hemodynamic) of development of PAH
- monitor these subjects’ clinical, functional, biological, echocardiographic and hemodynamic characteristics
- assess the risk of occurrence of PAH
- screen patients with PAH at an early stage of disease and offer them an early management
- constitute a collection of biological samples (0, 12, 24 months follow-up) of asymptomatic BMPR2 mutation carriers.

Eligibility

Ages Eligible for Study: 18 Years and older (Adult, Senior)
Sexes Eligible for Study: All
Accepts Healthy Volunteers: No
Sampling Method: Non-Probability Sample

Study Population
asymptomatic BMPR2 mutation carriers.

Criteria

Inclusion Criteria:
- men and women over 18 years old,
- carriers of a BMPR2 mutation without known PAH,
- having given his informed consent

Exclusion Criteria:
- men and women under 18 years old,
- patients with a known PAH,
- pregnant women,
- adults protected,
- detainees,
- people in emergencies,
- people refusing or unable to give informed consent,
- no affiliation to a regime of social security.
Genetic testing and counseling: Pros and Cons

**Pros**
- Assess heritability
- Reproductive issues
- Genetic modifiers
- Environmental modifiers
- Research Database
- Personalized therapy (?)
- Personalized prognosis

**Cons**
- No current clinical impact (?)
- ‘Guilt of heritability’
  - Psychological impact
- Genetic discrimination (?)
  - Insurability
  - Employment
- Cost (?)
  - $1000-2500 if mutation unknown
  - Provider time
Acknowledgements

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De Jesus Perez Group
Mark Orcholski
Ke Yuan
Elya Shamskhou
Ellen Hong
Nils Nickel
Monica Romero
Erin Stewart
Halley Tsai
Charlotte Rajasingh

PH Genetics Program
Vinicio de Jesus Perez
Roham T. Zamanian
Mark Krasnow
Mark Nicolls
Marlene Rabinovitch
Jeffrey Feinstein
Kyla Dunn
Michelle Ogawa
Allyson Rupp

Adult Clinic
Roham T. Zamanian
Edda Spiekerkoetter
Kristina Kudelko
Andrew Sweat
Yon K. Sung
Cyrus Kholdani
Andrew Hsi
Patricia Del Rosario
Allyson Rupp
Juliana Liu
Genomics
Mutations
Ethnicity
Ancestry

PAH Patient

Imaging
Cardiac MRI
TTE
Molecular Imaging

Proteomics
Cytokine Profile
Exosomes
Autoantibodies

Socialome
Community

Health Disparities

Microbiome
Airway flora
Gut Flora

Stem Cells
iPSCS
Gene editing
Drug Screening

PAH
Patient
Genetic Testing for PH-associated mutations

(BMPR2, ALK1, ENG, KCNK3, CAV1)

• No current Rx to correct mutations
• May inform severity of disease and risk of progression
  – may influence Rx choices
• Family value w/ regard to etiology of PH
• Informative for other family members
  – Siblings
  – Future pregnancy attempts
• Familial and idiopathic PAH forms
  – Genetic counseling critical
    • ‘guilt of heritability’
    • May not account for alternative genetic and non genetic modifiers
      of disease expression
Targeting BMPR2 Can Lead to Novel Therapies but is it going to Work for Everyone with Mutations?

What are we missing?
More genes?

Ataluren
Drake et al, AJRMB 2013

BMP-9

FK506
Spiekerkoetter et al, AJRCCM 2015
Spiekerkoetter, JCI 2013

Blueprint Genetics
Our diagnostics process

OS-Seq™
OS-Seq automates target enrichment, providing streamlined sample preparation and uniform sequencing result, hence driving down pricing

CN-Seq™

Bioinformatics
Fully automated bioinformatics requires no manual intervention. Includes high-sensitivity CNV, repeat, insertion, deletion and duplication analysis.

CLINT
IBM Watson – powered clinical interpretation automates majority of manual steps, enabling geneticists to focus on details of variant classification and clinical insight

Interpretation
Our interpretation process is focused to add maximal clinical value to the results report

Clinical report
The statement includes the genetic analysis completed with relevant findings and interpretation of the test results.

These technologies enable us to provide fast turnaround time, high diagnostic yield and most comprehensive test menu at an affordable price
PAH Severity is **Worse** in Patients with BMPR2 Mutations

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![Box Plot Showing Age at Diagnosis](image)

- **BMPR2 mutation non-carriers**
  - Females: n=188
  - Males: n=79
- **BMPR2 mutation carriers**
  - Females: n=81
  - Males: n=34

*p < 0.0001*

*NS*
BMPR2 Mutation Carriers with Higher Expression of the Healthy BMPR2 Allele are Protected from PAH

Updated from Hamid et al Hum Mutat 2009

P<.005

N=78  N=58
BMPR2 Protein is Reduced in Lungs of Patients with other forms of WHO Group 1 PAH