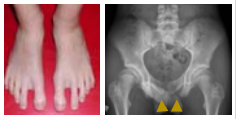
Genetics of Pulmonary Arterial Hypertension

Ernie Bongers

Clinical Geneticist, dept of Human Genetics
Radboud University Medical Center
Nijmegen, The Netherlands

Outline

Genetics of Pulmonary Arterial Hypertension (PAH)



- Classification of PAH
- Genetic causes: the past
- Genetic causes: at present? *W.S. Kerstjens-Frederikse: new gene*
- Genetic investigations: the future?

Classification of Pulmonary Arterial Hypertension (PAH)

PAH is group 1 in 5 groups of PH and has 5 subgroups:

- 1.1 idiopathic (IPAH)
- 1.2 heritable (HPAH)
  (*BMPR2, ACVRL1, BMPR1B, CAV1, ENG, SMAD9* or unknown)
- 1.3 associated with drugs or toxins
- 1.4 associated with connective tissue disorders or several other diseases
- 1.5 specific disease of the newborn (persistent PH)

Updated Clinical Classification of Pulmonary Hypertension Dana Point, 2008 (Simonneau, JACC 2009)

Classification of Pulmonary Arterial Hypertension (PAH)

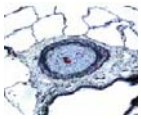
PAH is group 1 in 5 groups of PH and has 5 subgroups:

- 1.1 idiopathic (IPAH)
- 1.2 heritable (HPAH)
  (*BMPR2, ACVRL1, BMPR1B, CAV1, ENG, SMAD9* or unknown)
- 1.3 associated with drugs or toxins
- 1.4 associated with connective tissue disorders or several other diseases
- 1.5 a specific disease of the newborn (persistent PH)

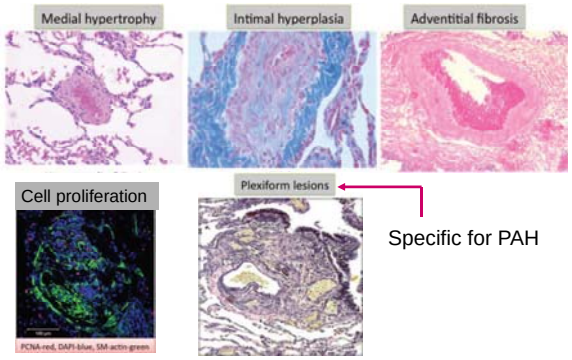
Updated Clinical Classification of Pulmonary Hypertension Dana Point, 2008 (Simonneau, JACC 2009)

Pulmonary Arterial Hypertension

- Intrinsic disease of the lung arteries leading to high pulmonary vascular resistance and increased pulmonary artery pressure
- Right heart failure with eventually death
  (mean 2.8 yrs after diagnosis without therapy)
- 3 yrs survival increased from 48% to 55% with therapy

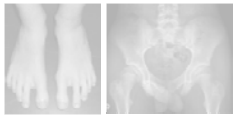


Histology in PAH



Outline

Genetics of Pulmonary Arterial Hypertension (PAH)



- Classification of PAH
- Genetic causes: the past
- Genetic causes: at present? *W.S. Kerstjens-Frederikse*
- Genetic investigations: the future?

umcg
university of groningen
Human Genetics Nijmegen

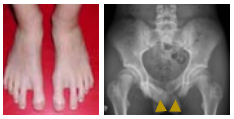
Genetics of PAH: the past

- 6-10% Familial PAH: 50-90% *BMPR2* mutations
- Sporadic PAH: 25% *BMPR2* mutations
- Reduced penetrance: 20% *BMPR2* mutation carriers PAH

- Infrequently mutations in *ACVRL1, BMPR1B, CAV1, ENG, SMAD9 (1% each gene)*
- **In childhood-onset PAH only 16% *BMPR2* mutations**

Outline

Genetics of Pulmonary Arterial Hypertension (PAH)



- Classification of PAH
- Genetic causes: the past
- Genetic causes: at present? *W.S. Kerstjens-Frederikse*
- Genetic investigations: the future?

umcg

university of groningen

Human Genetics Nijmegen

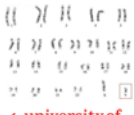
New genes underlying childhood-onset PAH

METHOD 1

Initial study group Mieke Kerstjens-Frederikse:

20 childhood-onset PAH patients

- Enrolled in National Referral Center PH in children, Groningen, the Netherlands between 2003-2010
- 6 Patients had mental retardation/dysmorphic features*
- Array CGH in these 6 patients
- Sequencing and MLPA of candidate genes



umcg

university of groningen

** van Loon et al. J Pediatrics 2009*

Results (1) gene identification of PAH: array CGH

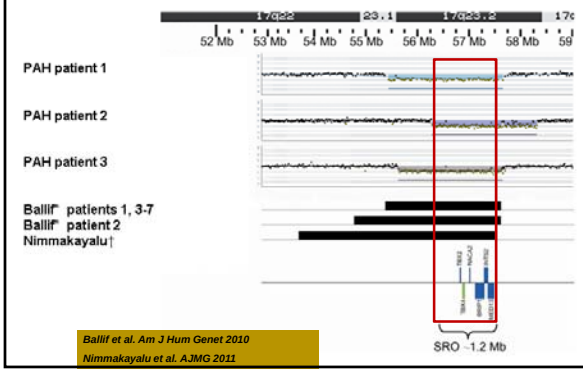
Patient 1:
mental retardation
deletion chromosome 17q23: 55,5 – 57,7 Mb

Patient 2:
mental retardation + dysmorphic features
deletion chromosome 17q23: 56,7 – 58,7 Mb

Patient 3:
mental retardation + microcephaly
deletion chromosome 17q23: 55,6 – 57,7 Mb

Results (2) gene identification of PAH: smallest region of overlap

Chromosome 17q32.2 deletions:



Ballif et al. Am J Hum Genet 2010
Nimmakayalu et al. AJMG 2011

Results (3) gene identification of PAH

Genes in smallest region of overlap

TBX2, TBX4, NACA2, BRIP1, INTS2 and *MED13*

Results (3) gene identification of PAH

Genes in smallest region of overlap

TBX2, TBX4, *NACA2, BRIP1, INTS2* and *MED13*

Results (4) gene identification of PAH

Sequencing *TBX2* and *TBX4*

Patient 4:
TBX4 exon 3 c.355_356 het_insA;p.Ile119Asn fsX6 *mat*

Patient 5:
TBX4 exon 8 c.1164_1165 het_insC;p.Arg389Gln fsX30 *pat*

Patient 6 (deceased):
TBX4 exon 8 c.1145A>C;p.Tyr382Ser *mat*

Results (5) gene identification of PAH

Patient 6:

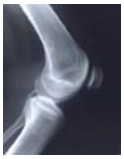
- c.1145A>C; p.Y382S
- **Highly conserved nucleotide** (score 1.0, [0-1]),
  Highly conserved amino acid (considering 11 species);
  Grantham distance 144;
  Polyphen score 0,531 (**possibly damaging**);
  SIFT score 0,00 (**not tolerated**).

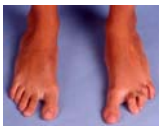
  Mutation is not detected in **1000 control chromosomes**.

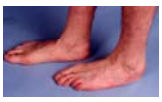
Conclusion: **probably pathogenic**

TBX4 mutations cause Small Patella Syndrome

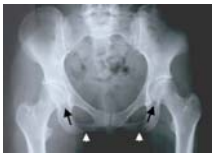
- Small patellae







- Large gap between 1st and 2nd toe
- Long toes with relatively short 4th and 5th rays

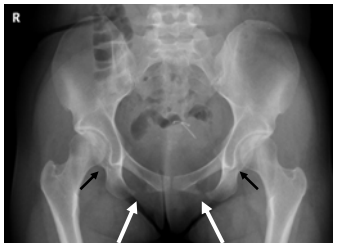


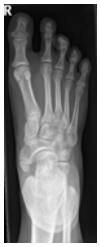
- Acetabular axe-cut notches
- Abnormal ossification of ischiopubic junction
- Long femoral necks

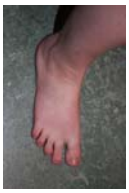
Bongers et al. Am J Hum Genet 2004

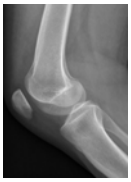
Results (6) gene identification of PAH

Small Patella Syndrome features in all patients and parent carriers

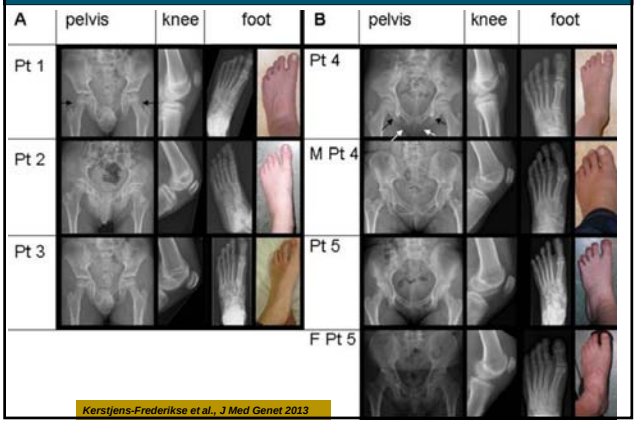








Small Patella Syndrome features in *TBX4* mutations carriers



Kerstjens-Frederikse et al., J Med Genet 2013

New gene underlying (childhood-onset) PAH?

METHOD 2

After finding mutations in *TBX4*, two additional cohorts were recruited:

- 49 adult-onset PAH patients for *TBX4* sequencing/MLPA

VUmc

- 23 Small Patella Syndrome patients with *TBX4* mutations for PAH screening

Radboudumc university medical center

Results *TBX4* sequencing in 49 adult onset PAH pt

- *Patient 1:*
  c.229T>C, p.W77R highly conserved nucleotide (score 1.0, [0-1]);
  Highly conserved amino acid (considering 11 species);
  Grantham distance 101;
  Polyphen score 1,00 (**probably damaging**);
  SIFT score 0,00 (**not tolerated**).
  Mutation is not detected in **1000 control chromosomes**.
  Conclusion: **probably disease causing.**

- 1/49 adult onset PAH patients
  vs 6/20 childhood onset PAH patients

Results echocardiographic screening
23 Small Patella Syndrome patients

- 22 adults, one 11 year old
- Including 2 carrier parents from initial cohort
- 3 possible PH, 2 due to COPD or LV dysfunction,
  one had RHC that showed no PAH

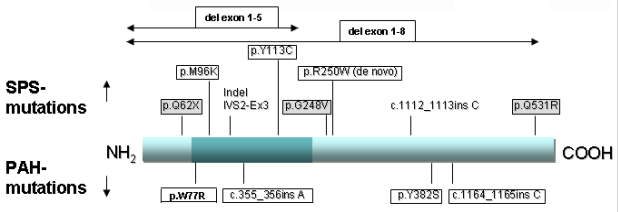
Conclusion: no PAH in (adult) SPS patients

Summary of results

- *TBX4* mutations in 6/20 children with PAH (30%)
- *TBX4* mutations in 1/49 adults with PAH (2%)
- No PAH in 23 (adult) Small Patella Syndrome patients

Kerstjens-Frederikse et al., J Med Genet 2013

TBX4 gene mutations
in PAH and Small Patella syndrome



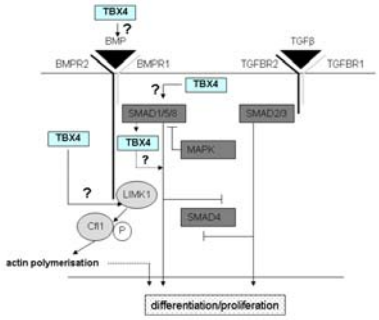
Kerstjens-Frederikse et al., J Med Genet 2013

TBX4 in animals

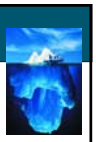
- *TBX4* expression in developing limbs and lungs in chicken and mouse
- Related to FGF, BMP and Wnt pathways
- Knock-out mice embryonic lethal; PAH postnatal disease

Chapman DL et al. Dev Dyn 1996;206:379-390.
Arora et al. PLoS Genet 2012
Cebra-Thomas JA et al. Dev Dyn 2003;226:82-90
Sekine K et al. Nat Genet 1999;21:138-141.
Sountoulidis A et al. PLoS One 2012;7:e41460

TBX4 in the pathogenesis of PAH



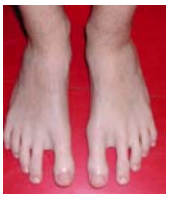
TBX4 conclusions



- *TBX4* mutations are associated with childhood-onset PAH (6/20; 30%)
- Small patella syndrome is often unrecognised in these patients
- *TBX4* mutations are less frequently found in adult-onset PAH (1/49; 2%)
- *TBX4* mutations might contribute to PAH through a decreased activation of the BMP pathway

Kerstjens-Frederikse et al., J Med Genet 2013

Limitations *and* Recommendations



Limitations

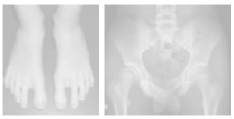
- Small group: replication
- No functional data

Recommendations:

- Clinical investigation (feet!) of PAH patients for signs of Small Patella Syndrome for decision on genetic testing
- Screening Small Patella Syndrome patients, especially young patients for PAH (echocardiography)

Outline

Genetics of Pulmonary Arterial Hypertension (PAH)



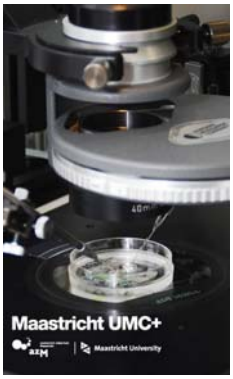
- Classification of PAH
- Genetic causes: the past
- Genetic causes: at present? *W.S. Kerstjens-Frederikse*
- Genetic investigations: the future?

umcg

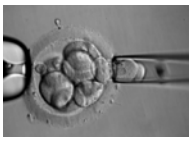
university of groningen

Human Genetics Nijmegen

Preimplantation Genetic Diagnosis

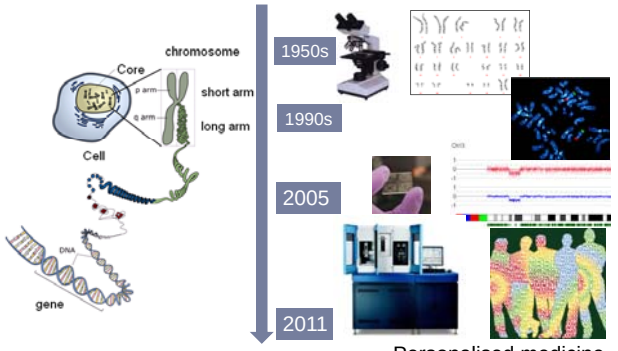


- Testing of pre-implantation stage embryos for genetic defects (to prevent the birth of children with severe genetic disorders)
- *For example:*
  Chromosome abnormalitis, CF, Huntington disease



Maastricht UMC+ umcg

New development; exome sequencing



Personalised medicine

New development; non-invasive prenatal testing

Testing of fetal DNA in maternal blood

Acknowledgements

Mieke W.S. Kerstjens-Frederikse
Rianne Kinds
Lennart Johansson
Eddy de Boer
Pieter Neerincx
Marcus T.R. Roofthooft
Edward M. Leter
J. Menno Douwes
Arie Van Dijk

Anton Vonk-Noordegraaf
Krista K. Dijk-Bos
Lies H. Hoefsloot
Elke S. Hoendermis
Johan J.P.Gille
Birgit Sikkema-Raddatz
Robert M.W. Hofstra
Rolf M.F. Berger

Ernie.Bongers@radboudumc.nl