ALTERNATIVE APPROACHES TO IDENTIFY ADDITIONAL VARIANTS/GENES IMPLICATED ON COMPLEX DISEASES

Use of plasma analytes values as a quantitative endophenotype for genetic studies of stroke risk and outcomes

hopecenter
for neurological disorders

Genomics
RecoXery
Study
Linking Genetics Rehabilitation

Washington University in St. Louis

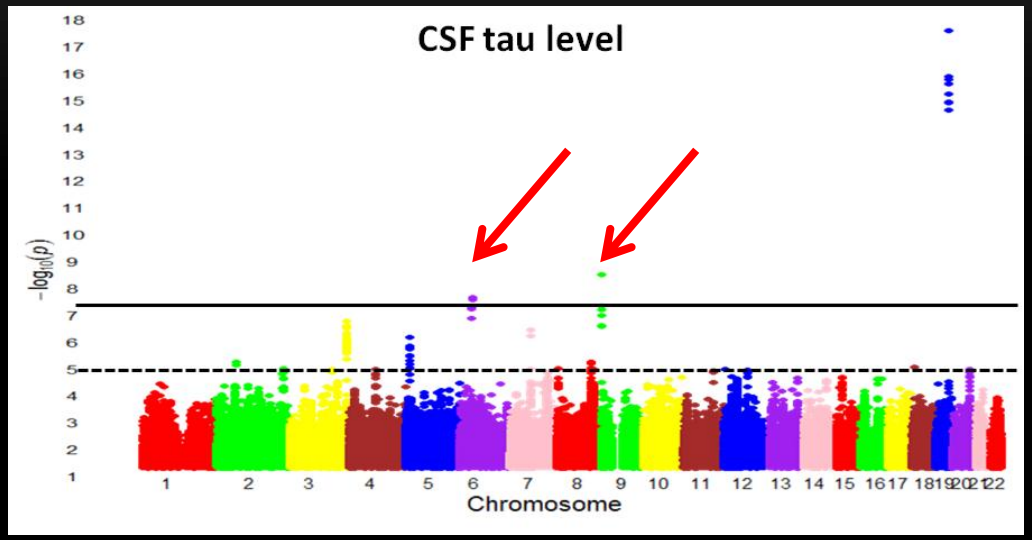
- P-value threshold in GWAS is very stringent: $5x10^{-8}$
- Some of the signals with lower p-value may be real
- There is a need of alternative approaches to identify such signals

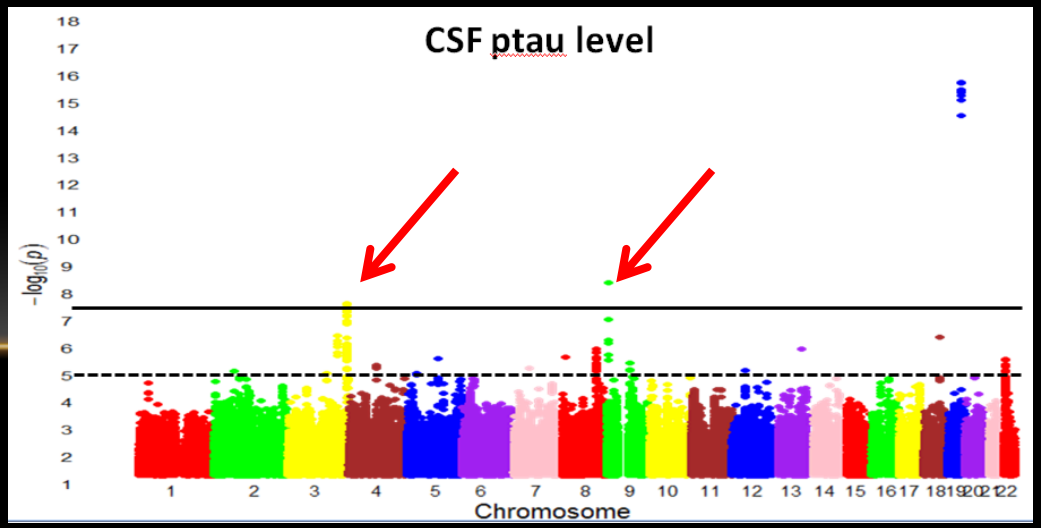
Intermediate phenotypes

- Intermediate endophenotypes may provide more power than regular phenotypes
- More directly affected by genetic variation
- provide a biological model relating the genetic variation with the trait

INTERMEDIATE PHENOTYPES: AN EXAMPLE

- Cerebrospinal Fluid (CSF) tau and ptau levels have emerge as a promising biomarkers for Alzheimer's disease
- CSF tau and ptau levels present great interindividual variability
- We did performed a GWAS (common variants) in 1300 individuals with CSF data





Intermediate phenotypes: Stroke

- We do have access to plasma levels of 180 different analytes (RBM Discovery Map panel) in more than 800 people
- Several of these analytes may be important to stroke risk and outcomes and were selected for genetics studies
- We also have access to GWAS data as well as exome-chip or whole-exome data

Available datasets

Table 8: Demographics WU-ADRC and ADNI samples

	Study	Sample #	Age (range)	Male (%)	*APOE* ε4+ (%)
WU-ADRC	controls	239	71±7 (53-91)	35	32
	AD	100	74±7 (53-90)	46	42
ADNI	controls	85	76±5 (62-92)	50	24
	AD	481	75±6 (57-89)	66	57

Age represents age at lumbar puncture

Intermediate phenotypes: Stroke

List GWASed analytes (55):

Alpha 1-Antichymotrypsin(AACT)
Alpha-1 Antitrypsin(AAT)
Angiotensin Converting Enzyme (ACE)
Adiponectin(Adipo)
Apolipoprotein E (ApoE)
Beta-2-Microglobulin (B2M)
Brain-Derived Neurotrophic Factor (BDNF)
B-Lymphocyte Chemoattractant (BLC)
Bone Morphogenetic Protein 6 (BMP6)
Complement C3 (C3)
Clusterin (CLU)
Ciliary Neurotrophic Factor (CNTF)
Complement Factor H(CFHR1)
C-Reactive Protein (CRP)
Cystatin C(CystC)
Epidermal Growth Factor (EGF)
Epidermal Growth Factor Receptor (EGFR)
E-Selectin(Esel)
Factor VII(Factor7)
FASLG Receptor (FAS)
Fas-Ligand(FASL)
Fibroblast Growth Factor 4 (FGF4)
Fibrinogen(Fibrino)
Ferritin(FRTN)
Heparin-Binding EGF-Like Growth Factor(HBEGF)
Intercellular Adhesion Molecule 1 (ICAM1)
Interleukin-6 receptor(IL6r)
Insulin
Leptin
Monocyte Chemotactic Protein 1 (MCP1)
Monocyte Chemotactic Protein 2 (MCP2)
Monocyte Chemotactic Protein 3 (MCP3)
Monocyte Chemotactic Protein 4 (MCP4)
Macrophage-Derived Chemokine (MDC)
Monokine Induced by Gamma Interferon(MIG)
Macrophage Inflammatory Protein-1 alpha(MIP1a)
Macrophage Inflammatory Protein-1 beta(MIP1b)
Macrophage Inflammatory Protein-3 alpha(MIP3a)
Matrix Metalloproteinase-2 (MMP2)
Matrix Metalloproteinase-9 (MMP9)
Matrix Metalloproteinase-9- total (MMP9tot)
Neuronal Cell Adhesion Molecule (NrCAM)
Plasminogen Activator Inhibitor 1 (PAI1)
Platelet-Derived Growth Factor BB(PDGFBB)
Resistin(Resist)
Thrombospondin-1(TP1)
Tissue Inhibitor of Metalloproteinases 1(TIMP1)
Tumor Necrosis Factor alpha(TNFa)
Tumor Necrosis Factor Receptor-Like 2(TNFR2)
Thrombopoietin(TMBP)
Vascular Cell Adhesion Molecule-1(VCAM1)
Vascular Endothelial Growth Factor (VEGF)
Protein S
von Willebrand Factor(vWF)
Brain Natriuretic Peptide (NT-proBNP)

Available datasets

Table 9: Similar plasma levels between AD and controls

	Controls	AD cases	p-value
α2-Macrogobulin (mg/ml)	1.18 ± 0.64 (0.73-6.5)	1.17 ± 0.23 (0.63-2.6)	0.80
PAI-1 (ng/ml)	60.2 ± 38.3 (12-190)	55.8 ± 41 (7-500)	0.37
IL-6 (pg/ml)	3.31 ± 1.83 (1.2-12)	3.76 ± 3.43 (1.1-35)	0.41
CRP (ug/mL)	3.19 ± 4.35 (0.17-22)	3.23 ± 6.95 (0.10-52)	0.95

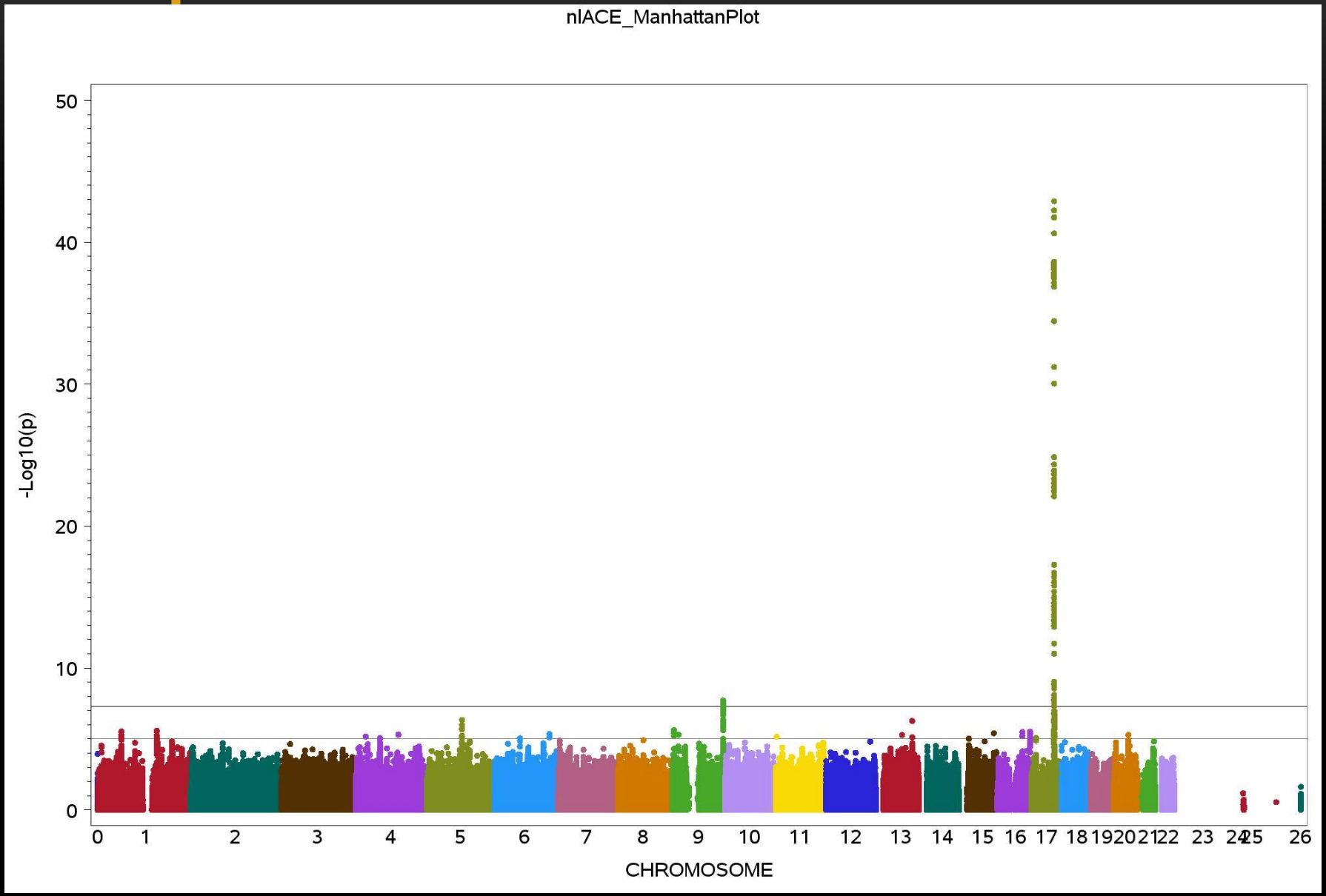
Intermediate phenotypes: Stroke

- **Results:**
  - **We did find genome-wide significant hits for 24 analytes: AAT ACE ApoE BLC CFHR1 CRP CystC EGFR Esel Factor7 FGF4 IL6r Leptin MCP2 MCP4 MDC MIP1a MIP1b NrCAM PDGFBB TMBP VCAM1 VEGF vWF**

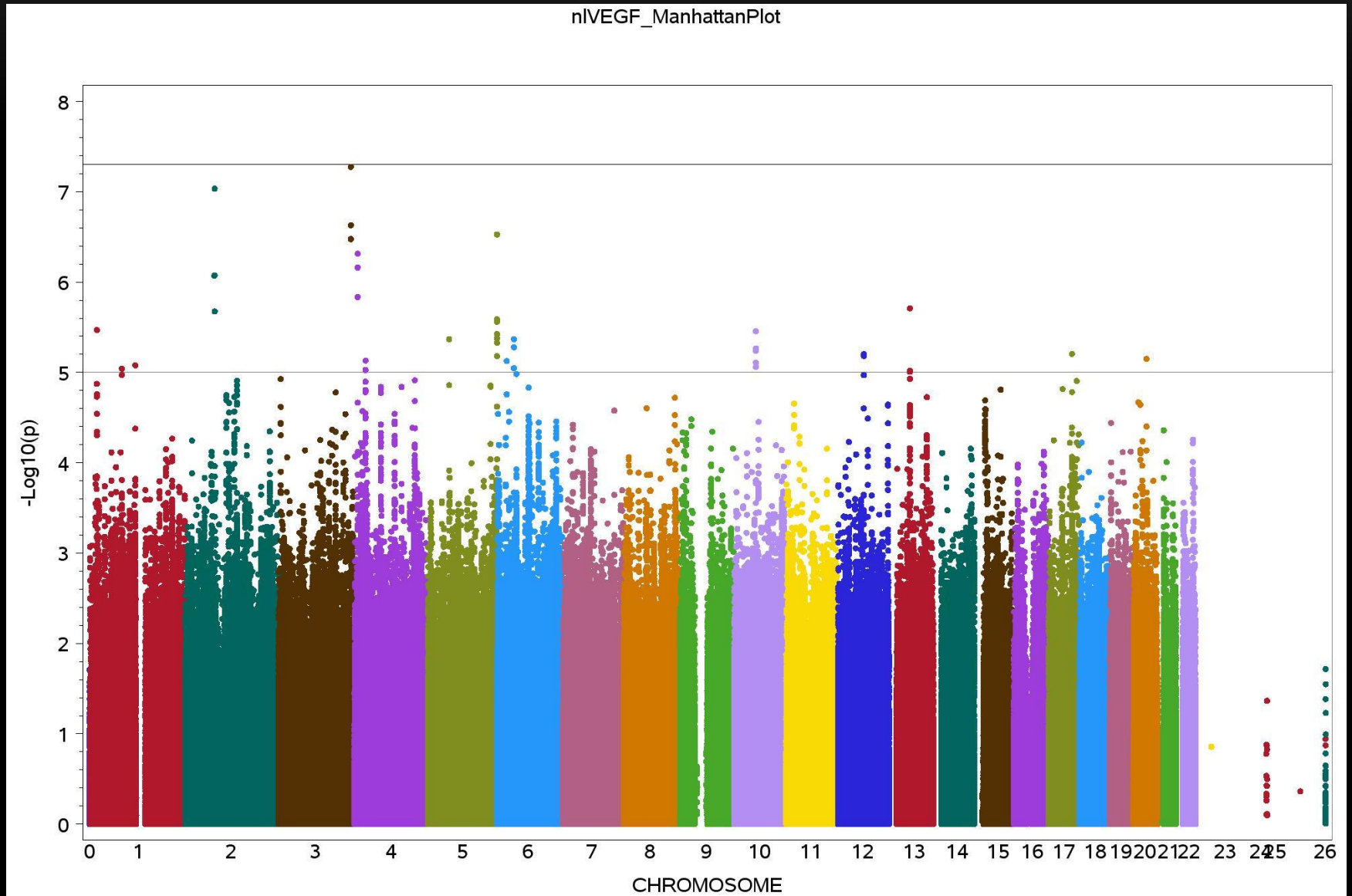
Results GWAS plasma levels

Analytes	GWAS hit gene	p-value
AAT	PPP4R4,SERPINA1, 6, 9, 10, 11	6.95E-12
ACE	ABO,ACE	1.28E-43
ApoE	APOE	3.01E-30
BLC	CACNG2,DDAH1	2.36E-08
CFHR1	CFH	5.52E-142
CRP	APOE	8.99E-12
EGFR	SLC35F4	6.14E-08
Esel	ABO	4.48E-53
Factor7	WDR11-AS1	2.45E-26
FGF4	GALNTL6	4.54E-10
IL6r	IL6R	4.65E-105
Leptin	LOC387723,CTAGE7P,GLRX3	7.99E-09
MIP1b	CCR3	1.89E-10
NrCAM	NRCAM	2.59E-10
TMBP	DHX8,ARL10	9.84E-09
VCAM1	RAMP1,UBE2F	2.55E-08
VEGF	LPP	5.29E-08
vWF	ABO	4.63E-08
CystC	CST3,CST4,CST9,CST9L	2.66E-08
MCP2	CCL8	2.45E-13
MCP4	DIAPH3,LINC00311,MIR5093	7.55E-10
MDC	Hrh4	8.94E-08
MIP1a	CCL18	3.80E-15
PDGFBB	PAG1	7.46E-08

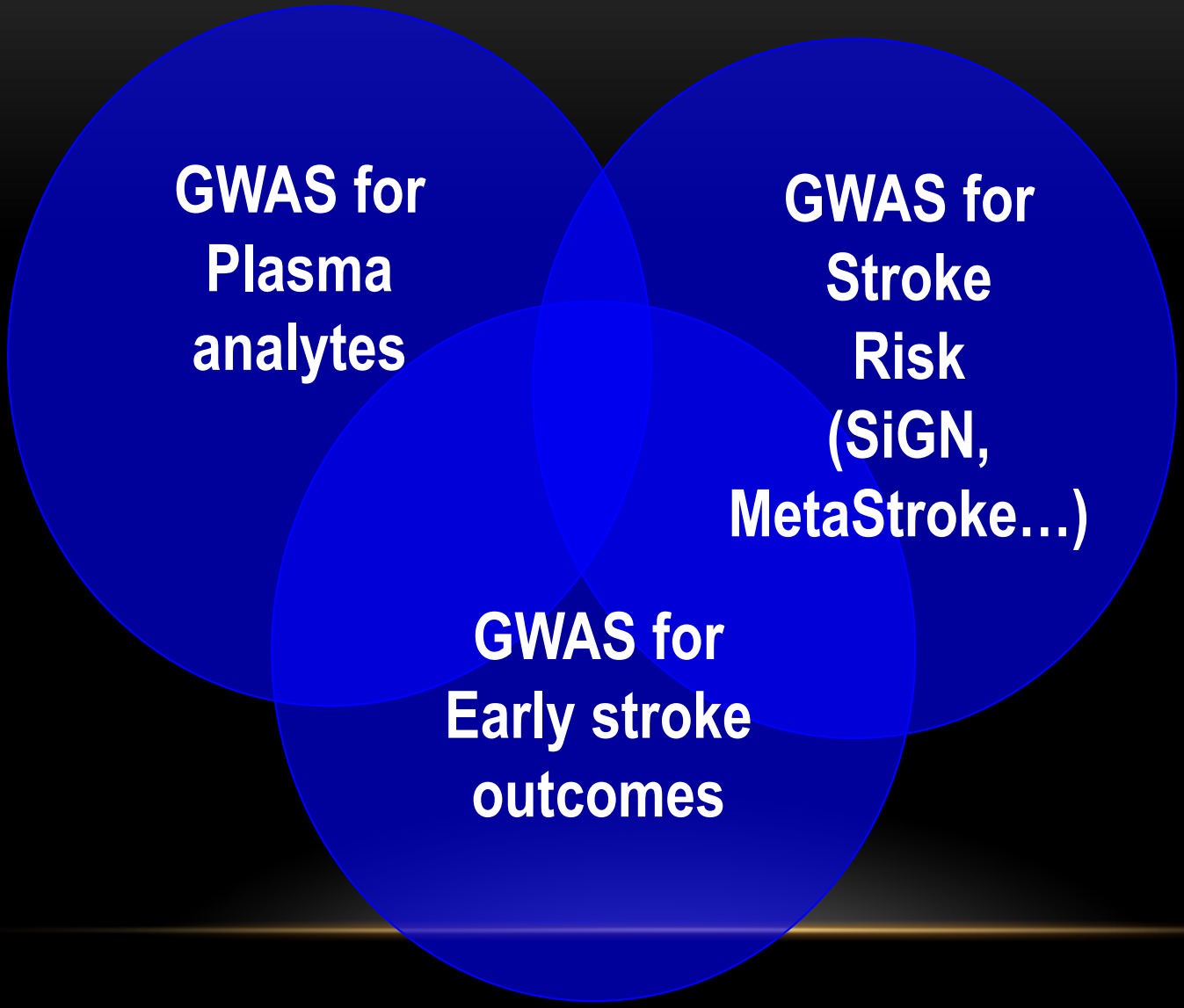
GWAS plasma ACE



GWAS plasma VEGF

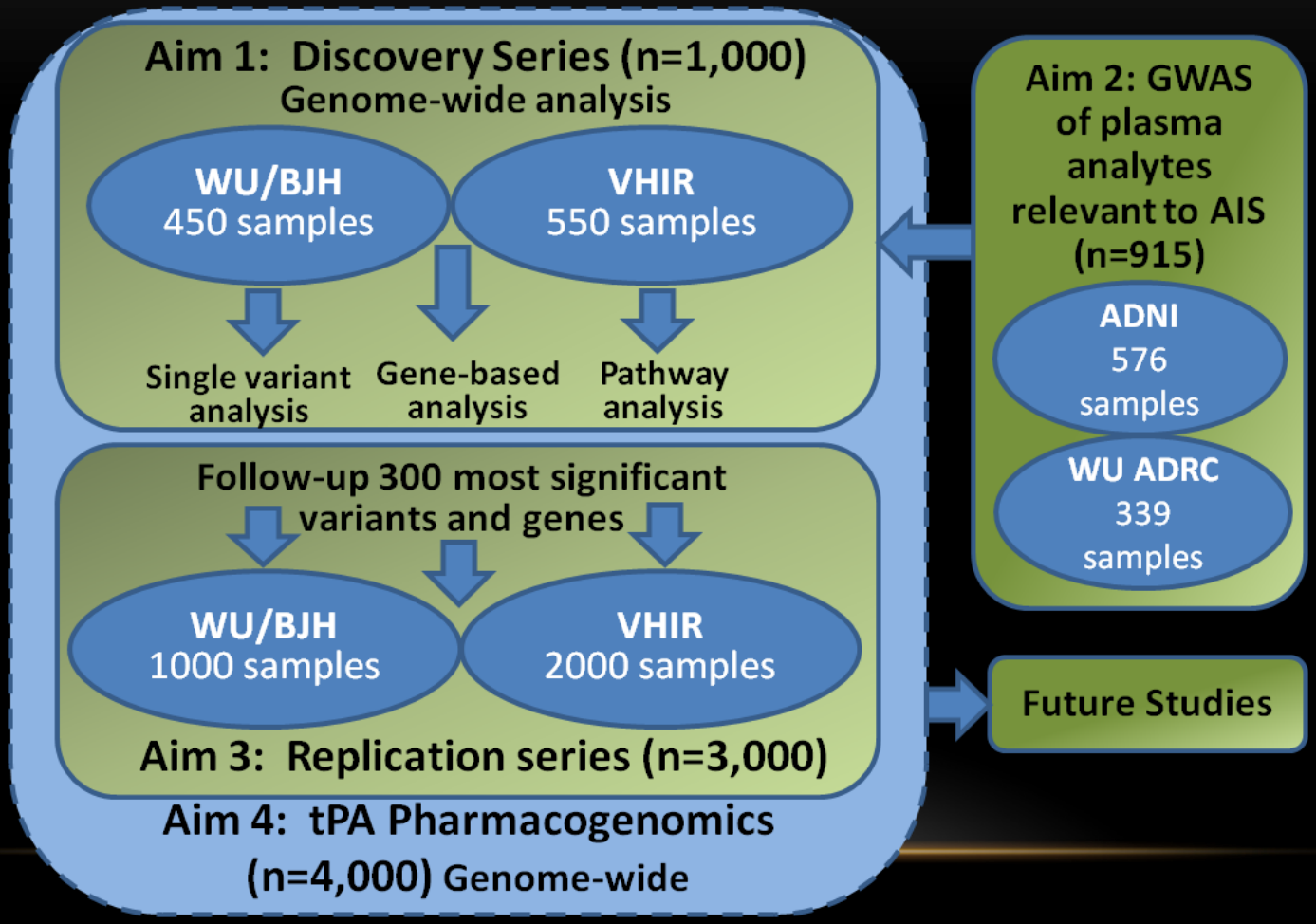


Proposal


GWAS for Plasma analytes
GWAS for Stroke Risk (SiGN, MetaStroke…)
GWAS for Early stroke outcomes

Study design for GWAS of Stroke Outcome

Figure: Overall Scheme



Discussion

- We now have genome-wide significant loci for 24 analytes relevant to stroke risk
- We will also perform rare variant analyses to find additional signals
- Proposal: Examine the impact of these eQTLs on stroke risk using SiGN's data
- Thoughts?

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Postdoc position available: ccruchaga@wustl.edu
https://sites.google.com/site/cruchagalab/