CSER and Beyond: Challenges and Opportunities in Interpreting Variants and Actionability

Gail Jarvik, MD, PhD
Motulsky Chair of Medicine and Genome Sciences
Head, Medical Genetics
University of Washington, Seattle

Stipulate that for optimal care we need 1)germline variants pathogenicity annotated with justification 2) to be consistent in these

- Average person's WGS has >3.5 million variants*
  - 0.6 million are rare or novel
  - 400 genes have rare or novel, nonsynonymous variants in conserved regions
- Total unique variant submissions to ClinVar with clinical assertions = 111,446 (Sept 21, 2015) http://www.ncbi.nlm.nih.gov/clinvar/submitters/
  - If multiple assertions, 17% contradict (per H. Rehm)

cser Clinical Sequencing Exploratory Research

**Kohane, Tsing, Kong, Genet Med 2012, PMID 22323072*

CSER bake-off 2: Intra-laboratory Usual vs. ACMG Classification Comparison of 99 variants*

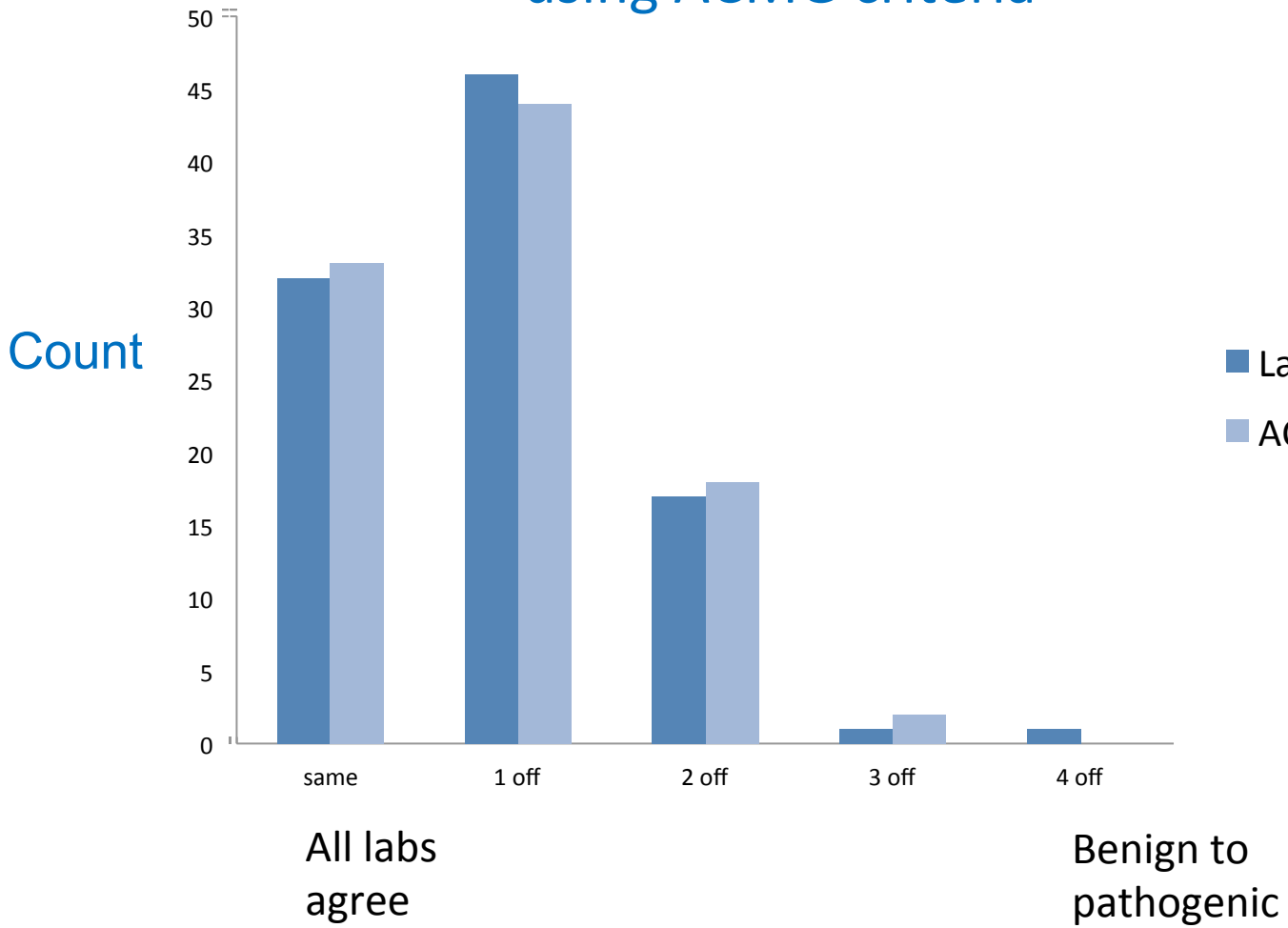
ACMG Class	Lab Class: P	LP	VUS	LB	B	Total
P	62	8	5	0	0	75
LP	12	55	4	0	0	71
VUS	2	6	94	17	4	123
LB	0	0	3	34	7	44
B	0	0	0	4	30	34
Total	76	69	106	55	41	**347**

Cramér's V = 0.75

- 275/347 (79%) concordant
- 11/347 (3.2%) shift by >1 class
- If discordant, 45/67 (67%); ACMG less certain (e.g. VUS)
- Labs call more things benign, likely benign.

*Bakeoff:
99 germline variants
-9 classified by 9 sites
-90 classified by 3 sites
by ACMG and own rules

Inter-laboratory Concordance of 97 variants did not improve using ACMG criteria



P=NS

1.Pathogenic
2.Likely pathogenic
3.VUS
4.Likely benign
5.Benign

Difference in classifications across labs

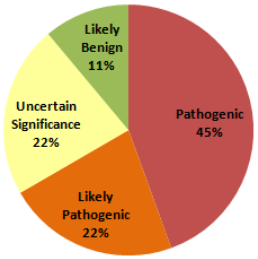
cser
Clinical Sequencing Exploratory Research

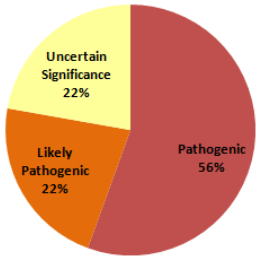
Resolving Variants with Disagreement

SPG7:c.1529C>T (p.Ala510Val)

- 0.8% people carry; ExAC
- AR, late-onset, +/- reduced penetrance, spastic paraplegia

Laboratory classification





ACMG Classificatio

- **32/61 (52%) of discordants discussed to date are resolved**
- **To ClinVar**

Cosegregation

MAF > disease frequency

Functional evidence

Missense gene with low rate of

Computational

Pt phenotype specific for gene

Laboratory class	ACMG Rules	PP3	PS3	PM3	PP1	PS1	PS4	PP5	PM2	BS1	PP2	PP4	ACMG lines of evidence
Pathogenic	Pathogenic	X	X	X			X	X					PS3,PS4,PM3,PP3,PP5
Pathogenic	Pathogenic	X	X	X	X	X		X					PS1, PS3, PM3, PP1, PP3, PP5
Pathogenic	Pathogenic	X	X	X	X	X	X						PS1, PS3(moderate) ,PS4, PM3, PP1, PP3
Pathogenic	Pathogenic			X	X								PM3 (strong), PP1 (strong)
Likely Pathogenic	Likely Pathogenic	X	X	X	X		X		X				PP1, PP3, PM2, PM3, PS3(weak), PS4
Likely Pathogenic	Likely Pathogenic	X				X		X					PS1, PP3, PP5
Uncertain Significance	Pathogenic	X	X						X		X	X	PS3, PM2, PP2, PP3, PP4
Likely Benign	Uncertain Significance		X			X	X			X			PS1, PS3, PS4, BS1
Uncertain Significance	Uncertain Significance	X			X					X			PP1, PP3 BS1
		7	6	5	5	4	4	3	2	2	1	1	

Opportunities: Variant sorting

- CSER efforts to improve variant pathogenicity classification must continue.
- Continue to
  - identify sources of variation
  - clarify and improve ACMG guidelines
  - deposit to ClinVar
- Expand to:
  - non-CSER labs
  - low penetrance variants
    - no system now

cser
Clinical Sequencing Exploratory Research

Opportunities

Maximize variants classified:

- Exome/genome level data optimal
- Re-phenotype based on genotype
  - Public variant database/outside requests and local interest (LoF)
  - Putative pathogenic variants that are frequent
- Re-analyzed sequence if
  - phenotype changes
  - new genes for condition reported
- High throughput functional partners

cser
Clinical Sequencing Exploratory Research

Re-phenotype based on genotype

- *ACVRL1* c.1249A>T, p.Ile417Phe found in 56 yo
- Hereditary hemorrhagic telangiectasia (AD)
  - Cutaneous, membrane, organ telangiectasias
  - Nose and GI bleeds
  - Arteriovenous malformations of lungs (50%), liver (30–70%) and the brain (10%)
- Disease 1/5000, SNV 1/5000 EU
- Published report of affected patient with this SNV
- On return – normal skin exam, no hx epistaxis
  - Data to ClinVar
  - Advantage if exome/genome

Photo: Herbert L. Fred, MD and Hendrik A. van Dijk - Case 115

cser
Clinical Sequencing Exploratory Research

Opportunities

- Tumor sequence reanalysis and correlate with outcomes
- EHR integration
- Training, including diversity
- Clinician and patient report/communication
  - Do clinicians reinterpret variants?
  - Outside academic centers
  - Underserved and Non-European ancestry

cser
Clinical Sequencing Exploratory Research

Gene lists

For primary indication:

+ Focuses efforts / saves time
  Reduces VUS

- Limits discovery

Opportunity: Cost/benefit not evaluated?

For incidental findings:

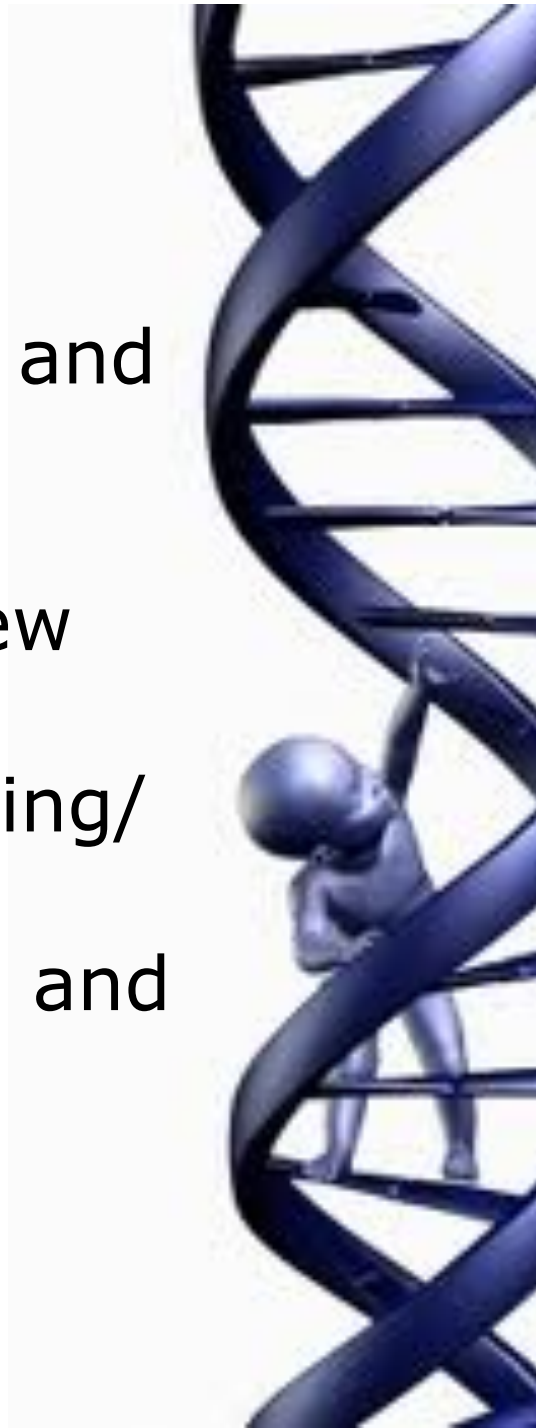
Generally support actionable genes

Opportunities: Consensus list? List of lists?
Methods of consent and education?

cser
Clinical Sequencing Exploratory Research

Major Themes

- Determination of pathogenicity and sources of variability in interpretation
- Reanalysis of data in light of new information
- What are the implications of using/ not using a gene list?
- Results reporting and physician and patient perspective?



cser
Clinical Sequencing Exploratory Research

Acknowledgements

CESR members involved in bakeoff: Jarvik GP, Amendola LA, Leo, M, McLaughlin H, Milosavljevic A, Horton C, Ghosh R, Dorschner M, Punj S, Pak C, Akkari Y, Salama J, Cooper, G, Biesecker L, Conlin LK, Biswas S, Dulik M, Ghazani A, Strande NT, Yang Y, Van Allen E, Wagle N, Green RC, Krantz I, Chinnaiyan A, Berg JS, Evans JP, Garraway L, Goddard KAB, Spinner N, Plon SE, Richards S, and Rehm HL

The whole CSER team; esp. UO1 PIs: Sharon Plon, Will Parsons, Robert Green, Leslie Biesecker, Ian Krantz, Nancy Spinner, Levi Garraway, Pasi Janne, Richard Myers, Katrina Goddard, Ben Wilfond, Arul Chinnaiyan, Jim Evans, Gail Jarvik, Wylie Burke, Debbie Nickerson, and Peter Tarczy-Hornoch

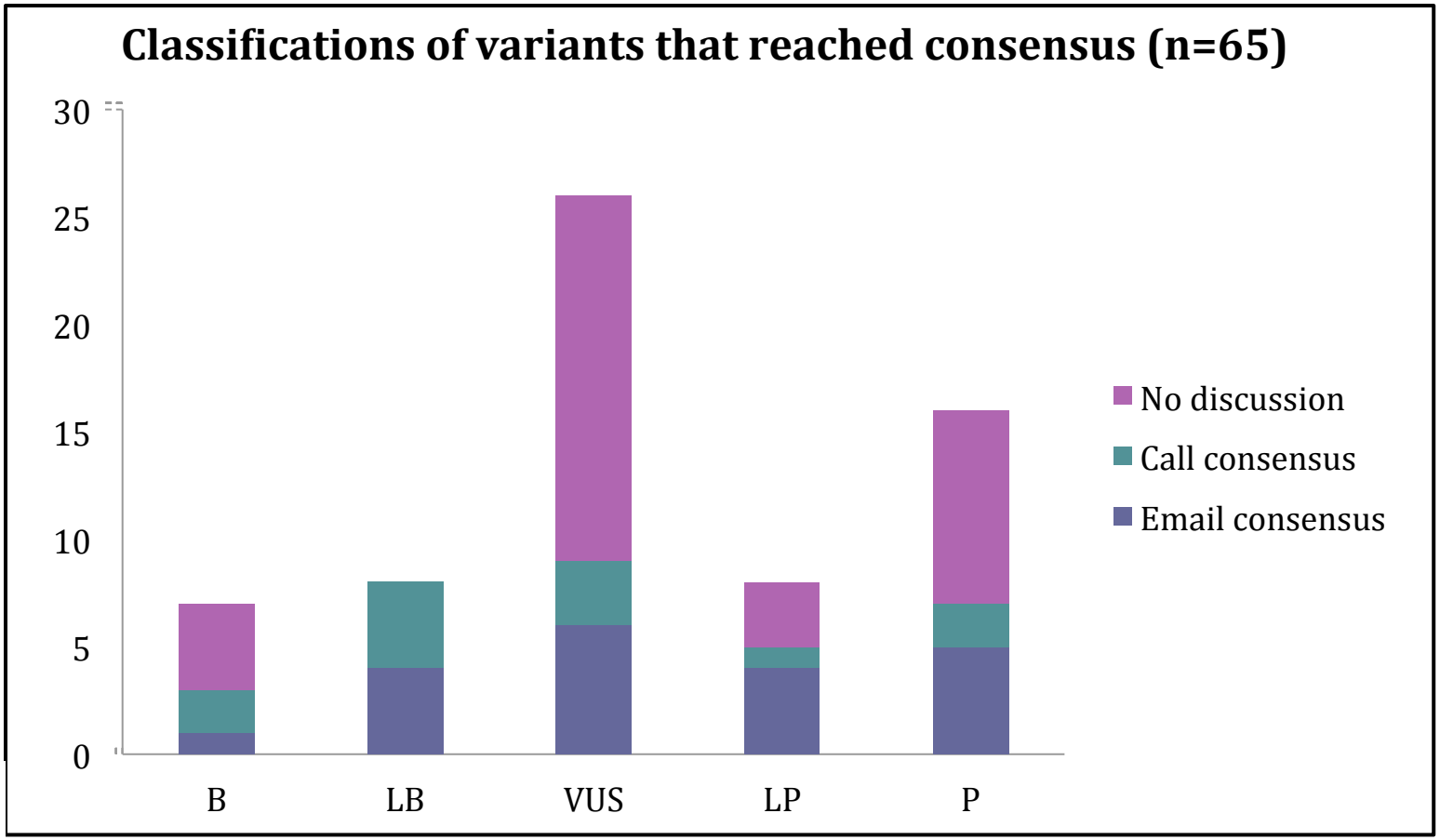
Funding: NHGRI & NCI (including U01HG006507, U01HG006375)

cser
Clinical Sequencing Exploratory Research

Extra slides

32 of 61 (52%) of ACMG discordants resolved

- 61/65 variants with discordant ACMG classifications reviewed to date
  - 12/19 (63%) 'call consensus' variants resolved; 7 calls **(5.5 hours!)**
  - 20/42 (48%) 'email consensus' variants resolved



cser
Clinical Sequencing Exploratory Research

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

ACMG Standard Recs
Richards et al GIM 2015
PMID:25741868