Protein-altering and regulatory genetic variants implicated in BAV

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Disclosure of Interest

I do not have any potential conflicts of interest

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What study design issues do we need to account for?

- Discovery of BAV is usually dependent on disease presentation – CAVD, AI or TAAD
- Strong age dependence of disease severity
- Complex inheritance pattern
- Complex embryogenesis – associated with BAV type?

- Strong probability of bias due to other genetic confounders related to clinical presentation
  - *LPA* for tricuspid aortic stenosis
    - (Thanassoulis et al. NEJM 2013)
  - *FBN1* for TAAD
    - (LeMaire et al. Nature Genetics 2011)
Penetrance

Disease and Allele Frequency

High

Intermediate

Modest

Low

Family-based

Sporadic disease

Genetic Contribution to BAV

Same genes $\Rightarrow$ common pathways

LDS

Marfan Turner

Disease and Allele Frequency

Very rare

Rare

Uncommon

Common
LPA gene

Genetic Associations with Valvular Calcification and Aortic Stenosis

Table 1 Meta-analysis results for aortic valve stenosis variants

<table>
<thead>
<tr>
<th>Cases/controls</th>
<th>PALMD intergenic rs7543130 [A/C] EAF = 51.2%</th>
<th>TEX41 intronic rs1830321 [T/C] EAF = 37.5%</th>
<th>LPA intronic rs10455872 [G/A] EAF = 6.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Iceland</td>
<td>1.23 (1.15-1.31)</td>
<td>6.8 × 10^{-10}</td>
<td>1.20 (1.12-1.28)</td>
</tr>
<tr>
<td>Sweden (MDCS)a</td>
<td>1.14 (0.98-1.33)</td>
<td>0.092</td>
<td>1.21 (1.05-1.39)</td>
</tr>
<tr>
<td>Sweden, Stockholm</td>
<td>1.25 (0.98-1.59)</td>
<td>0.068</td>
<td>1.18 (0.89-1.56)</td>
</tr>
<tr>
<td>UK Biobank</td>
<td>1.25 (1.17-1.33)</td>
<td>3.8 × 10^{-11}</td>
<td>1.14 (1.06-1.22)</td>
</tr>
<tr>
<td>Norway (HUNT)</td>
<td>1.13 (1.05-1.22)</td>
<td>0.0012</td>
<td>1.11 (1.02-1.20)</td>
</tr>
<tr>
<td>USA, Michigan</td>
<td>1.15 (0.96-1.39)</td>
<td>0.13</td>
<td>1.01 (0.85-1.24)</td>
</tr>
<tr>
<td>Combined</td>
<td>1.20 (1.16-1.25)</td>
<td>1.2 × 10^{-22}</td>
<td>1.15 (1.11-1.20)</td>
</tr>
</tbody>
</table>

Results are shown for the discovery and follow-up datasets and the joint analysis (combined). The effect allele is the first allele in brackets [effect allele/non-effect allele]. The EAF is for the Icelandic population. P value from logistic regression analysis. Results from the different study groups were combined using a Mantel-Haenszel model.

EAF effect allele frequency, OR allelic odds ratio, 95% CI 95% confidence interval, MDCS Malmö Diet and Cancer study.

a The association results for the rs10455872 variant in the MDCS included 613 cases and 28,109 controls.
Table 2: Association of aortic valve stenosis variants with other cardiovascular traits

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Bicuspid aortic valve</td>
<td>1.26 (0.99, 1.60)</td>
<td>0.059</td>
<td>1.31 (1.03, 1.67)</td>
<td>0.025</td>
</tr>
<tr>
<td>Sweden-Stockholm</td>
<td>1.27 (1.06, 1.52)</td>
<td>0.0098</td>
<td>1.20 (0.99, 1.46)</td>
<td>0.063</td>
</tr>
<tr>
<td>USA-Houston</td>
<td>1.29 (0.99, 1.67)</td>
<td>0.057</td>
<td>1.25 (0.97, 1.60)</td>
<td>0.085</td>
</tr>
<tr>
<td>USA-Boston</td>
<td>1.27 (1.09, 1.48)</td>
<td>0.002</td>
<td>1.10 (0.95, 1.28)</td>
<td>0.21</td>
</tr>
<tr>
<td>USA-Michigan</td>
<td>1.31 (1.14, 1.51)</td>
<td>1.2 × 10^−4</td>
<td>1.00 (0.86, 1.16)</td>
<td>0.97</td>
</tr>
<tr>
<td>Combined BAV</td>
<td>1.28 (1.19, 1.39)</td>
<td>6.6 × 10^−10</td>
<td>1.12 (1.04, 1.22)</td>
<td>3.3 × 10^−3</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>1.23 (1.07, 1.42)</td>
<td>3.9 × 10^−3</td>
<td>1.22 (1.06, 1.41)</td>
<td>5.9 × 10^−3</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>1.23 (1.07, 1.42)</td>
<td>4.8 × 10^−3</td>
<td>1.04 (0.90, 1.21)</td>
<td>0.59</td>
</tr>
<tr>
<td>Coronary artery disease N</td>
<td>1.00 (0.97, 1.02)</td>
<td>0.74</td>
<td>1.05 (1.03, 1.08)</td>
<td>9.3 × 10^−5</td>
</tr>
<tr>
<td>Phenotype (qtl)</td>
<td>0.065 (0.01)</td>
<td>1.3 × 10^−8</td>
<td>β (SE) Value</td>
<td>0.16</td>
</tr>
<tr>
<td>Aortic root diameter</td>
<td></td>
<td></td>
<td>−0.017 (0.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.12 (0.72, 1.75)</td>
<td>0.61</td>
<td>1.19 (0.93, 1.54)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>1.02 (0.92, 1.12)</td>
<td>0.77</td>
<td>1.07 (0.98, 1.16)</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>0.96 (0.51, 1.81)</td>
<td>0.91</td>
<td>1.15 (0.87, 1.52)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>1.44 (1.08, 1.93)</td>
<td>0.014</td>
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<td>0.014</td>
</tr>
<tr>
<td></td>
<td>1.19 (0.93, 1.54)</td>
<td>0.17</td>
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<td>0.17</td>
</tr>
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<td>0.014</td>
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<td></td>
<td>1.31 (1.47, 6.81)</td>
<td>3.2 × 10^−3</td>
<td>4.40 (2.14, 9.07)</td>
<td>5.7 × 10^−5</td>
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<tr>
<td></td>
<td>3.17 (1.47, 6.81)</td>
<td>3.2 × 10^−3</td>
<td>4.40 (2.14, 9.07)</td>
<td>5.7 × 10^−5</td>
</tr>
<tr>
<td></td>
<td>1.21 (1.00, 1.48)</td>
<td>0.056</td>
<td>1.21 (1.00, 1.48)</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>1.07 (0.98 – 1.16)</td>
<td>0.13</td>
<td>1.07 (0.98 – 1.16)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Association of aortic valve stenosis variants with cardiovascular phenotypes is shown for Icelandic samples. Follow-up and joint analysis (combined BAV) is also provided for the association with BAV. The effect allele is the first allele in brackets [effect allele/non-effect allele]. The effect (β) for aortic root diameter is given in standardized units. Logistic (cc) or linear (qtl) regression analyses were used for association testing. Results from the different study groups were combined using a Mantel-Haenszel model.

Cc case-control, Qtl quantitative trait, OR allelic odds ratio, 95% CI 95% confidence interval, BAV bicuspid aortic valve, SE standard error.

PALMD - 1p21.2, abundant in heart and skeletal muscle cytosolic protein involved in lipid rafts, other roles
Genetic Associations between BAV and TAAD

- Syndromic-TAAD (ACTA2, ELN, FBN1, FLNA, SMAD3/6, TGFB2/3, TGFBR1/2)
- Non-syndromic-TAAD (ACTA2, FBN1, LOX, MAT2A, NOTCH1, SMAD3/6, TGFB2, TGFBR1/2)

- But fewer associations with BAV alone
  - NOTCH1 (Familial associations) PMIDs 23578328, 16025100
  - KCNJ2 (Anderson syndrome)
  - Nkx2-5 (Rare variants)
  - GATA5 (Rare variants) PMIDs 24796370, 22641149, 24638895
  - GATA4 (Common and rare variants) PMIDs 28541271, 29325903
  - GATA6 (Rare variants) PMIDs 29653232
Association with largest aortic dimension in BAV

Manhattan-Plot for Aortic dimension GWAS analysis - additive linear model adjusted for age & gender
7,694,440 SNPs with 404 samples (101 females + 303 males)
1q41; P=1.5×10^{-8}
4-dimensional tissue

Aortic Cusp Cross-section

- Endocardial Cells
- Endocardial-Neural Crest Derived Tissue Boundary
- Neural Crest Derived Cells
- Valve Elongation

VEC

300 – 700 μm thickness

- VECs
- Fibroblasts

- Collagen
- Elastin
- GAG’s
- Collagen/Elastin

Fibrosa (~45%)

Pulmonary

Aortic

AC
LC
RC

LCC
RCC
NCC
Protein-altering and regulatory genetic variants near GATA4 implicated in bicuspid aortic valve

Bo Yang1,2,*, Wei Zhou3,*, Jiao Jiao3,*, Jonas B. Nielsen4, Michael R. Mathis5, Mahyar Heydarpour6, Guillaume Lettre2,8, Lasse Folkersen9,10, Siddharth Prakash11, Claudia Schurmann12, Lars Fritsch13,14, Gregory A. Farmar3, Xiaoxuan Lin4, Mohammad Othman15, Whitney Hornsby2, Anisa Driscoll2, Alexandra Levasseur2, Marc Thomas2, Linda Farhat7, Marie-Pierre Dubé2,8, Eric M. Isselbacher6, Anders Franco-Cereceda16, Dong-chuan Guo1, Erwin P. Bottiger17, G. Michael Deeb1,2, Anna Booher2,4, Sachin Kheterpal5, Y. Eugene Chen2,7,4, Hyun Min Kang13, Jacob Kitzman18,17, Heather J. Cordell18, Bernard D. Keenan19,20, Judith A. Goodship18, Santhi K. Ganesh4,17, Gonçalo Abecasis13, Kim A. Eagle2,4, Alan P. Boyle3,17, Ruth J.F. Looe2,21, Per Eriksson7,8,9,9, Jean-Claude Tardif7,8,9, Chad M. Brummett5,8,9, Dianna M. Miliewicz11,1*, Simon C. Body9,12,15, & Cristen J. Willer2,3,4,17,15.

Table 1 | Genetic variants associated with BAV.

<table>
<thead>
<tr>
<th>Chr:posrsid</th>
<th>Protein change</th>
<th>Freq Case/ Ctrl (%)</th>
<th>N Case/ Ctrl</th>
<th>OR</th>
<th>P value</th>
<th>N Case/ Ctrl</th>
<th>Freq Case/ Ctrl (%)</th>
<th>OR</th>
<th>P value</th>
<th>N Case/ Ctrl</th>
<th>OR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:11778803rs6601627</td>
<td>Intergenic</td>
<td>8.2/3.7</td>
<td>466/4,660</td>
<td>2.38 (1.81-3.13)</td>
<td>1.5 × 10^-10</td>
<td>1,021/5,357</td>
<td>7.2/4.2</td>
<td>1.73 (1.42-2.12)</td>
<td>1.1 × 10^-7</td>
<td>1,487/10,017</td>
<td>1.93 (1.64-2.27)</td>
<td>3.0 × 10^-15</td>
</tr>
<tr>
<td>8:11614575rs3729856</td>
<td>p.S377G GATA4</td>
<td>18.2/14.1</td>
<td>466/4,660</td>
<td>1.39 (1.17-1.66)</td>
<td>3.2 × 10^-4</td>
<td>1,326/8,103</td>
<td>15.3/12.7</td>
<td>1.28 (1.14-1.45)</td>
<td>5.3 × 10^-5</td>
<td>1,792/12,763</td>
<td>1.31 (1.19-1.45)</td>
<td>8.8 × 10^-8</td>
</tr>
<tr>
<td>16:72146374rs137867582</td>
<td>p.T1221M DHX38</td>
<td>0.9/0.1</td>
<td>466/4,660</td>
<td>13.14 (5.39-32.04)</td>
<td>1.5 × 10^-8</td>
<td>720/5,831</td>
<td>0.37/0.15</td>
<td>2.87 (1.82-2.22)</td>
<td>5.0 × 10^-2</td>
<td>1,186/10,491</td>
<td>7.13 (3.63-14)</td>
<td>1.2 × 10^-8</td>
</tr>
</tbody>
</table>

BAV, bicuspid aortic valve; Ctrl, control; Freq, frequency; OR, odds ratio.
Functional role of GATA4

- Tempting to invoke its cofactors and ligands in an embryonic mechanism for BAV, notably Cd2, Cdk4, Fog2/3, Tbx5, Sox17, Nkx2-5
- Identified variants are both coding and transcriptional sites
**GATA4 is not a complete story**

- Accounts for <2% heritability of BAV
- Numerous mouse models of BAV occurring with GATA4/5/6 knockouts, NOTCH1, etc.
- Several human sequencing and EWAS studies have identified other putative variants

- GATA4 variants are probably not TAAD or CAVD-causing variants in patients with BAV
- But why don’t BAV GATA4 variants cause ASD or other outflow tract variants?
Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations

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Prem Shekar
Tsuyoshi Kaneko
J. Daniel Muehlschlegel
Jasmine Shahram
Thy Nguyen

BAVCon, Michigan and deCode
Cristen Willer
Bo Yang
Wei Zhou

Aikawa Lab
Elena Aikawa
Mark Blaser