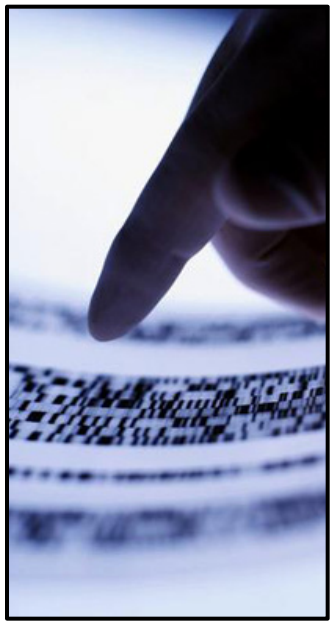
Building a Pulmonary Hypertension Genetics Program at Stanford: Challenges and Opportunities



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 thrombothic microangiopathy, fibrosing mediastinitis, 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

Altered Metabolism

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

Environment

Drugs and toxins
Hypoxia
Viruses

Fibrosis and Matrix

Fibroblast
Proliferation
Collagen Production
Elastase

Inflammation

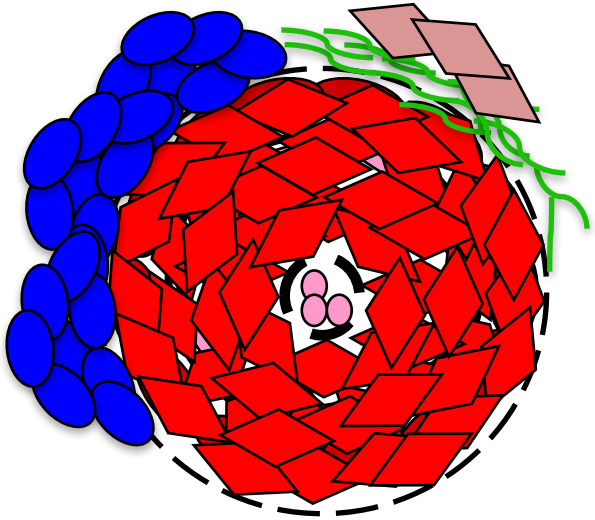
Reduced Tregs
Increased macrophage
B Lymphocytes
NK cells
Tertiary Lymphoid Follicles
Mast Cells
Dendritic Cells
Neutrophils
Autoantibodies
Cytokines

epigenetics

miRNAs
DNA methylation

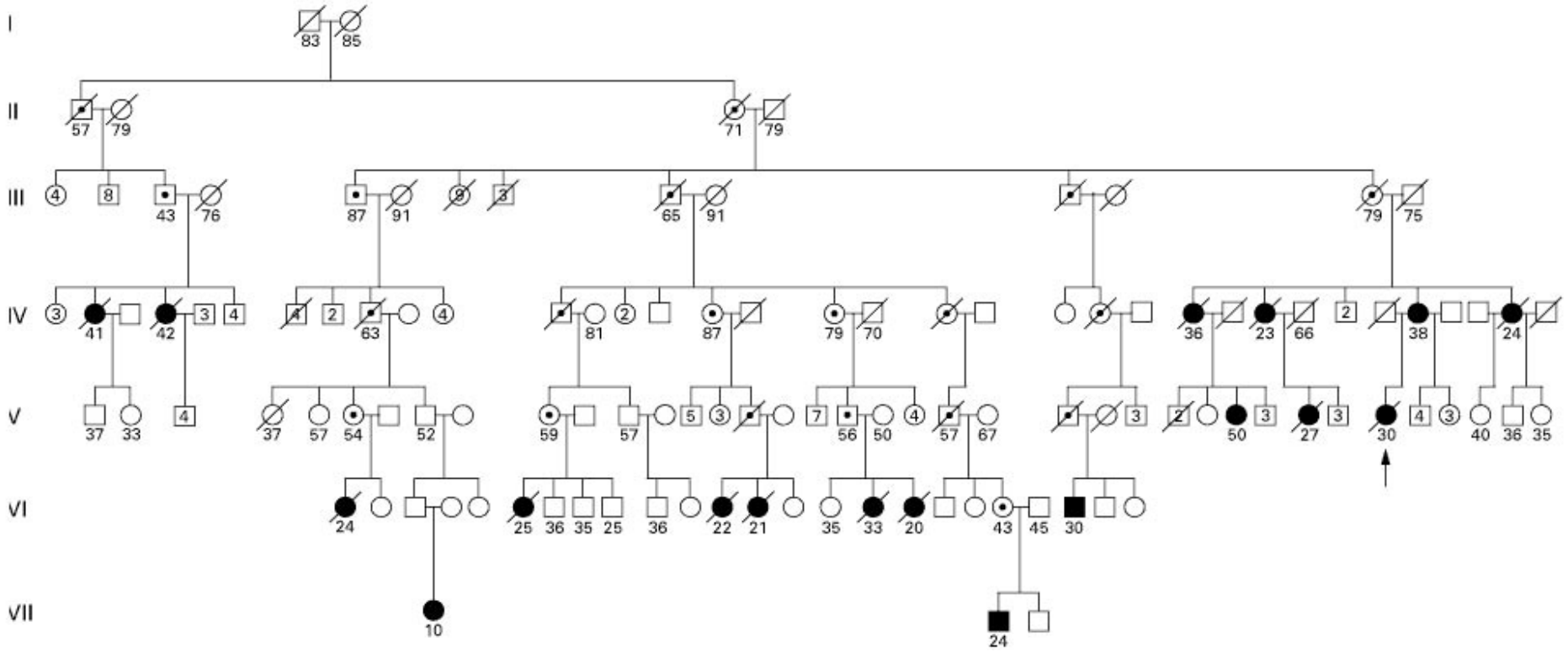
angiogenesis

Small vessel loss
Impaired angiogenesis



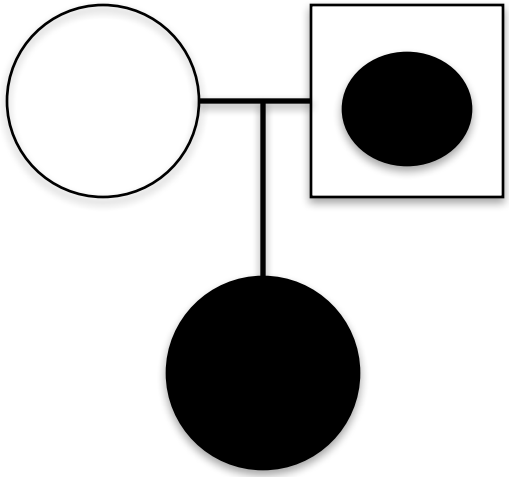
De Jesus Perez, Heart Failure Rev, 2016

Abbreviated Pedigree of a Large Kindred Comprising Five Subfamilies over Seven Generations



Newman JH et al. N Engl J Med 2001;345:319-324.

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

Larkin *AJRCCM* 2012

PAH families worldwide ~ 500

- 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

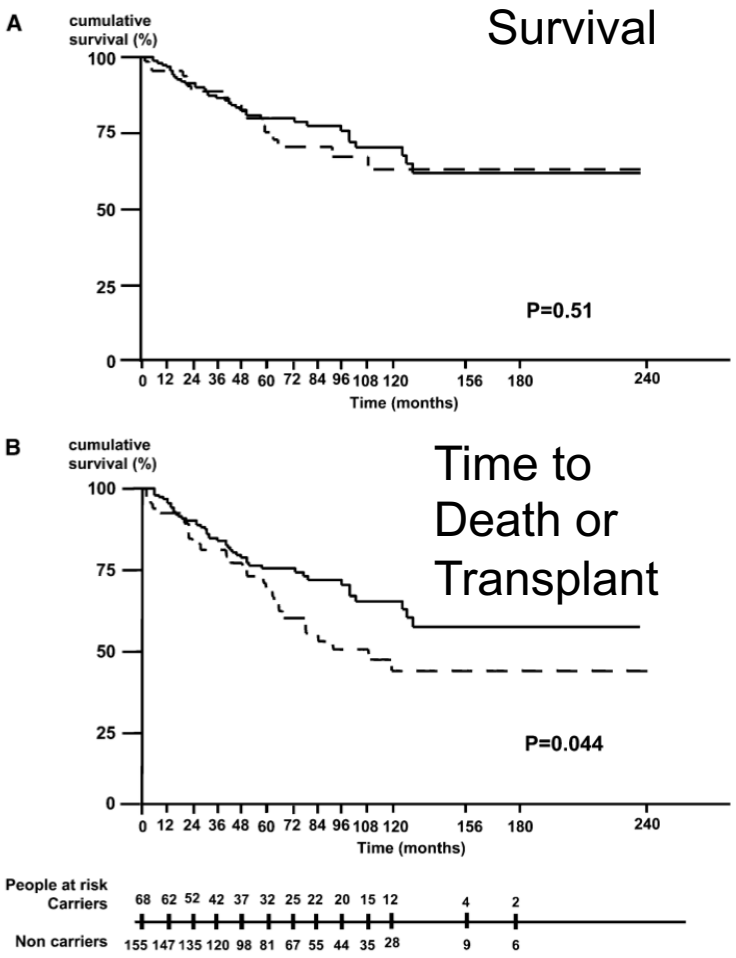
Information courtesy of Drs. Eric Austin, Marc Humbert and Greg Eliot; circa 2012

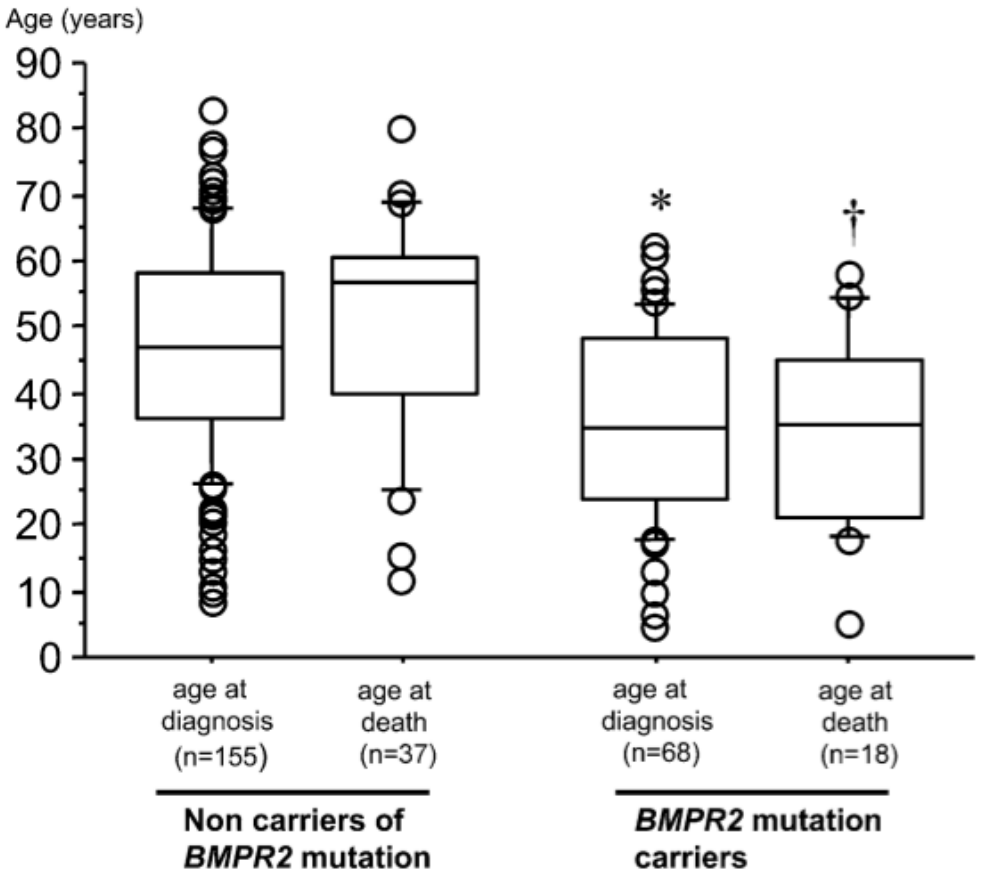
Clinical Outcomes of Pulmonary Arterial Hypertension in Carriers of *BMPR2* Mutation

Benjamin Sztrymf[1], Florence Coulet[2], Barbara Girerd[1], Azzedine Yaici[1], Xavier Jais[1], Olivier Sitbon[1], David Montani[1], Rogério Souza[1], Gerald Simonneau[1], Florent Soubrier[2], and Marc Humbert[1]

[1]Université 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-Béclère, Assistance Publique des Hôpitaux de Paris, Clamart, France; and [2]Université Pierre et Marie Curie–Paris 6, Laboratoire d'Oncogénétique et Angiogénétique Moléculaire, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

(AJRCCM, 2008)





Relationship of *BMPR2* Mutations to Vasoreactivity in Pulmonary Arterial Hypertension

C. Gregory Elliott, MD; Eric W. Glissmeyer, BS; Gregory T. Havlena, BS; John Carlquist, PhD; Jason T. McKinney, MS; Stuart Rich, MD; Michael D. McGoon, MD; Mary Beth Scholand, MD; Miryoung Kim, BS; Robert L. Jensen, PhD; Jon W. Schmidt; Kenneth Ward, MD

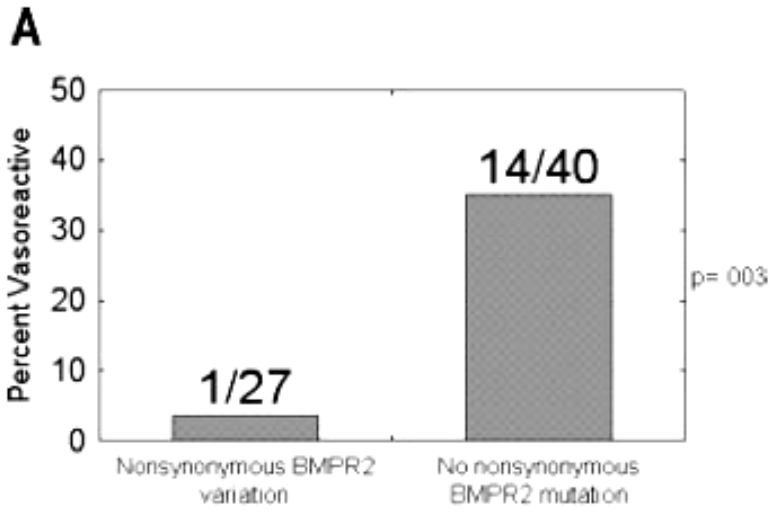
(Circulation, 2000)

TABLE 4. Clinical Characteristics of Patients With Idiopathic or Familial PAH Based on Their Acute Response to a Vasodilator*

	Vasoreactive (n=15)	Nonreactive (n=52)	*P*
Age, y	35.8±11.2	38.3±11.4	0.46
Sex, M/F	1/14	13/39	0.08
Family history, n (%)	1 (7)	14 (37)	0.04
Baseline			
MPAP, mm Hg	52.7±7.7	60.0±11.4	0.02
MRAP, mm Hg†	6.3±1.1	11.0±6.9	0.02
PVR, Wood units	10.5±3.7	14.0±6.1	0.04
CO, L/min	4.6±1.0	4.0±1.3	0.07
CI, $L \cdot min^{-1} \cdot m^{-2}$	2.7±0.6	2.1±0.6	<0.002
NYHA functional class			
II, n (%)	0 (0)	1 (2)	...
III, n (%)	15 (100)	50 (96)	...
IV, n (%)	0 (0)	1 (2)	...

*Vasoreactivity was defined according to recent international consensus guidelines.[8,9]

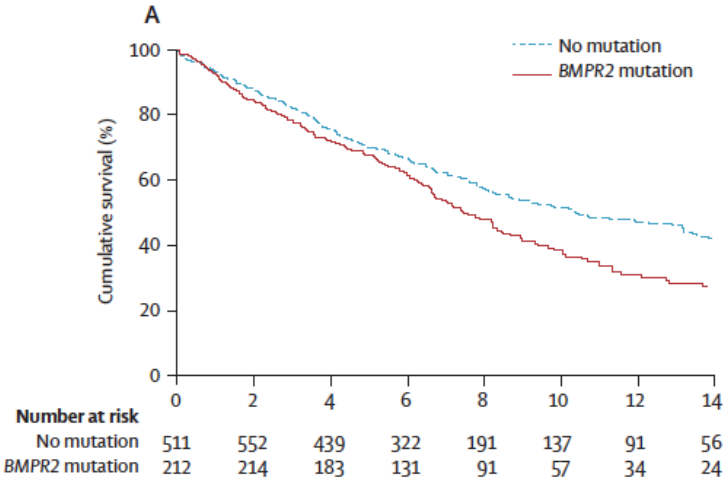
†n=64; right atrial pressure was not recorded for 3 of 67 patients.



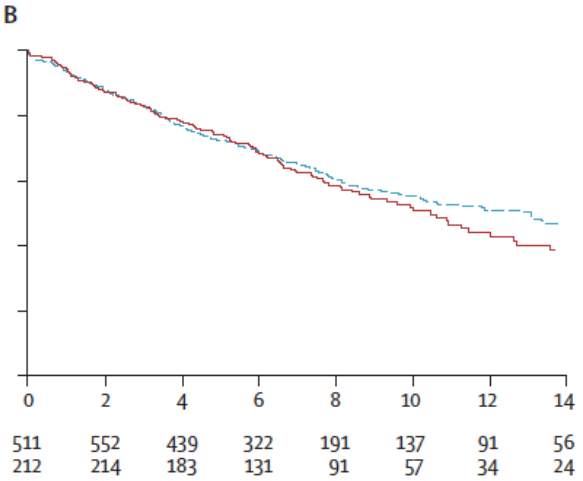
BMPR2 mutation carriers have less vasoreactivity and severe disease compared to noncarriers.

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

Transplant-Free Survival



Survival



Evan JW et al, Lancet Resp Med, 2016

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 a 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.

Evan JW et al, Lancet Resp Med, 2016

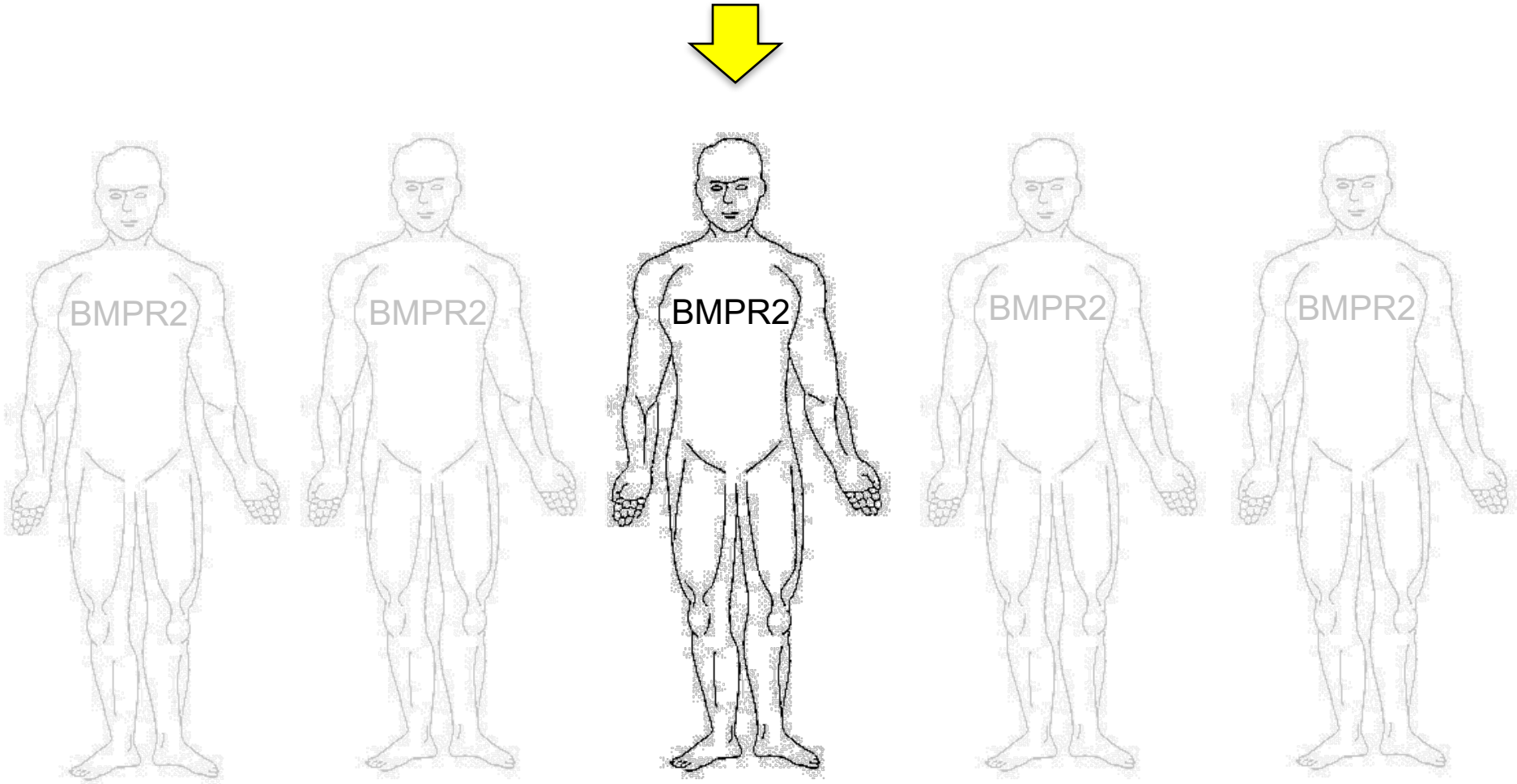
BMPR2 mutation are Not Limited to Familial PAH: Implications to Outcome and Therapeutic Response?

Type of PAH	Reported Mutation Prevalence
Familial PAH	> 75%
Idiopathic PAH	20%
Congenital heart disease	6%
Scleroderma	Not detected
HIV	Not detected
Hemolytic disease	Not reported

Slide courtesy of Dr. Eric Austin

Not All BMPR2 Mutation Carriers Develop Pulmonary Arterial Hypertension

Penetrance of BMPR2 Mutations is ~20%



Hereditary Hemorrhagic Telangiectasia (HHT) and PAH

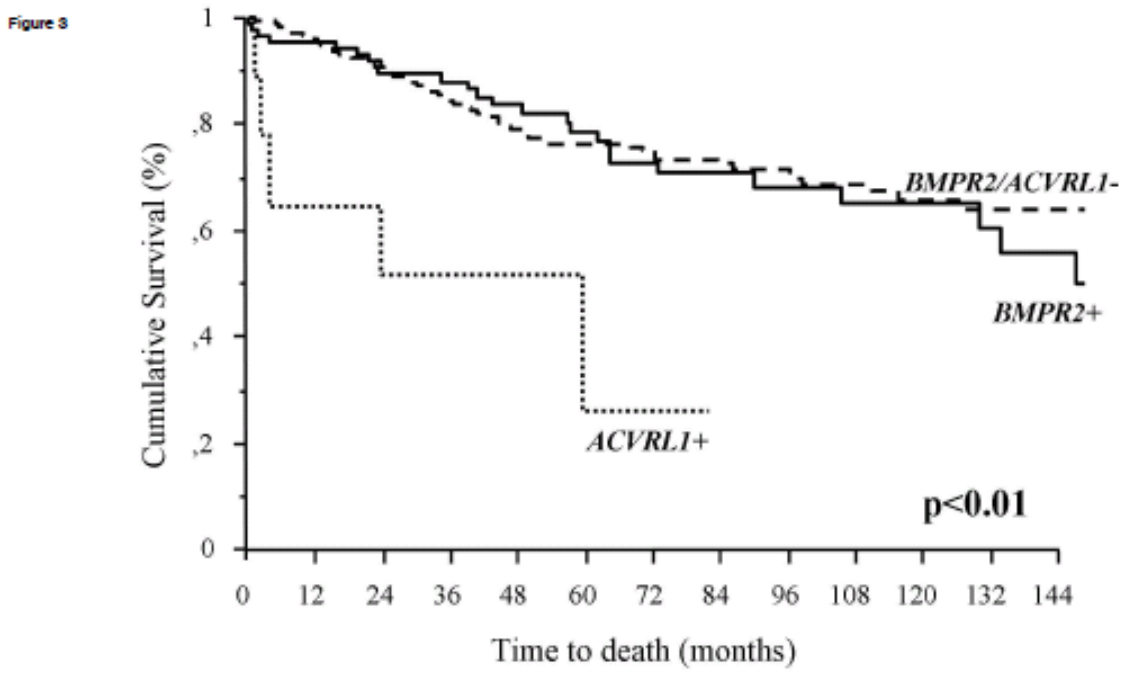
- Autosomal dominant vascular disease
  - Mucocutaneous telangiectasias
  - Multiorgan AVMs
  - PAH rare, may precede HHT dx

- ALK1: TGFβ type I receptor (≤ 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
  - < *BMPR2* mutation
- Less severe hemodynamics



BMPR2/ACVRL1-	277	225	193	166	134	114	99	84	73	56	40	33	26
BMPR2 +	91	82	70	63	50	44	37	30	25	23	18	13	9
ACVRL1 +	9	5	4	4	3	1	1	0					

Girerd *AJRCCM* 2010

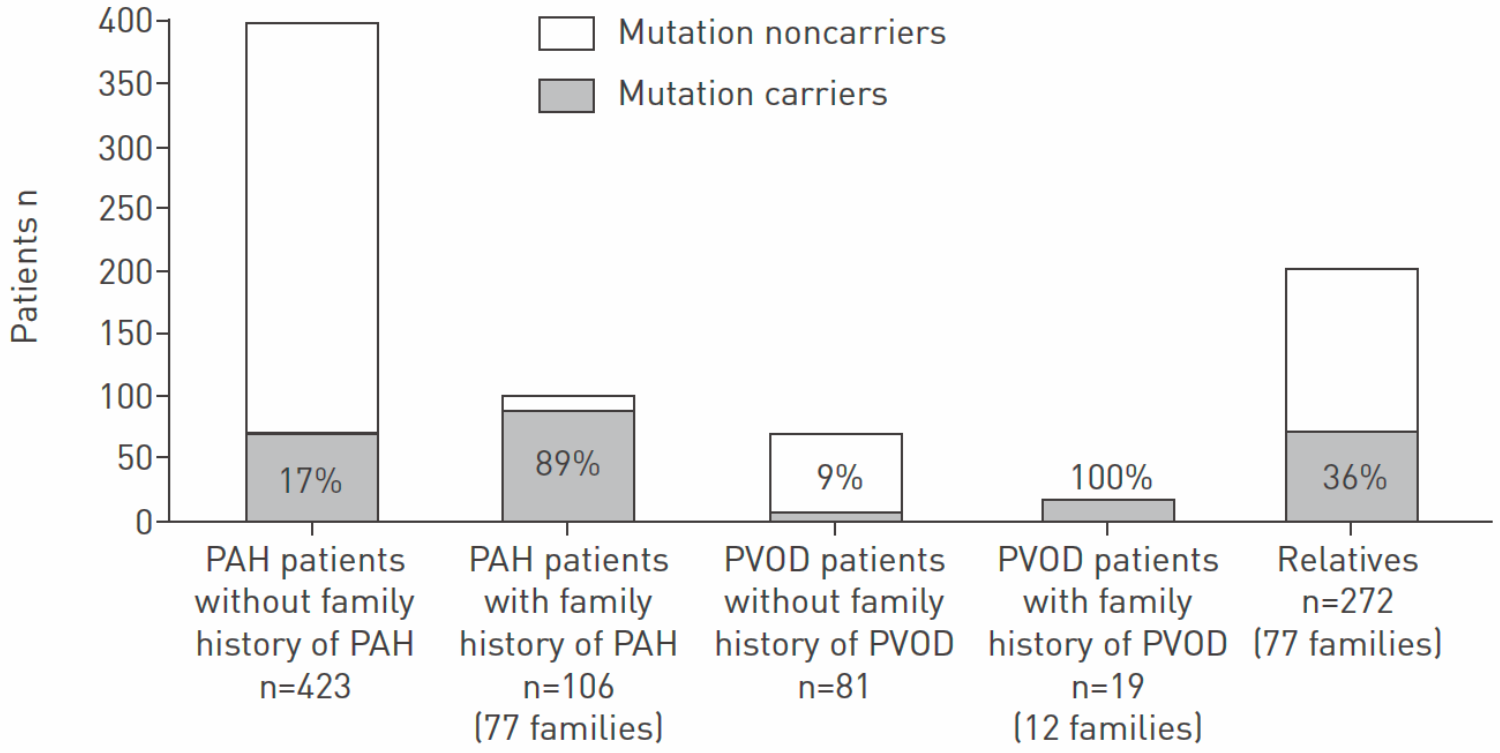
Beyond BMPR2: Recently Discovered Mutations

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

Exact estimates of frequency among PAH patients TBD

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



BMPR2 mutation	62	89 (65 families)	0	0	96
ACVRL1 (ALK1) mutation	9	3 (2 families)	–	0	1
ENG mutation	1	0	–	0	–
KCNK3 mutation	–	2 (2 families)	–	–	2
EIF2AK4 mutations	–	0	7	19 (12 families)	–
Total mutations	72	94	7	19	99

Girerd B et al, ERJ 2016

Structure of a PH Genetic Program

Clinician/Geneticist

- MD expert in PH
- Diagnosis and Management
- Follow-up

PH Center

- Diagnostic tools
- Access to therapy
- Patient Registries
- Clinical Trials

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

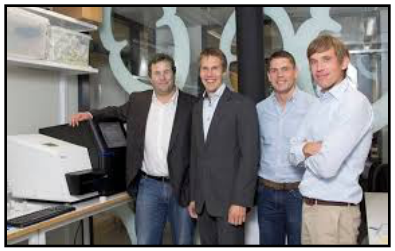
- CLIA Certificate
- Full sequencing capability
- Communication with care team

Barbara Girerd
Nick Morrell
Greg Eliot
Eric Austin

OS-Seq™ Technology: The Stanford PH Gene Screen Strategy

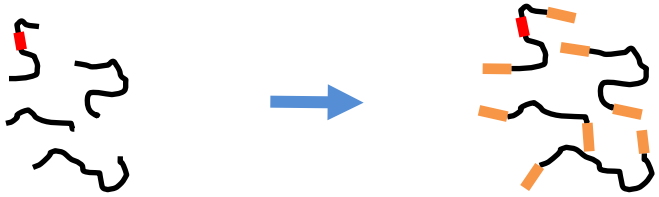
DNA Source: Saliva or PBMC

Turnaround: 21 days



Blueprint Genetics

SEQUENCING LIBRARY



RANDOM SHEARING → SEQUENCING LIBRARY

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

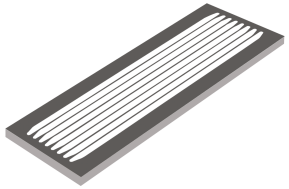
TARGET ENRICHMENT & SEQUENCING

Sequencing library →

Oligonucleotide library →

PREPARATION

Flow Cell: All 9 PAH genes



PROCESSING

Sequencing on Illumina system

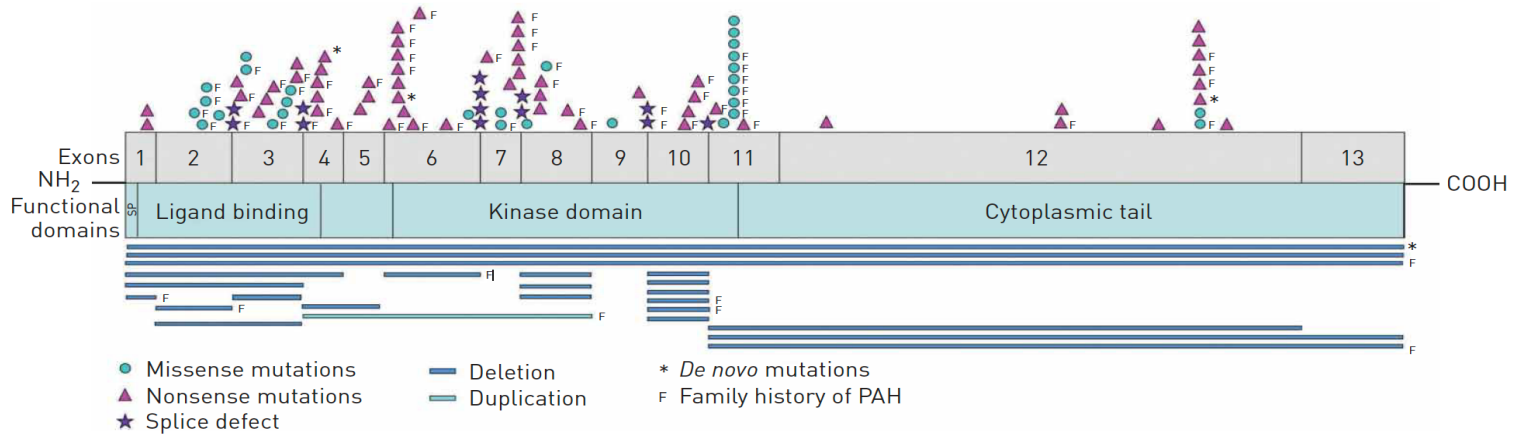
SEQUENCING

Bioinformatic Analysis

↓

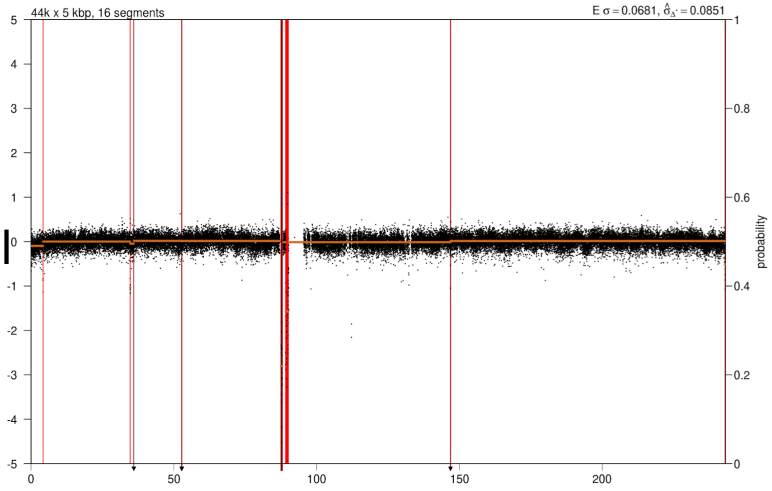
Final Report

OS-Seq Helps Identify Mutations Across the Entire Gene

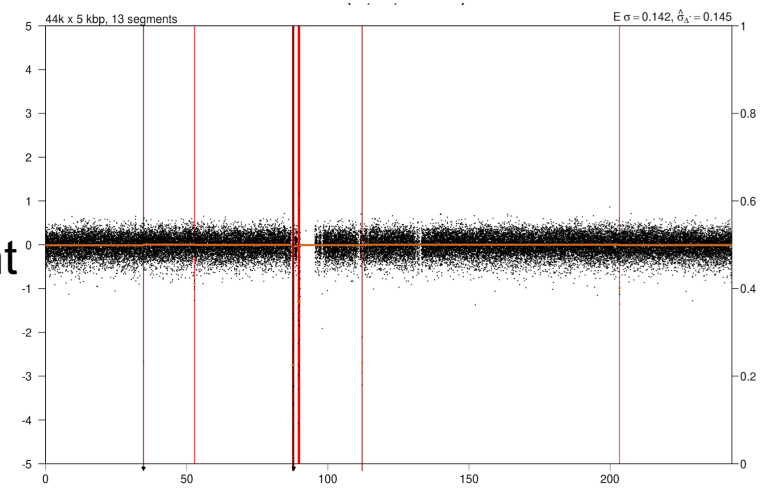


55kb deletion deleting most of the **BMPR2** gene: **203325001 - 203380000**

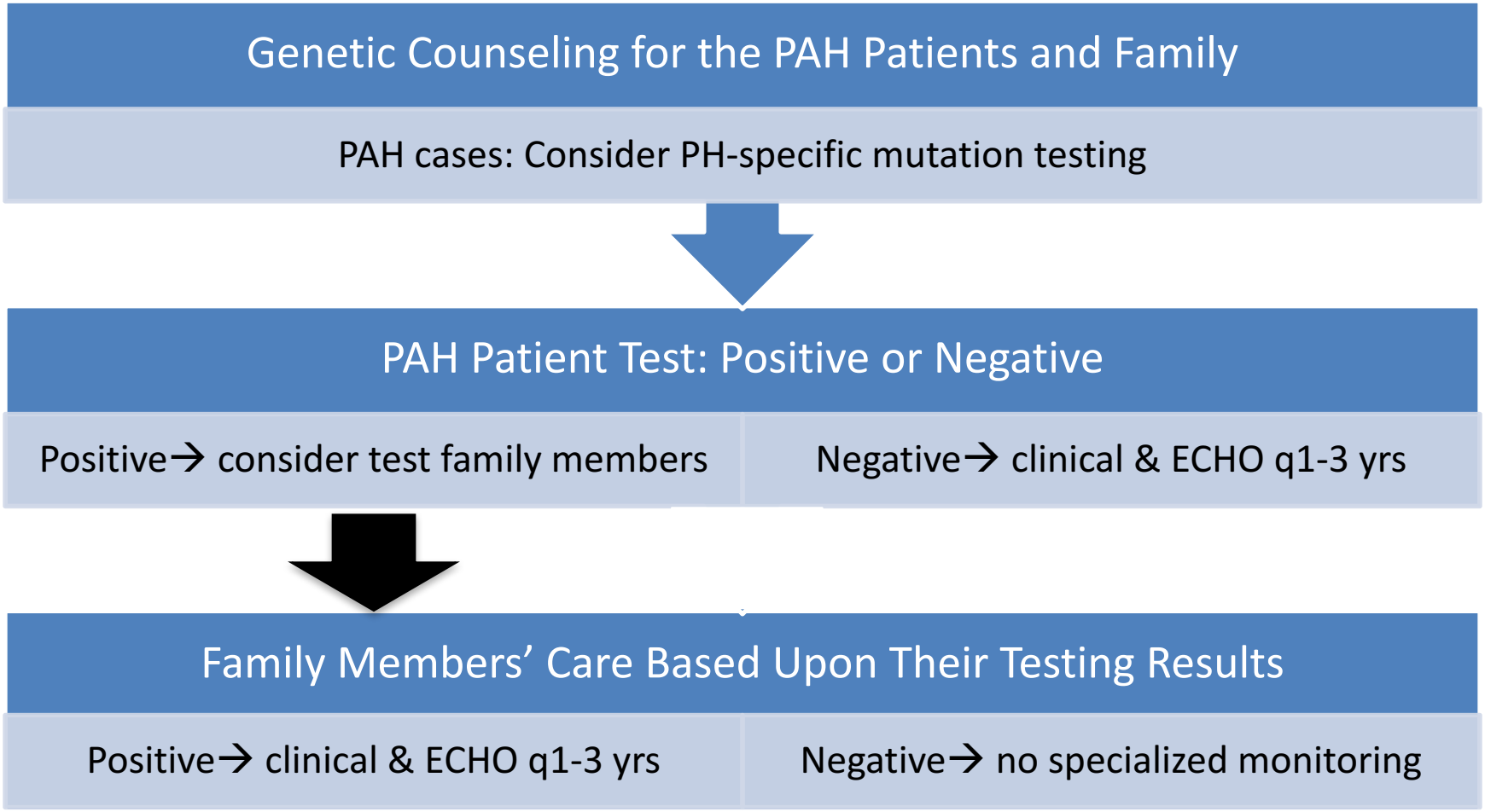
Control



Patient



After Genetic Testing: Surveillance and Treatment



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
History of Changes

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

Adult Clinic

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Collaborators

Euan Ashley
Nicholas Morrel
Eric Austin
Marc Humbert
C. Gregory Eliot
Paul Bollyky
Kurt Stenmark
Grazyna Kwapiszewska
Tero-Pekka Alastalo

De Jesus Perez Group

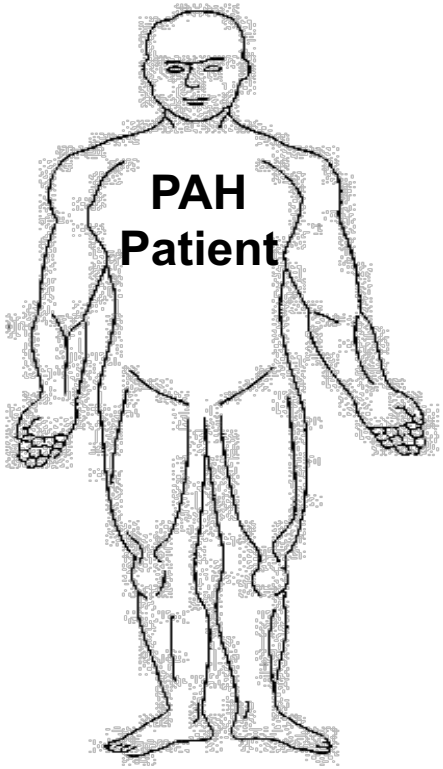
Mark Orcholski
Ke Yuan
Elya Shamskhou
Ellen Hong
Nils Nickel
Monica Romero
Erin Stewart
Halley Tsai
Charlotte Rajasingh
Christian Westoo
Justin Li

PH Genetics Program

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

Genomics

Mutations
Ethnicity
Ancestry



Genetic Testing for PH-associated mutations (*BMPR2*, *ALK1*, *ENG*, *KCNK3*, *CAV1*)

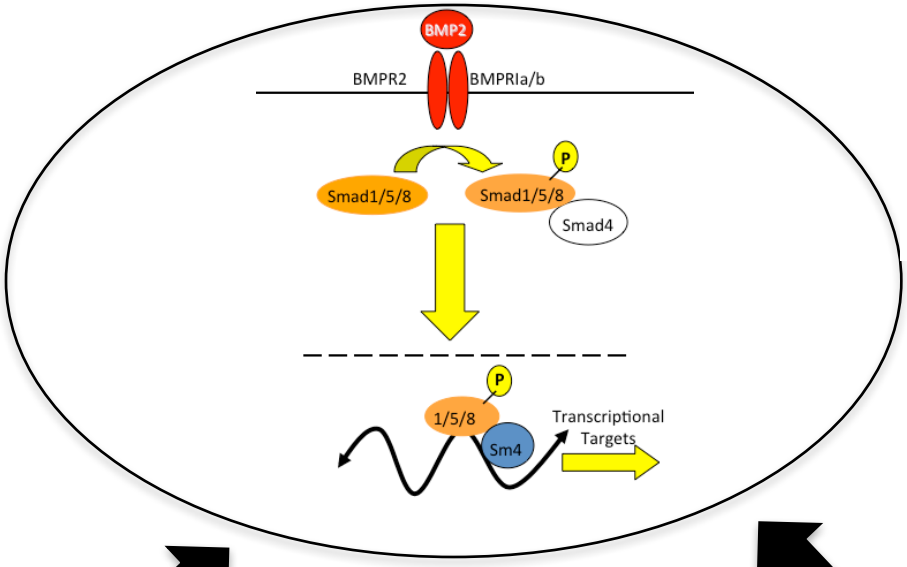
- No current Rx to correct mutations
- May inform severity of disease and risk of progression
  - may influence Rx choices
- Family value w/ regard to etiology of PH
- Informative for other family members
  - Siblings
  - Future pregnancy attempts
- Familial and idiopathic PAH forms
  - Genetic counseling critical
    - 'guilt of heritability'
    - May not account for alternative genetic and non genetic modifiers of disease expression

Austin and Loyd *Circ Research* 2014 *in press*

Targeting BMPR2 Can Lead to Novel Therapies but is it going to Work for Everyone with Mutations?

Ataluren

Drake et al, AJRMB 2013



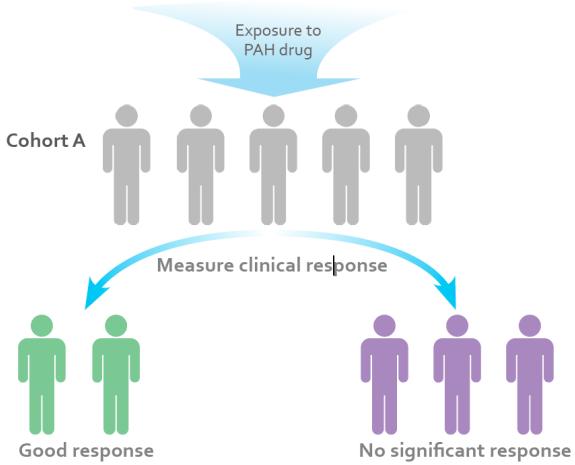
BMP-9

Long et al, Nature Medicine 2015

FK506

Spiekerkoetter et al, AJRCCM 2015
Spiekerkoetter, JCI 2013

BMPR2 Mutation

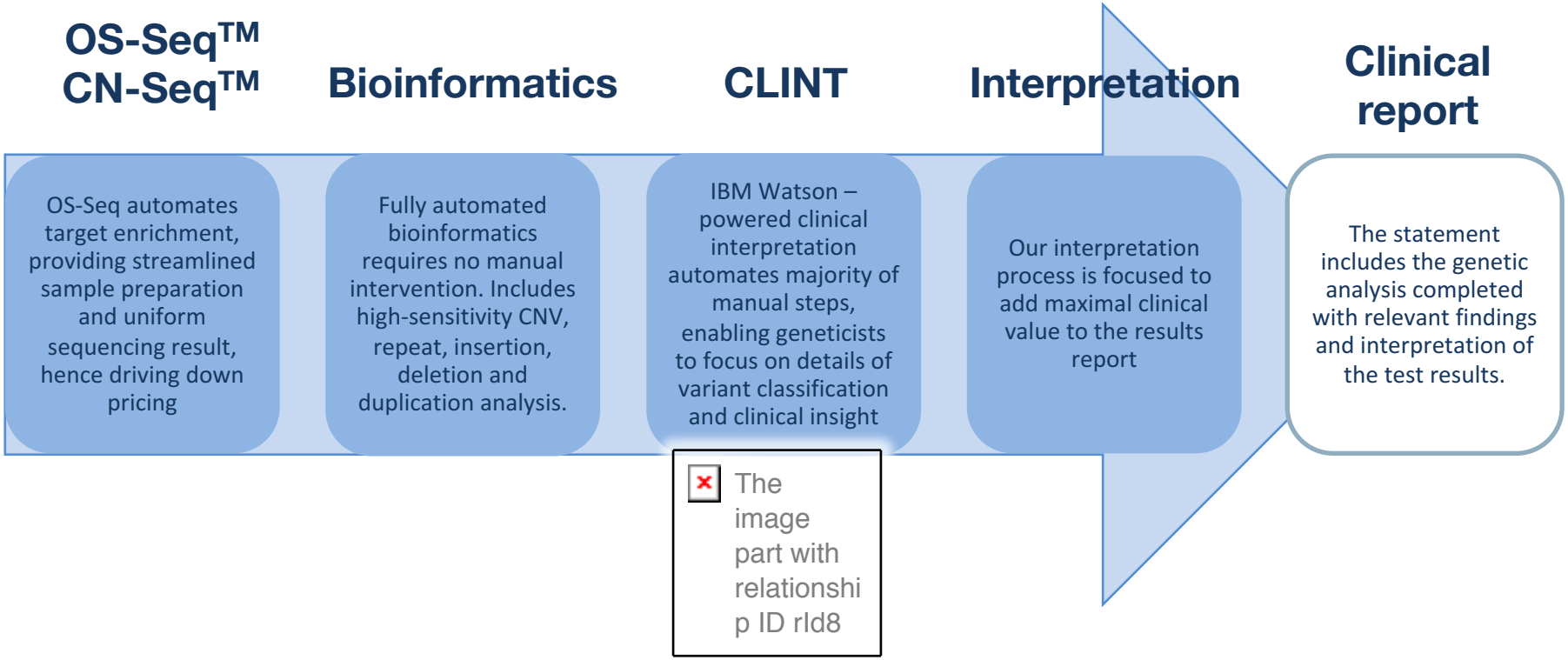


What are we missing?

More genes?

Blueprint Genetics

Our diagnostics process



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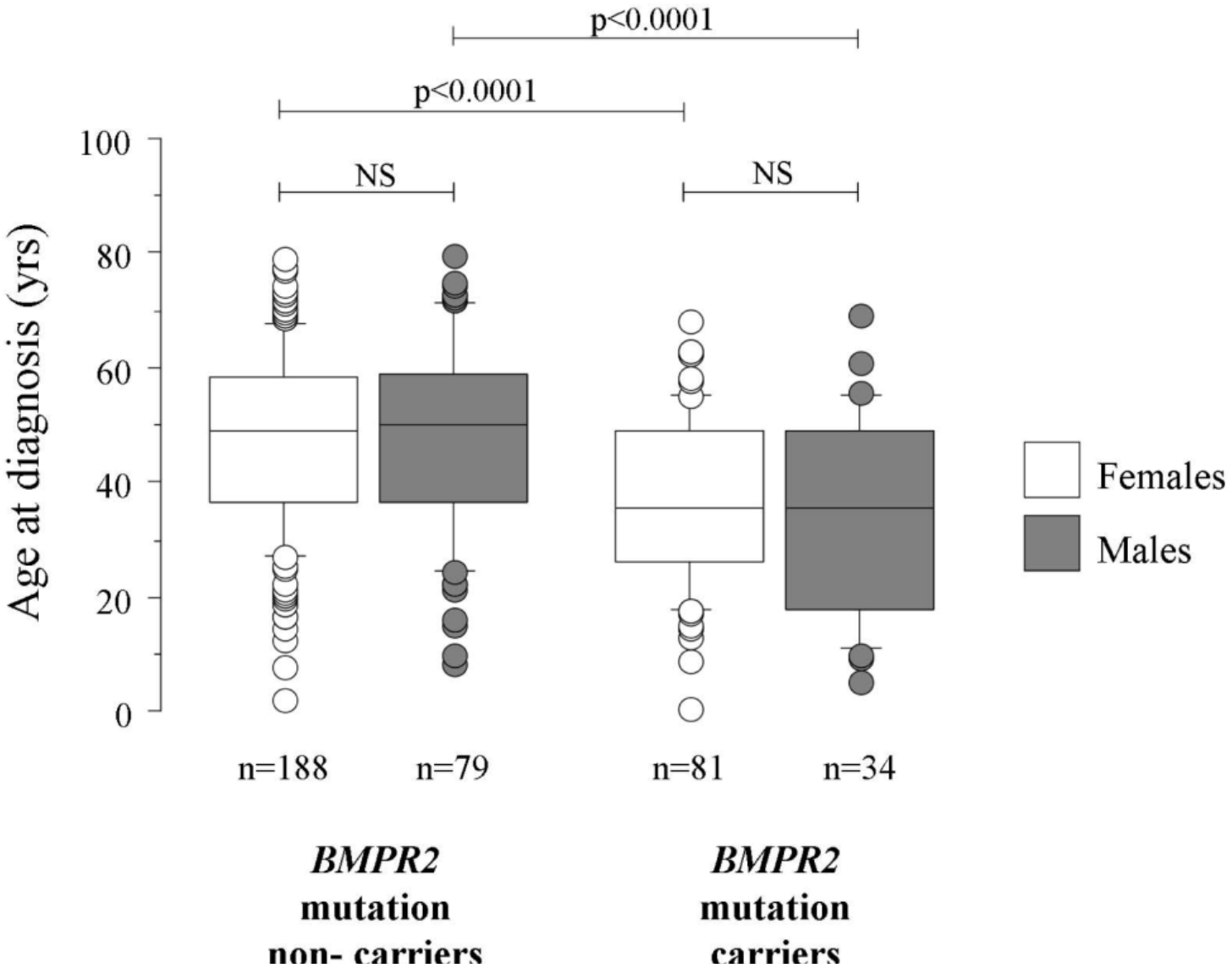
These technologies enable us to provide fast turnaround time, high diagnostic yield and most comprehensive test menu at an affordable price

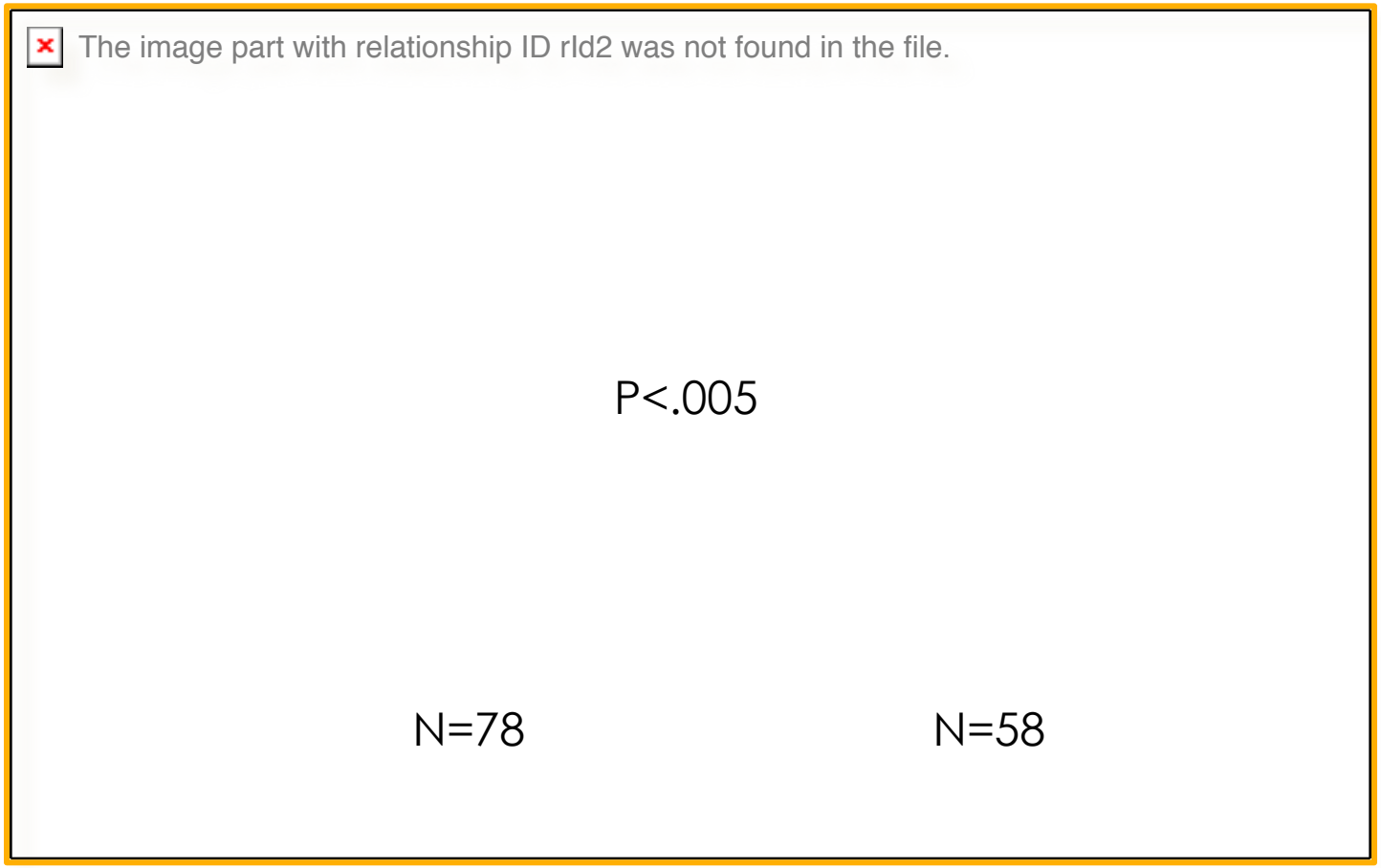
Blueprint Genetics

PAH Severity is Worse in Patients with BMPR2 Mutations



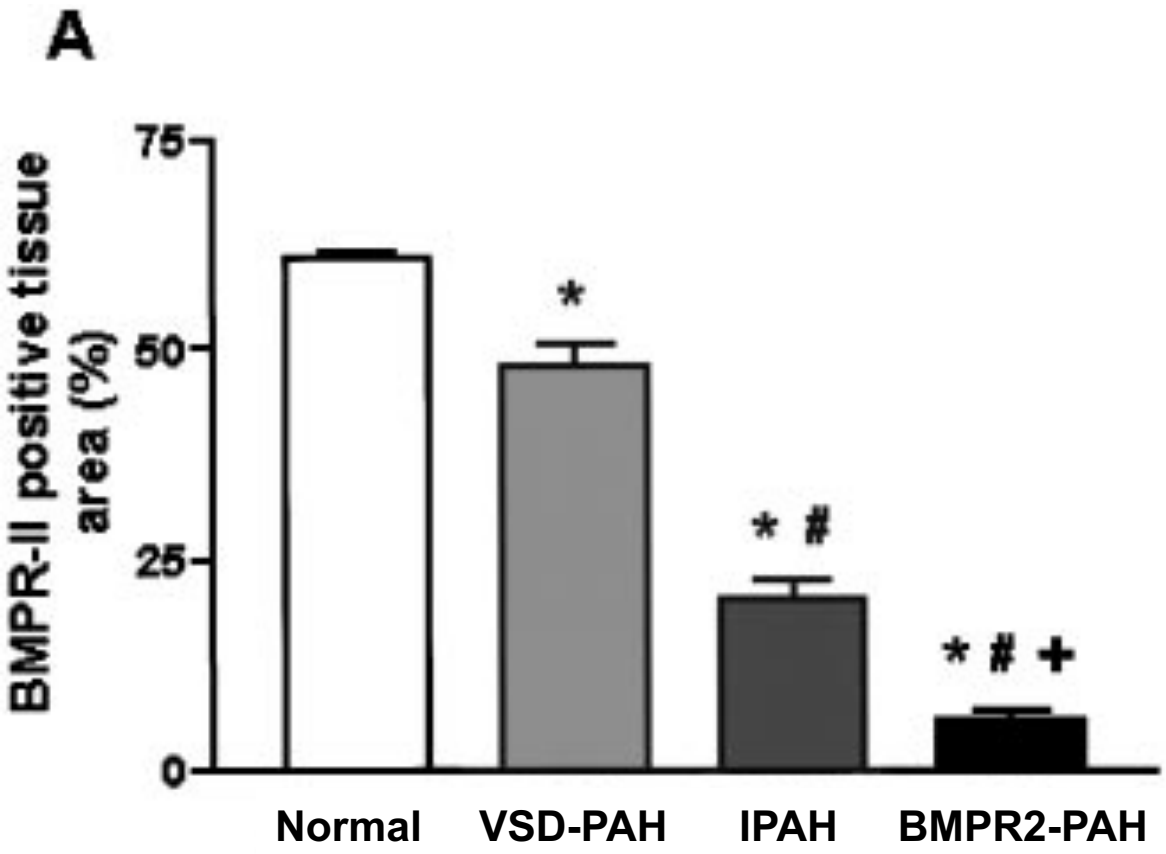
Austin *Resp Res* 2009

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



Updated from Hamid et al Hum Mutat 2009

BMPR2 Protein is Reduced in Lungs of Patients with other forms of WHO Group 1 PAH



Atkinson *Circ Res* 2009