November 18, 2021

Yale School of Medicine
Genetics Clinical Grand Rounds

NIH StrokeNet Grand Rounds Webinar

Emerging Analysis Methods Related to Genetics and Personalized Medicine

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Disclosures / Conflicts of interest

- Conflicts of Interest
  - None

- Disclosures / Funding
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  - Neurocritical Care Society Research Fellowship

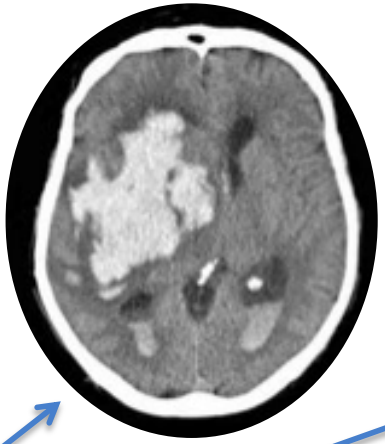
Roadmap

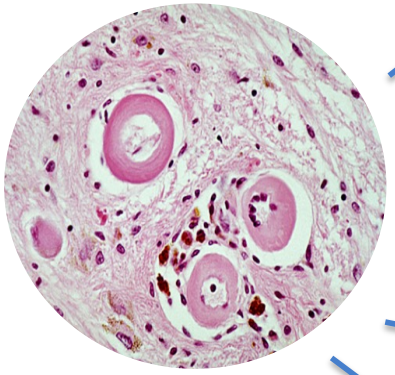
- What we do
- Concrete examples related to stroke genomics
  - Pathway discovery
  - Prediction
- Ongoing and future plans to bring population genetics to the bedside of stroke patients
  - Clinical trials as a platform
  - Returning polygenic risk score results to treating clinicians

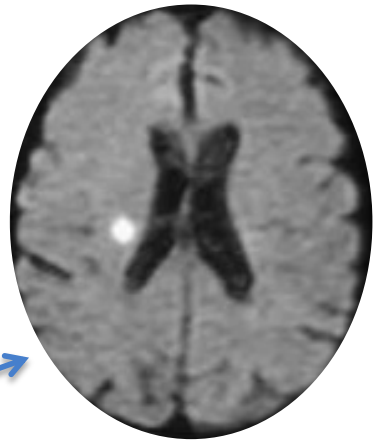
Part I
What do we do? How do we do it?

Cardiovascular & Cerebrovascular Disease + Stroke

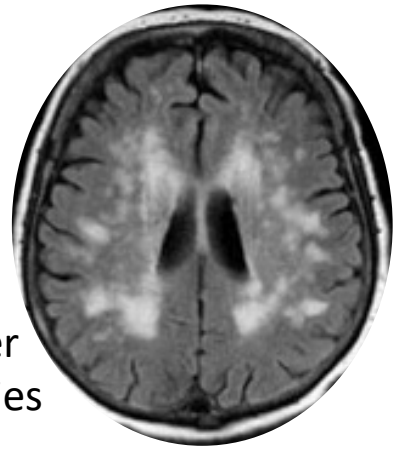
Spontaneous intracerebral hemorrhage



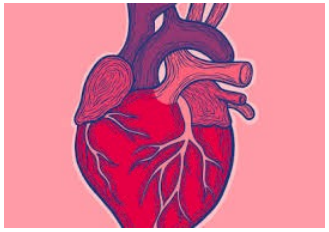




Small vessel ischemic stroke



White matter hyperintensities



Disability

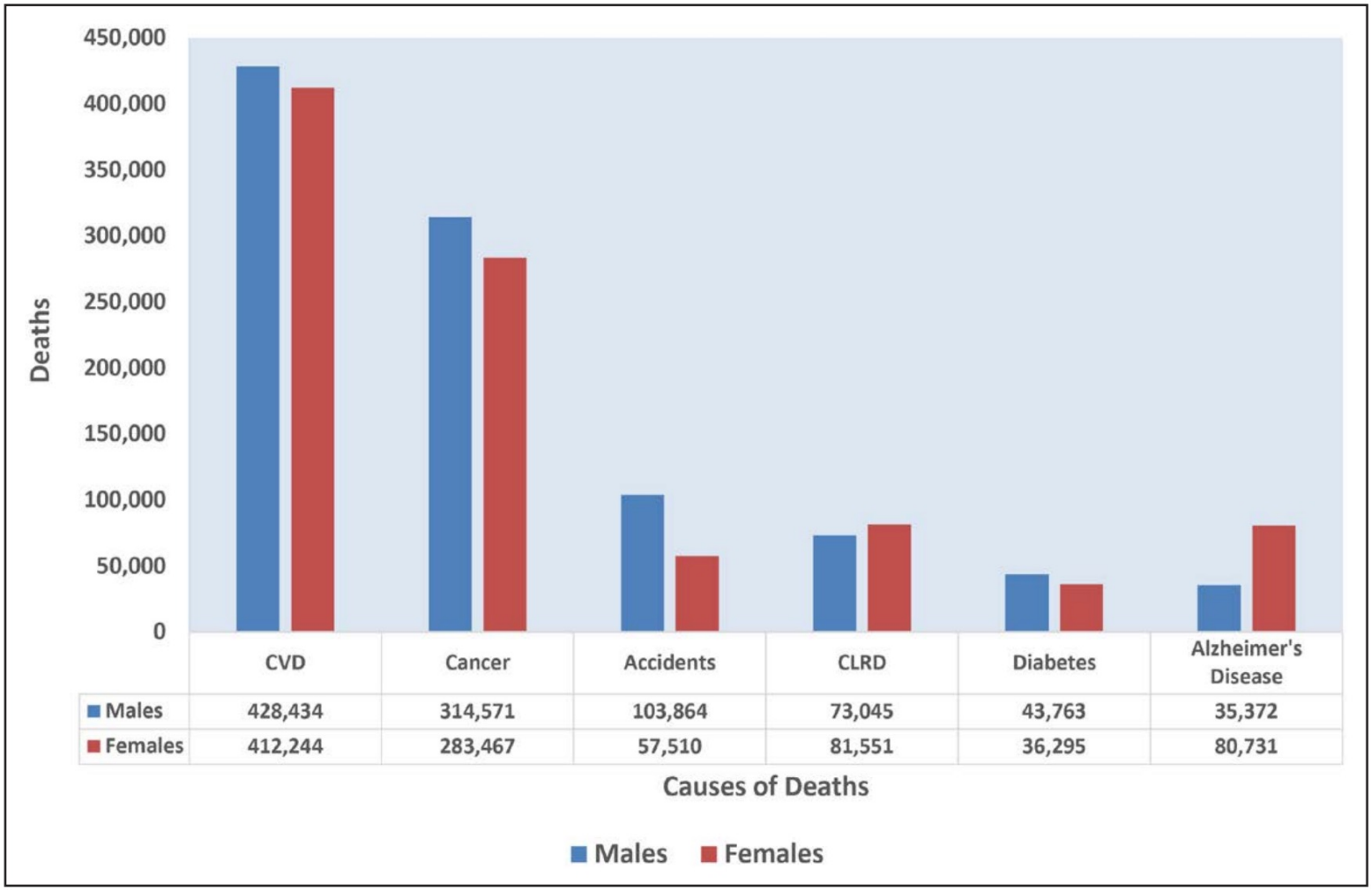
Gait impairment

Cognitive Decline & Dementia

Lancet Neurol. 2014 Apr;13(4):405-18.

Cardiovascular & Cerebrovascular Disease + Stroke

CVD and other major causes of death for all males and females (United States, 2016).



Heart Disease and Stroke Statistics—2019 Update

Cardiovascular & Cerebrovascular Disease + Stroke

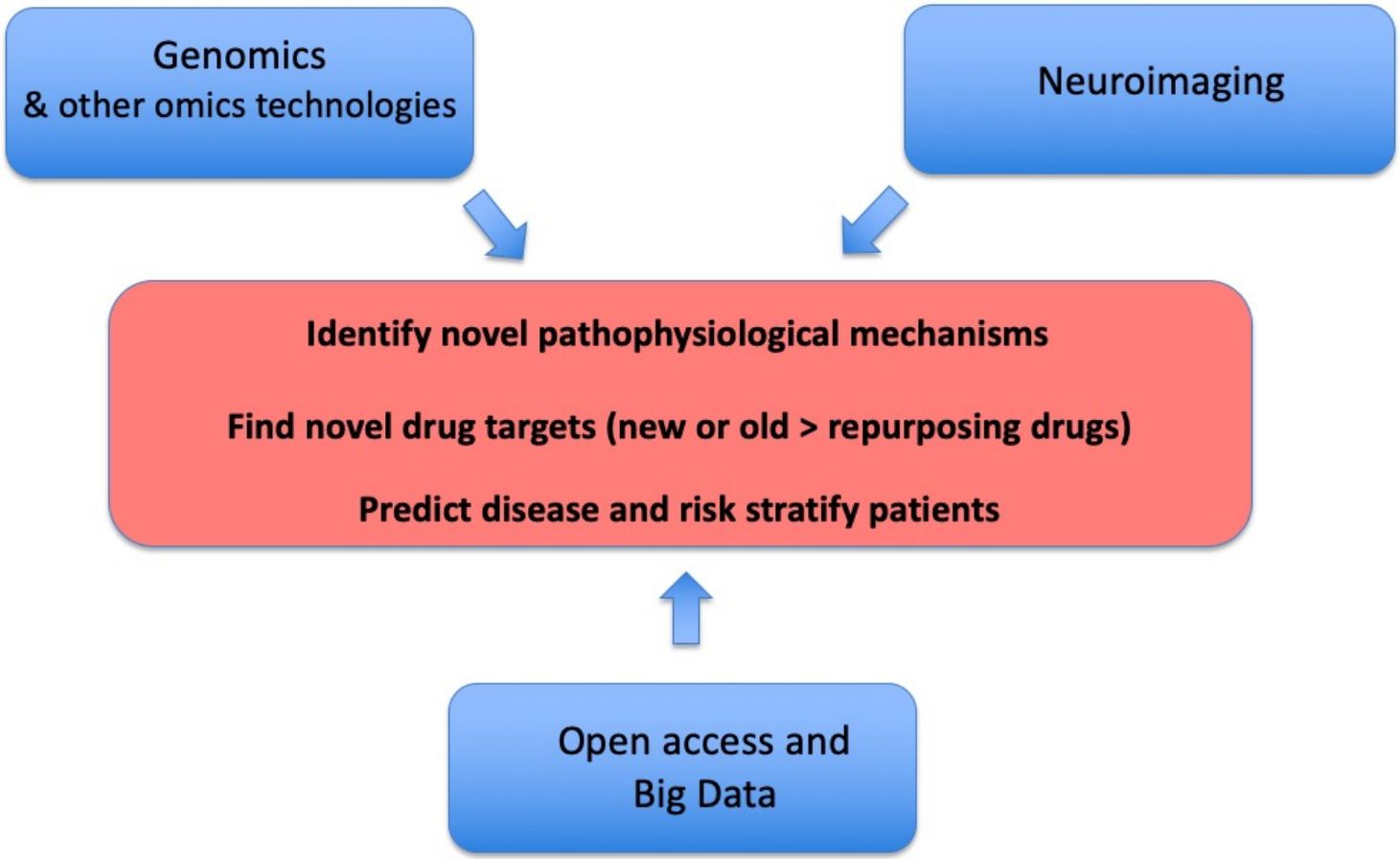
CAD / MI
Stroke
Dementia

Cognitive decline
Frailty

Heart Disease and Stroke Statistics—2019 Update

Mission statement
Bring genomic medicine to stroke patients

Research strategy



Part II

Causal inference & pathway discovery

Genetically-Determined Lipid Levels and Risk of Hemorrhagic Stroke

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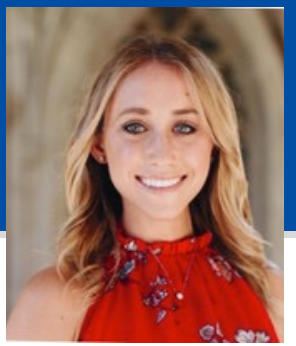
Prior evidence



Elayna Kirsch

- Elevated LDL is bad in general, however …
- Evidence from observational studies
  - Total cholesterol and LDL: inverse association with ICH
- Evidence from randomized clinical trials
  - Post-hoc analysis of the SPARCL trial (secondary prevention)
    - atorvastatin is associated with increased ICH risk

Ann Neurol. 2020 Jul;88(1):56-66.

Hypothesis and Goals

- Investigate whether the cumulative burden of genetic variants related to lipid levels influences ICH risk
- Investigate whether the association above is mediated by specific lipid (total cholesterol versus LDL versus HDL versus triglycerides)
- Evaluate the causal effect of genetically-instrumented lipids levels on ICH risk

Ann Neurol. 2020 Jul;88(1):56-66.

Methods

Stage 1

Identify lipid-related loci ($p<1x10E-8$)

Construct lipid fraction-specific polygenic risk scores (PRS)

Test for association between each PRS and its corresponding lipid fraction in the UK Biobank

Stage 2

Association between lipids PRS and risk of ICH
4 case/control genetic studies of ICH

Implement sensitivity analysis to test different building strategies for the GRS building

Stage 3

Utilize the effect estimates from Stage 1 and Stage 2 to conduct Mendelian Randomization analyses to estimate the effect of instrumented lipid levels and risk of ICH

Ann Neurol. 2020 Jul;88(1):56-66.

Population characteristics

	Effect of Lipid PRS on lipids	Effect of Lipid PRS on risk of intracerebral hemorrhage		
Characteristic	**UK Biobank**	**GOCHA**	**ISGC ICH Study**	**GERFHS**
Analytical stage	Association Cholesterol level ~ PRS	Association ICH Risk ~ PRS	Association ICH Risk ~ PRS	Association ICH Risk ~ PRS
Study design	Cohort	Case / Control	Case / Control	Case / Control
Study participants	316,428	277 / 248	563 / 523	446 / 490
Age, mean (SD)	68 (8)	73 (10) / 72 (8)	71 (14) / 66 (16)	70 (14) / 68 (13)
Female sex n, %	170,871 (54)	130 (47) / 123 (50)	252 (45) / 255 (49)	211 (47) / 235 (48)
Genotyping platform	Affymetrix UK Biobank array	Illumina HumanHap550	Illumina HumanHap550	Affymetrix 6.0
Genotyped SNPs	820,967	527,508	527,508	580,491
Imputed SNPs	73,355,667	7,965,700	7,965,700	7,967,430

Ann Neurol. 2020 Jul;88(1):56-66.

Lipid-based polygenic risk scores explain a significant proportion in the observed variation in lipids

Lipid Trait PRS	Independent SNPS in PRS	UK Biobank Effective sample size	Mean increase in cholesterol trait per 1-SD increase in PRS	Standard error	Variance explained	P
Primary analysis *						
Total Cholesterol	410	316,428	0.33 mmol/L	0.0018	9.33%	$<1x10^{-100}$
LDL Cholesterol	339	315,841	0.24 mmol/L	0.0014	8.38%	$<1x10^{-100}$
HDL Cholesterol	393	289,349	0.11 mmol/L	0.0006	8.17%	$<1x10^{-100}$
Triglycerides	317	316,174	0.22 mmol/L	0.0017	4.8%	$<1x10^{-100}$
Secondary analysis **						
Total Cholesterol	410	437,676	0.26 mmol/L	0.0017	5.21%	$<1x10^{-100}$
LDL Cholesterol	339	436,867	0.19 mmol/L	0.0013	4.72%	$<1x10^{-100}$
HDL Cholesterol	393	400,579	0.11 mmol/L	0.0005	8.04%	$<1x10^{-100}$
Triglycerides	317	437,331	0.23 mmol/L	0.0015	5%	$<1x10^{-100}$

Ann Neurol. 2020 Jul;88(1):56-66.

Lipid-based polygenic risk scores for total and LDL cholesterol and risk of intracerebral hemorrhage

Study	Total Cholesterol		LDL Cholesterol		HDL Cholesterol		Triglycerides	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
GOCHA	0.95 (0.80 - 1.1)	0.59	0.93 (0.78 - 1.1)	0.41	1.1 (0.94 - 1.3)	0.20	0.95 (0.80 - 1.1)	0.59
ISGC ICH Study	0.93 (0.82 - 1.1)	0.24	0.88 (0.77 - 0.99)	0.04	1.1 (1.0 - 1.3)	0.03	1.0 (0.90 - 1.1)	0.84
GERFHS	0.88 (0.77 - 1.0)	0.07	0.85 (0.75 - 0.97)	0.02	0.99 (0.86 - 1.1)	0.84	1.2 (1.0 - 1.4)	0.009
Metanalysis	0.92 (0.85 - 0.99)	0.03 Het-p 0.77	0.88 (0.81 - 0.95)	0.002 Het-p 0.75	1.10 (1.01 - 1.21)	0.06 Het-p 0.23	1.11 (0.98 - 1.23)	0.14 Het-p 0.08

Ann Neurol. 2020 Jul;88(1):56-66.

Genetically-elevated total and LDL cholesterol are associated with decreased risk of ICH

Mendelian Randomization Method	Instrument	Total Cholesterol		LDL Cholesterol	
		OR (95%CI)	P	OR (95%CI)	P
Ratio method	Polygenic risk score using on individual level data	0.77 (0.6 - 0.98)	0.03	0.59 (0.42 - 0.82)	0.002
IVW	Multiple SNPs using summary level data	0.84 (0.72 - 0.99)	0.04	0.65 (0.52 - 0.82)	<0.001
Weighted median	Multiple SNPs using summary level data	0.95 (0.72 - 1.30)	0.74	0.79 (0.56 - 1.10)	0.20
MR-Egger (Intercept)	Multiple SNPs using summary level data	1.0 (0.99 - 1.0)	0.81	1.0 (0.98 - 1.0)	0.59

Ann Neurol. 2020 Jul;88(1):56-66.

How about aneurysmal subarachnoid hemorrhage? (the other frequent form of hemorrhagic stroke)

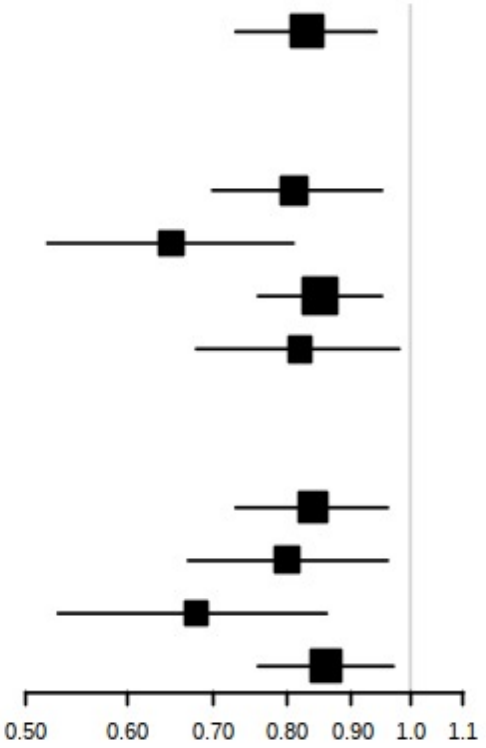
Julian Acosta

- Two sample Mendelian Randomization
- Instruments
  - Independent (r2 <0.1) SNPs associated with circulating LDL-C at genome-wide levels (p<5e-8)
  - Total: 117 SNPs
- Primary analysis:
  - Europeans + Asians
  - Composite of aneurysm presence or aneurysmal subarachnoid hemorrhage
- Secondary analyses:
  - Europeans only
  - Aneurysmal subarachnoid hemorrhage

Ann Neurol. 2021 Oct 28. Online ahead of print. PMID: 34709661

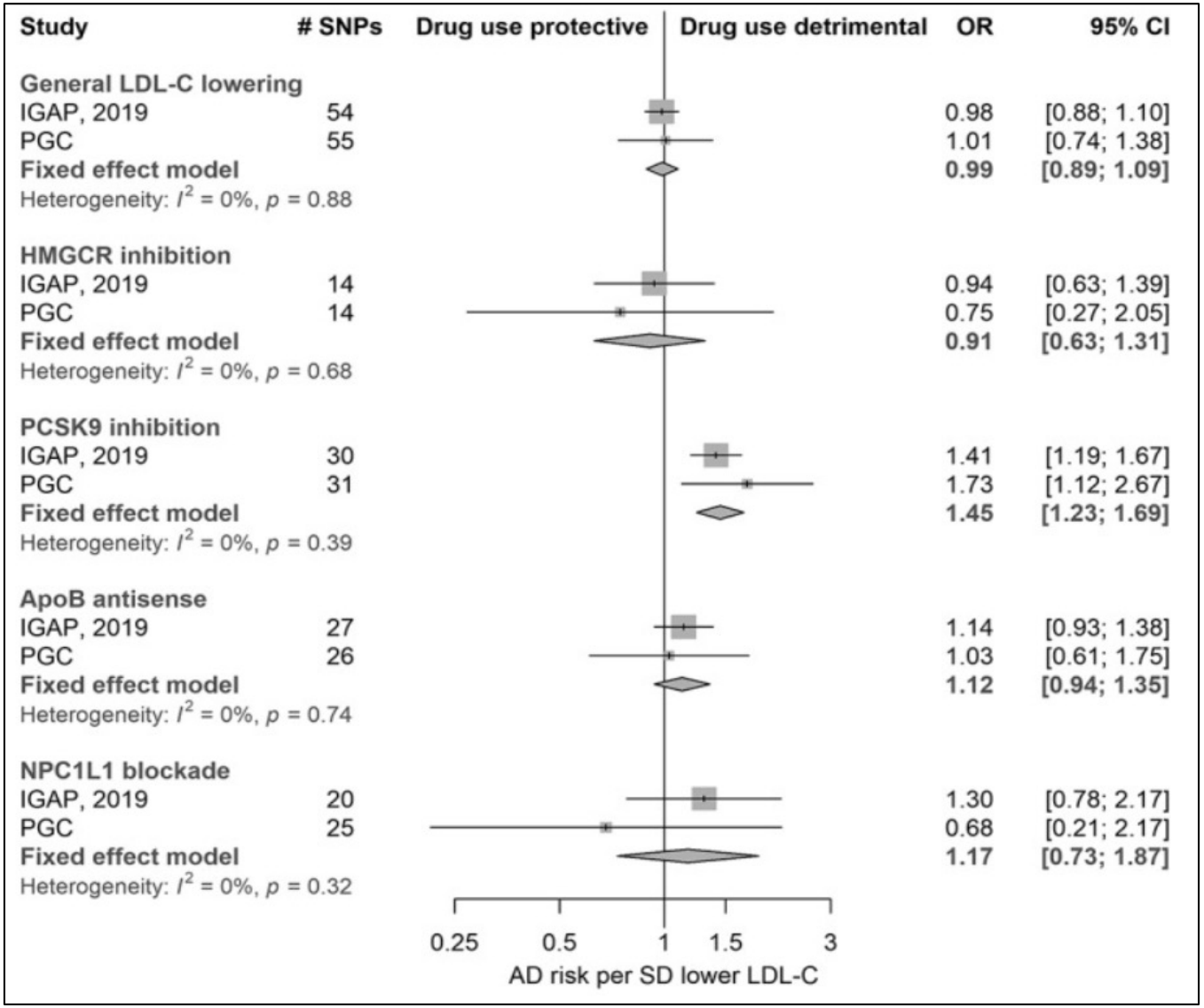
How about aneurysmal subarachnoid hemorrhage? (the other frequent form of hemorrhagic stroke)

MR Method		OR (95%CI)	p
Primary analysis			
IVW	117	0.83 (0.73-0.94)	0.004
Secondary analyses			
WM	117	0.81 (0.7-0.95)	0.008
MR-Egger	117	0.65 (0.52-0.81)	<0.001
MR-PRESSO	116	0.85 (0.76-0.95)	0.005
LDL-C specific	39	0.82 (0.68-0.98)	0.03
European ancestry only			
IVW	129	0.84 (0.73-0.96)	0.01
WM	129	0.8 (0.67-0.96)	0.02
MR-Egger	129	0.68 (0.53-0.86)	0.002
MR-PRESSO	128	0.86 (0.76-0.97)	0.01



Ann Neurol. 2021 Oct 28. Online ahead of print. PMID: 34709661

More on CNS and LDL lowering
PCSK-9 inhibition and risk of Alzheimer's Disease



Ann Neurol. 2020 Jan; 87(1): 30–39.

Possible mechanisms?

- **BBB integrity**
  - Adequate lipid level is essential for maintaining normal membrane fluidity and vessel integrity -> low LDL-C leads to increased blood-brain barrier permeability with increased vessel wall smooth muscle necrosis.

- **Two hits hypothesis**
  - Lipid lowering (first hit) plus additional stressors (blood thinners, hypertension, inflammation/infection) lead to BBB permeability

Stroke. 2009 Feb;40(2):454-61.
Atherosclerosis. 2018 Mar;270:191-192.

Some answers are coming

- SATURN Randomized clinical trial
  - STATINS USE IN INRACEREBRAL HEMORRHAGE PATIENTS
- Funded by the NINDS
- PROBE design
  - pragmatic, prospective, randomized, open-label, and blinded end-point assessment
- 140 sites in the US and Canada
- 1500 survivors of intracerebral hemorrhage with an indication for a statin
- Randomized to continuing or stopping statins

Part IV

The present is exciting, the future is even better

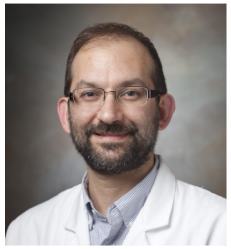
How do we bring stroke genomics to the bedside?

AHA Bugher Network of Centers for ICH Research
$11M gift from the Bugher Foundation

Yale University
School of Medicine

UCSF
University of California
San Francisco

Yale/AHA Bugher Center for ICH Research



Legend
- Basic
- Clinical
- Population

Yale AHA/ASA Bugher Center of Excellence in Hemorrhagic Stroke

External Advisory Collaborators
Jonathan Rosand, MD (MGH)
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Project 3 (Population)
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Michael Murray, MD
Kevin Sheth, MD
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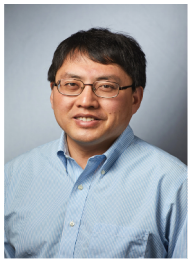
Polygenic Susceptibility to Hypertension in ICH: from Population Studies to Precision Medicine at the Bedside

Hypothesis:
Polygenic susceptibility to hypertension influences the clinical trajectory of ICH survivors

Main goal
Evaluate clinical applications of genomic data and bring population genetics to the bedside of ICH survivors



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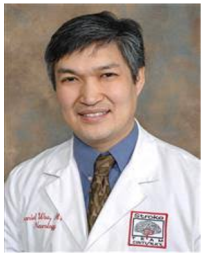
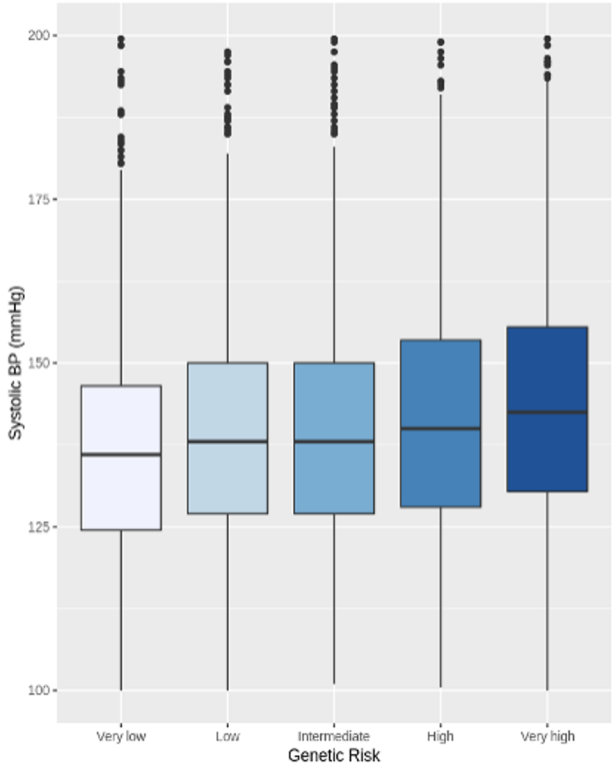


Daniel Woo
U of Cincinnati

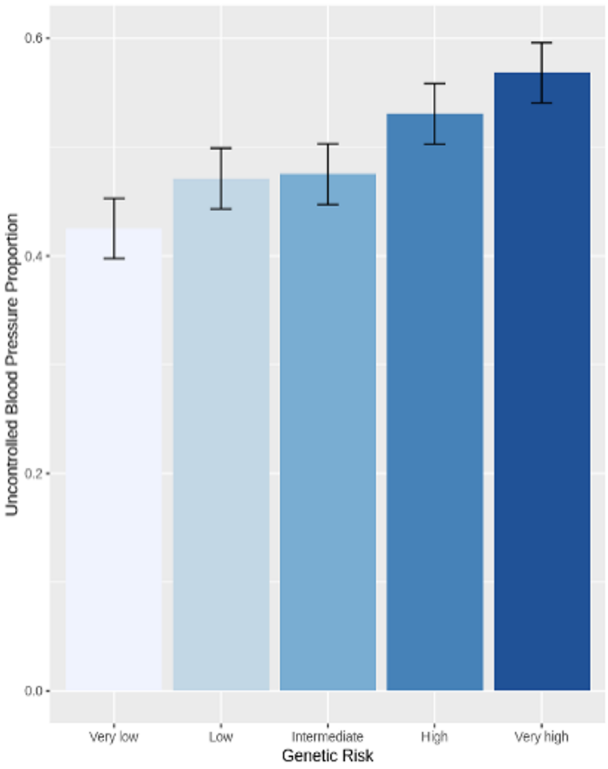
Ashkan Shoamanesh
McMaster

Polygenic susceptibility to hypertension and blood pressure trajectories after stroke
4,652 ischemic and hemorrhagic strokes enrolled in the UK Biobank

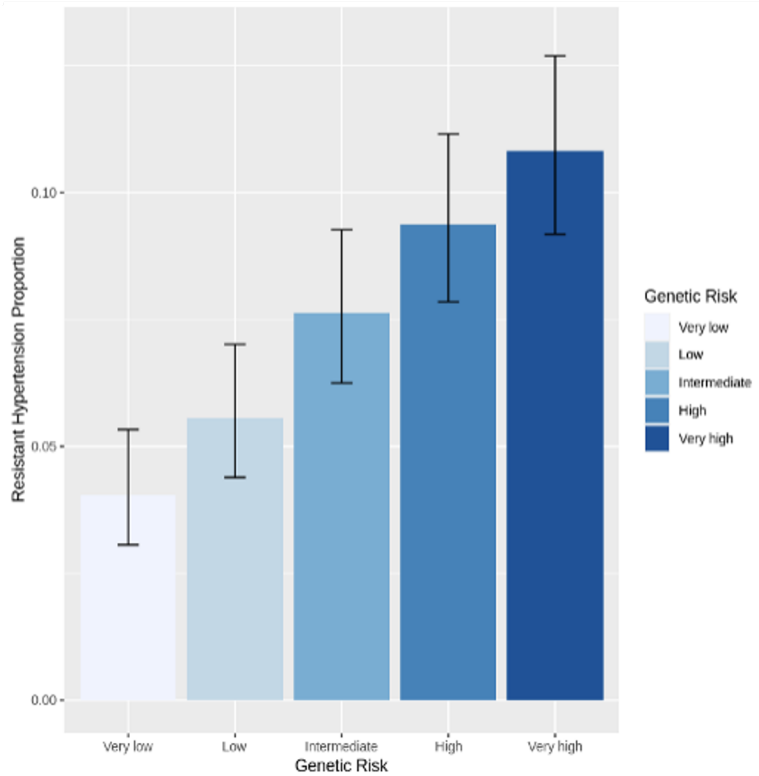
Observed systolic blood pressure



Uncontrolled blood pressure



Resistant blood pressure



Use of oral anticoagulation is a major clinical dilemma in care of ICH patients with atrial fibrillation

Currently available evidence:

Thrombosis Risk
- Ischemic stroke
- Systemic Thromboembolism

Hemorrhage Risk
- Recurrent ICH
- Systemic Bleeding

Mortality

Genetic risk factors for hypertension

Stroke genomics at the bedside

Polygenic susceptibility to hypertension in survivors of intracerebral hemorrhage with afib

Use of oral anticoagulation is a major clinical dilemma in care of ICH patients with atrial fibrillation

Currently available evidence:

Thrombosis Risk
- Ischemic stroke
- Systemic Thromboembolism

Hemorrhage Risk
- Recurrent ICH
- Systemic Bleeding

Mortality

Two ongoing clinical trials

1) ASPIRE (US)

2) ENRICH-AF (Canada)

3) Combined sample size: 2,000

Collecting DNA samples

Genotype at the YCGA using the GDA

Focus on blood pressure genomics

Stroke genomics at the bedside

Return of genomic information related to blood pressure to patients and doctors

Generations Project

GENERATIONS

To learn more about Generations and how you can participate, please contact us at:

1-877-978-8343 | helpusdiscover@yale.edu

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Michael F. Murray, MD
Principal Investigator

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Generations Project

- Led by Yale Center for Genomic Health.
- Formal launch September 2019.
- Plan is to enroll ≥ 100K volunteers across the Yale Health System
  - Genome-wide genotyping using Illumina's GDA array
  - Whole exome sequencing
- Link their electronic health record (EHR) to their DNA sequence data in a EHR-DNA dataset that enables both research and clinical care.
- Collaboration between: Yale New Haven Health System & Yale School of Medicine

Generations Project

1. De-identified Data for Research
   - DNA sequence linked to EHR

2. Banked Bio-specimens for Research and Clinical
   - Germline DNA, other

3. Participants for New Studies
   - Available for re-contact (clinical trials, surveys, deep phenotyping, other)

4. Precision Medicine Clinical Results Delivered
   - Genomic reports into EHR and implementation of care

Stroke genomics at the bedside

Return of genomic information related to blood pressure to patients and doctors

Generations Project

- Enroll 500 stroke survivors in Generations
- Calculate polygenic risk score using ~600 independent genetic risk variants for elevated blood pressure
- Use All of Us to calculate the ancestry-specific distribution of this blood-pressure related polygenic risk score
- Assign low, intermediate or high genetic risk based on tertiles of the polygenic risk score
- Return the information on genetic risk to doctors and patients
  - Interpretability
  - Willingness to act on this information
- Enrolled to far: 60

In summary

- Cardiovascular / Cerebrovascular disease poses a complex challenge
  - Clinically evident events is only the tip of the iceberg
  - Cognitive decline, dementia and disability are becoming the focus of research

- Concrete example (Causal inference / new targets)
  - Mendelian randomization analysis
  - Elevated LDL levels associated with decreased risk of intracerebral hemorrhage

- Concrete example (Risk prediction)
  - Stroke and MI in middle aged adults without risk factors

- Genomic medicine is here
  - Yale/AHA Bugher Center > bring genomic medicine to stroke survivors
  - Project 1: Blood pressure genomics in stroke survivors who also have afib > anticoagulation?
  - Project 2 via Generations: Return genetic information on blood pressure to stroke survivors

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