Genetics of bicuspid aortic valve related aortopathy

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IMAD, Liege, september 2014
1-2% of population; M/F: 3/1
Three subtypes: >> RL (70%), > RNC, < LNC
Bicuspid aortic valve - aneurysm

Aortic aneurysm: 7.5 - 79% of BAV
BAV: 9-fold higher risk for aortic dissection
22% of aortic dissection < 40 yrs: BAV related

Main risk factor dissection: aortic size > 45mm
Aneurysm in BAV patients historically considered as hemodynamic but arguments pro genetic etiology are:

1. High heritability 89%
2. BAV and TAA occur independent in same family
3. Aortic aneurysm progresses after BAV replacement
4. Aortic aneurysm occurs in BAV patients with normal valve function or AR
5. Aortic valve and ascending aorta share embryological origin
Bicuspid aortic valve - genetics

• Affected relatives
  9% prevalence in first degree relative (Huntington et al, 1997)
  24% prevalence if > 1 person affected (Glick et al, 1994)

• Variable clinical expression
  • BAV only
  • BAV/TAAD (heritability unknown but 23% dilatation in first degree relative)
  • BAV/CoArc (heritability 60%)
  • BAV/coronary anomalies

• Incomplete penetrance

• Heritability: 0.89 (Cripe et al, JACC, 2004) – 0.61 (Calloway et al, 2011)

• BAV subtype does not segregate in family (Calloway et al, 2011)
  • 76% concordance (22/29 pairs) (59% expected by chance)
Bicuspid aortic valve - genetics

**Syndromic BAV forms**

- Turner syndrome: $45,X0$
- Andersen syndrome: $KCNJ2$
- Loeys-Dietz syndrome: $TGFBR1/2, SMAD3, TGFβ2$
- X-linked valve disease: $FLNA$
- Marfan syndrome: $FBN1$
- Bosley-Salih-Alorainy: $HOXA1$

**Non-syndromic BAV forms**

- Frequent TAA:
  - sarcomeric: $ACTA2, MYH11$
  - $MYLK, PRKG1$
  - TGFβ: $TGFBR1, TGFBR2$
- Rare TAA:
  - $NOTCH1$
  - $GATA5$
  - $Nkx2.5$
- Loci: 5q21-22, 13q33, 15q25-26.6, 18q22.1
Turner syndrome: 45,X0

Cardiovascular features:
• Occur in 1/3 of Turner girls
• Most commonly: left sided anomalies
  • Bicuspid aortic valve
  • Coarctation of aorta
  • Aortic atresia
  • Aortic aneurysm/dissection
• Correlations:
  • 50% of neck webbing: CHD
  • BAV/coarctation

<table>
<thead>
<tr>
<th></th>
<th>TAV (n = 176)</th>
<th>BAV (n = 74)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>28.8 ± 15.2</td>
<td>26.6 ± 14.0</td>
<td>0.22</td>
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<tr>
<td>Height (cm)</td>
<td>143.6 ± 11.1</td>
<td>143.3 ± 12.3</td>
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<td>BSA kg/m²</td>
<td>1.44 ± 0.3</td>
<td>1.42 ± 0.3</td>
<td>0.58</td>
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<tr>
<td>Sys BP (mm Hg)</td>
<td>115 ± 11</td>
<td>114 ± 12</td>
<td>0.9*</td>
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<tr>
<td>Dias BP</td>
<td>69 ± 9</td>
<td>70 ± 9</td>
<td>0.4*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>86 ± 11</td>
<td>88 ± 11</td>
<td>0.4*</td>
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<tr>
<td>Peak flow (m/s)</td>
<td>1.30 ± 0.21</td>
<td>1.62 ± 0.66</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Annulus (cm)</td>
<td>1.88 ± 0.35</td>
<td>2.07 ± 0.49</td>
<td>0.001*</td>
</tr>
<tr>
<td>Sinuses (cm)</td>
<td>2.61 ± 0.39</td>
<td>2.80 ± 0.49</td>
<td>0.003*</td>
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<tr>
<td>Sinotubular junction (cm)</td>
<td>2.11 ± 0.35</td>
<td>2.31 ± 0.46</td>
<td>0.001*</td>
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<tr>
<td>Ascending aorta (cm)</td>
<td>2.34 ± 0.38</td>
<td>2.62 ± 0.63</td>
<td>0.0005*</td>
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<tr>
<td>Dilated aorta†</td>
<td>9/169 (5.3%)</td>
<td>17/69 (25%)</td>
<td>0.002</td>
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<tr>
<td>Neck webbing</td>
<td>43/176 (24%)</td>
<td>33/74 (45%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Aortic coarctation</td>
<td>9/176 (5%)</td>
<td>16/74 (22%)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Sachdev et al, 2008
Turner syndrome - BAV phenotype

- up to 1/3 of all Turner patients (MR study - Oliveiri et al, 2013)
- 95% fusion of R/L coronary cusps
- aortic regurgitation: 55% trivial, 30% mild, 15% moderate/severe
- aortic stenosis: 10%
- 25% have sinus dilatation (> P95) (versus 5% in TAV group)
- 1-2% risk of aortic dissection

- Underlying genetic defect:

<table>
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<tr>
<th></th>
<th>BAV</th>
<th>TAV</th>
<th>Totals</th>
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<tr>
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<td>101</td>
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<td>Qdel</td>
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</table>

-> suggests locus on short arm of X-chromosome
Turner syndrome: genotype/phenotype

PAR1 – SHOX
Visuospatial/perceptual abilities

Lymphedema gene?

POF2 – DIAPH2 gene

POF1

PAR2
Haplo-insufficiency for genes that normally escape from X-inactivation: mostly in PAR1 (n=24) and PAR2 (n=5) region, but also other genes do escape X-inactivation
Candidates: ASMTL, PPP2R3B and CSF2RA

Uncovering mutations on the remaining X

60-80% of cases: paternal chromosome is absent
Syndromic BAV

**Andersen syndrome:**

- **KCNJ2**
  1. Periodic paralysis
  2. Heart rhythm disturbances
  3. Congenital anomalies

**Loeys-Dietz syndrome:**

- **TGFBR1/2, SMAD3, TGFBR2**
  1. Hypertelorism
  2. Bifid uvula/cleft palate
  3. Aneurysm and tortuosity
Syndromic BAV - FLNA

Periventricular nodular heterotopia with EDS features
Otopalatodigital syndrome
X-linked cardiac valvular dysplasia

- ascending aortic aneurysm
- valvular dystrophy: mostly mitral valve
- occasional bicuspid aortic valve
- rare dysplastic pulmonary valve

Increased expression of filamin in BAV-aortic wall
(Pilop et al, 2011)

Kyndt et al, 2007; Norris et al, 2010
Identification of fibrillin 1 gene mutations in patients with bicuspid aortic valve (BAV) without Marfan syndrome

Guglielmina Pepe\textsuperscript{1,2}, Stefano Nistri\textsuperscript{1,3}, Betti Giusti\textsuperscript{1,2}, Elena Sticchi\textsuperscript{1,2}, Monica Attanasio\textsuperscript{1,2}, Cristina Porciani\textsuperscript{1,2}, Rosanna Abbate\textsuperscript{1,2}, Robert O Bonow\textsuperscript{4}, Magdi Yacoub\textsuperscript{5} and Gian Franco Gensini\textsuperscript{1,2,6}

BMC Med Genet, 2014

2/10 BAV/TAA patients: \textit{FBN1} mutations
- Pat 1: p.Arg529Gln

Own experience: 3/30 BAV BAV/TAA patients
- Pat 1: p.Arg1170His
- Pat 2: p.Ala986Thr
- Pat 3: p.Val2234Met

All mutations previously described in Marfan patients

-> hypothesis: BAV provokes TAA in patients with mild \textit{FBN1} mutations
Syndromic BAV – *ELN*

New insights into the pathogenesis of autosomal dominant cutis laxa with report of five *ELN* mutations

Bert Callewaert¹,², Marjolijn Renard¹, Vishwanathan Huchlagowder², Beate Albrecht³, Ingrid Hausser⁴, Edward Blair⁵, Cristina Dias⁶, Alice Albino⁷, Hiroshi Wachi⁸, Fumiaki Sato⁹, Robert P. Mechan⁹, Bart Loeys¹, Paul J. Coucke¹, Anne De Paepe¹,†, and Zsolt Urban²,¹⁰,†,*

Hum Mut, 2011

• 3/5 patients had aortic root dilatation
• 2/5 had bicuspid aortic valve (*ELN c.2333delC (CL-2), c.2137delG (CL-3)*)

Co-incidence or pathogenetic clue?
Elastin gene disorders

Gain of function mutation
AD – cutis laxa

Loss of function mutation
AD – supravalvular aortic stenosis (SVAS)
Autosomal dominant SVAS

Lack of elastin

Compensatory proliferation of vascular smooth muscle cells
ELN triplication – supravalvular aneurysm

Guemann et al, Cardiol Young, 2014
Bosley-Salih-Alorainy – HOXA1

- autosomal recessive
- = Athabascan brainstem dysgenesis
- Clinical features:
  - Duane retraction syndrome
  - neurosensorial hearing loss
  - delayed motor development
  - interrupted aortic arch type B, aberrant subclavian artery, VSD, TOF, BAV

- Pathogenesis: transcription factor important for cardiac neural crest development

Tischfield et al, Nat Genet, 2005
Non-syndromic BAV: frequent TAA

Autosomal dominant inheritance
Incomplete penetrance

Additional features:
*ACTA2*: premature stroke
*MYH11*: patent ductus arteriosus
*MYLK, PRKG1*: hypertension

Risk of aortic dissection at diameters < 50 mm

vascular smooth muscle cell contractile apparatus:

*ACTA2, MYH11*
*MYLK, PRKG1*
Non-syndromic BAV: rare TAA

**NOTCH1 clinical phenotype**
- Bicuspid aortic valve
- Left ventricular outflow tract obstruction
- Hypoplastic left heart
- Early valve calcification

Variable clinical expression and incomplete penetrance
Circa 5% of familial cases, 1% of sporadic

**NOTCH1 Pathogenesis**
Role in Endothelial Mesenchymal Transition
& neural crest function
NOTCH-1 BAV/TAA mutations

Garg, 2005  
Moham, 2006  
McKellar, 2007  
McBride, 2008

- R1108X  
- His1505del  
- T596M  
- R1796H  
- A1343V  
- P1390T

Other variants with population frequencies:

-> difficult distinction polymorphism and causal mutations
Non-syndromic BAV: rare TAA

**GATA-5**

4/100 patients with BAV  
(Padang et al, 2012)

2/78 patients with BAV/coarc  
(Bonachea et al, 2012) (p.Gln3Arg; p.Leu233Pro)

4/320 patients with CHD:  
ASD, VSD, TOF, DORV, AS  
(Jiang et al, 2012)

2/110 patients with AF  
(Gu et al, 2012)

2/130 patients with TOF  
(Wei et al, 2013)

**Nkx2.5**

ASD, VSD, AVSD, TOF, SVAS,  
LVNC, PA, PS, PDA, MV  
anomalies, AV conduction  
defects, DORV, PAPVR, TAPVR,  
heterotaxy, TGA  
<<BAV (Majundar et al, 2006)
Animal models

• Syrian hamster: 58% BAV, 56% R-L
  -> neural crest driven OFT septation defect

• Mouse models
  – Wild type mice: 1-2% have bicuspid aortic valve
  – Notch-1 -/-: no BAV
  – Gata-5 +/-: 26% BAV; all R-N
  – Nkx2.5 +/-: 11% BAV; all R-N
  – eNos +/-: 32% BAV, all R-N
  – Hoxa1 -/-: 24% BAV, subtype ?
  – Hoxa3 driven FGF8 +/-: 23% BAV, all R-N
  – Gata5 driven Alk2 +/-: 78% BAV, all R-N
  -> EMT defect of OFT cushion before septation
Lessons from mouse models

Padang et al, Circ, 2012
Added value of array analysis

Contribution of Global Rare Copy-Number Variants to the Risk of Sporadic Congenital Heart Disease

Rachel Soemedi,1 Ian J. Wilson,1 Jamie Bentham,2 Rebecca Darlay,1 Ana Töpf,1 Diana Zelenika,3,4 Catherine Cosgrove,2 Kerry Setchfield,5 Chris Thornborough,6 Javier Granados-Riveron,5 Gillian M. Blue,7 Jeroen Breckpot,8 Stephen Hellens,9 Simon Zwochinski,9 Elise Glen,1 Chrysovalanto Mamasoula,1 Thahira J. Rahman,1 Darroch Hall,1 Anita Rauch,10 Koenraad Devriendt,8 Marc Gewillig,11 John O’ Sullivan,12 David S. Winlaw,7 Frances Bu’Lock,6 J. David Brook,5 Shoumo Bhattacharya,2 Mark Lathrop,3,4 Mauro Santibanez-Koref,1 Heather J. Cordell,1 Judith A. Goodship,1,* and Bernard D. Keavney1,*

AJHG, 2012

Rare genetic deletions contribute 4% of sporadic CHD

Rare Copy Number Variants Contribute to Congenital Left-Sided Heart Disease

Marc-Phillip Hitz1,2, Louis-Philippe Lemieux-Perreault3, Christian Marshall4, Yassamin Feroz-Zada3, Robbie Davies5, Shi Wei Yang1, Anath Christopher Lionel4, Guylaine D’Amours6, Emmanuelle Lemyre7, Rebecca Cullum7, Jean-Luc Bigras1, Maryse Thibeault1, Philippe Chetaille8, Alexandre Montpetit9, Paul Khairy3, Bert Overduin10, Sabine Klaassen11, Pamela Hoodless7, Mona Nemer12, Alexandre F. R. Stewart13, Cornelius Boerkoel14, Stephen W. Scherer4, Andrea Richter6, Marie-Pierre Dubé3, Gregor Andelfinger1*

Plos Genet, 2012

Cohort implies a strong effect of unique CNVs in 10% of left-sided CHD
Future perspectives

Karyotyping

FISH

Array

Sanger sequencing

Next generation sequencing: Exome sequencing

- exome: all coding parts of the genome
- only 2% of the genome
- cheaper and faster than whole genome analysis

LETTER

De novo mutations in histone-modifying genes in congenital heart disease

Histone modifying genes

<table>
<thead>
<tr>
<th>ID</th>
<th>Gene</th>
<th>Mutation</th>
<th>Dx</th>
<th>Other structural/neuro/ht-wt</th>
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<td>MLL2</td>
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<td>WDR5</td>
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<td>p.Gln1599*</td>
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<td>1-00230</td>
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<td>1-02020</td>
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</table>

De novo exome sequencing in a cohort of 362 severe congenital heart disease patients

Epigenetics

- Histone modification and methylation of promoter regions of genes under different stimuli, which can lead to silencing certain genetic information leading to a change in the biological function without altering the DNA sequence.

- So far, the decreases in miR-29, miR-26A, miR-30b and miR-195 have been shown to be associated with BAV disease and AA

Nigam et al, 2010
Conclusion

Prakash et al, 2014
Thank you!