Phenotypic characterization: Refining the denominator

David Valle, MD
McKusick-Nathans Institute of Genetic Medicine
1 May 2018

dvalle@jhmi.edu
For even the strongest variant, nearly all phenotypes exhibit variation in expressivity.

For nearly all phenotypes, heterogeneity of etiology is the rule.

Informed, rigorous, iterative phenotyping yields best data.

For all these reasons and more, phenotyping is difficult!
Box 1: Research strategies using rare, low frequency variants and structural variants*

# 3 - Using targeted sequencing judiciously, focusing on people with extreme or unusual phenotypes

# 5 - Focusing discovery efforts on well-phenotyped groups, accessible families with large sibships, and families that allow return to family members for iterative phenotyping

* Manolio et al., Nature, 2009
Centers for Mendelian Genomics

**Goal:** Identify all genes with high penetrance variants to produce a map of genotype/phenotype relationships for all genes in the genome – “integrative, whole organism phenotypes”

**Strategy:**
- Recruit well-phenotyped probands and families or cohorts from around the world
- Perform WES or WGS on relevant family members
- Use family relationships, allele frequency data, functional predictions, model organism results and functional studies to identify the responsible genes and variants
CMGs: current progress (6yrs)

- Start date 1 Dec 2011

<table>
<thead>
<tr>
<th>Gene discovery</th>
<th>Tier 1</th>
<th>Total</th>
<th>Novel</th>
<th>Known</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tier 2</td>
<td>1000</td>
<td>933</td>
<td>67</td>
</tr>
</tbody>
</table>

- 437 publications
- 5250 unique authors
- 74 countries
BHCMG Gene Discovery
2011 to present

Data directly from submitted quarterly reports

Shalini N Janghani
Donna M. Muzny
Corinne Boehm
Mendelian disease: current scorecard

- Mendelian phenotypes: ~8,500
- Genes with phenotypes: ~3,894 (~19% of total)
- Explained phenotypes: ~6,187
- Unexplained phenotypes: ~2,500

OMIM.org

OMIM®
Online Mendelian Inheritance in Man®
An Online Catalog of Human Genes and Genetic Disorders
Updated 1 May 2018

Advanced Search: OMIM, Clinical Synopses, OMIM Gene Map

~300 new phenotypes/yr
Some lessons learned

- Heterogeneity extensive
  - Allelic often with different phenotypes
  - Locus
- Atypical phenotypes - Multi-Mendels
- Genome/epigenome interactions
- Penetrance
Allelic heterogeneity and the Phenotype/Gene relationship*

Disease genes = 2779
Phenotypes = 4631
Phenos/dz gene = 1.67

* OMIM
Locus heterogeneity: Robinow syndrome and the Wnt-PCP pathway (Lupski et al.)

**Gene**
- **WNT5A**: Noncanonical Wnt ligand
- **ROR2**: Co-receptor for WNT5A ligand
- **DVL1**: Mediates canonical & noncanonical signals
- **DVL3**: Mediates canonical & noncanonical signals
- **FZD2**: Co-receptor for WNT5A ligand
- **RAC3**: Downstream GTPase effector

**Clinical evidence**
- Person *et al.* *Dev Dyn.* (2010)
- 5 families (current)
- 1 *de novo* variant identified

**BHCMG**
Internal Robinow database

<table>
<thead>
<tr>
<th>Robinow Gene</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROR2-recessive</td>
<td>3/34: 9%</td>
</tr>
<tr>
<td>WNT5A</td>
<td>1/34: 3%</td>
</tr>
<tr>
<td>DVL1</td>
<td>9/34: 26%</td>
</tr>
<tr>
<td>DVL3</td>
<td>6/34: 18%</td>
</tr>
<tr>
<td>FZD2</td>
<td>5/34: 15%</td>
</tr>
<tr>
<td>Gene in differential</td>
<td>4/34: 12%</td>
</tr>
</tbody>
</table>

**Cell polarity/cytoskeleton reorganization**
Recognition informs diagnosis, treatment, recurrence risk

Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation

Jennifer E. Posey, M.D., Ph.D., Tamar Harel, M.D., Ph.D., Pengfei Liu, Ph.D., Jill A. Rosenfeld, M.S., Regis A. James, Ph.D., Zeynep H. Coban Akdemir, Ph.D., Magdalena Walkiewicz, Ph.D., Weimin Bi, Ph.D., Rui Xiao, Ph.D., Yan Ding, M.D., Fan Xia, Ph.D., Arthur L. Beaudet, M.D., Donna M. Muzny, M.S., Richard A. Gibbs, Ph.D., Eric Boerwinkle, Ph.D., Christine M. Eng, M.D., V. Reid Sutton, M.D., Chad A. Shaw, Ph.D., Sharon E. Plon, M.D., Ph.D., Yaping Yang, Ph.D., and James R. Lupski, M.D., Ph.D., D.Sc.

2017
Epigenome Patterns in Kabuki Syndrome *

- Kabuki syndrome
  - ID, growth retardation, dysmorphic features
  - $KMT2D$ (~65%), $KDM6A$ (4%)

- Sequencing in 27 KS probands
  - 12 with variants in $KMT2D$
  - 6 with likely pathologic variants in other genes incl. $KMT2A$, $HCFC1$, $ZBTB24$, $KMT2B$, $DNMT3B$

* Sobreira, ….Bjornsson, EJHG, 2017
How do histone alterations produce disease in KS? *

- Examine DNA methylation patterns in 9 KS probands and 9 age, sex matched controls to identify differentially methylated regions (DMRs)

- Whole blood DNA samples

- Infinium HumanMethylation 450 Bead Chip

* Sobreira, ….Bjornsson, EJHG, 2017
DNA methylation patterns in KS? *

* Sobreira, ….Bjornsson, EJHG, 2017
How do histone alterations produce disease in KS? *

- Alterations in patterns of DNA methylation secondary to defects in genes encoding histone modifying enzymes
- Suggests “cross talk” between epigenome changes in histones and DNA methylation
- Patterns similar in KS secondary to KMT2D, KDM6A and to KMT2A (Wiedermann-Steiner) suggesting similar phenotype derives from similar epigenetic pattern
- Results point to a set of downstream genes that may be important for pathogenesis

* Sobreira, ....Bjornsson, EJHG, 2017
TBX6 Null Variants and a Common Hypomorphic Allele in Congenital Scoliosis

Nan Wu, M.D., Xuan Ming, B.S., Jianqi Xiao, Ph.D., Zhihong Wu, M.D., Xiaoli Chen, M.D., Ph.D., Marwan Shinawi, M.D., Yiping Shen, Ph.D., Guangju Yu, B.S., Jiaqi Liu, M.D., Hua Xie, M.D., Zoran S. Gucev, M.D., Ph.D., Sen Liu, M.D., et al.

NEJM, 2015
Two locus inheritance of non-syndromic midline craniosynostosis via rare SMAD6 and common BMP2 alleles

Andrew T Timberlake¹,²,³, Jungmin Choi¹,², Samir Zaidi¹,², Qiongshi Lu⁴, Carol Nelson-Williams¹,², Eric D Brooks³, Kaya Bilguvar¹,⁵, Irina Tikhonova⁵, Shrikant Mane¹,⁵, Jenny F Yang³, Rajendra Sawh-Martinez³, Sarah Persing³, Elizabeth G Zellner³, Erin Loring¹,²,⁵, Carolyn Chuang³, Amy Galm⁶, Peter W Hashim³, Derek M Steinbacher³, Michael L DiLuna⁷, Charles C Duncan⁷, Kevin A Pelphrey⁸, Hongyu Zhao⁴, John A Persing³, Richard P Lifton¹,²,⁵,⁹*
Craniosynostosis: Two locus model*

- WES of a cohort of 191 (132 trios, 59 additional probands)
- 13 (7%) rare damaging de novo or transmitted variants in \textit{SMAD6}
- \textit{SMAD6} variants showed ~60% incomplete penetrance
- Previous GWAS identified common variant (allele frequency of 0.35) 345 kb downstream of BMP2

<table>
<thead>
<tr>
<th>SMAD6/BMP2</th>
<th>Synostosis +</th>
<th>Synostosis -</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMAD6 +/BMP2 risk +</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>SMAD6 +/BMP2 risk -</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>SMAD6 -/BMP2 risk +</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

Timberlake et al., eLIFE, 2016
Phenotype risk scores identify patients with unrecognized Mendelian disease patterns

Lisa Bastarache,1 Jacob J. Hughey,1 Scott Hebbring,2 Joy Marlo,1 Wanke Zhao,3 Wanting T. Ho,3 Sara L. Van Driest,4,5 Tracy L. McGregor,5 Jonathan D. Mosley,4 Quinn S. Wells,4,6 Michael Temple,1 Andrea H. Ramirez,4 Robert Carroll,1 Travis Osterman,1,4 Todd Edwards,4 Douglas Ruderfer,4 Digna R. Velez Edwards,7 Rizwan Hamid,5 Joy Cogan,5 Andrew Glazer,4 Wei-Qi Wei,1 QiPing Feng,6 Murray Brilliant,2 Zhizhuang J. Zhao,3 Nancy J. Cox,4 Dan M. Roden,1,4,6 Joshua C. Denny1,4,*

- Phenotype risk scores (PheRSs) for > 1200 Mendelian traits from EHR
- Map back to ~21,000 genotyped individuals at Vanderbilt
- Identify associations with rare variants suggesting candidate responsible gene and variants
Thanks for your attention!

Acknowledgements:
CMGs and especially BHCMG colleagues