FIA Whole Exome Sequencing Project International Stroke Genetics Consortium

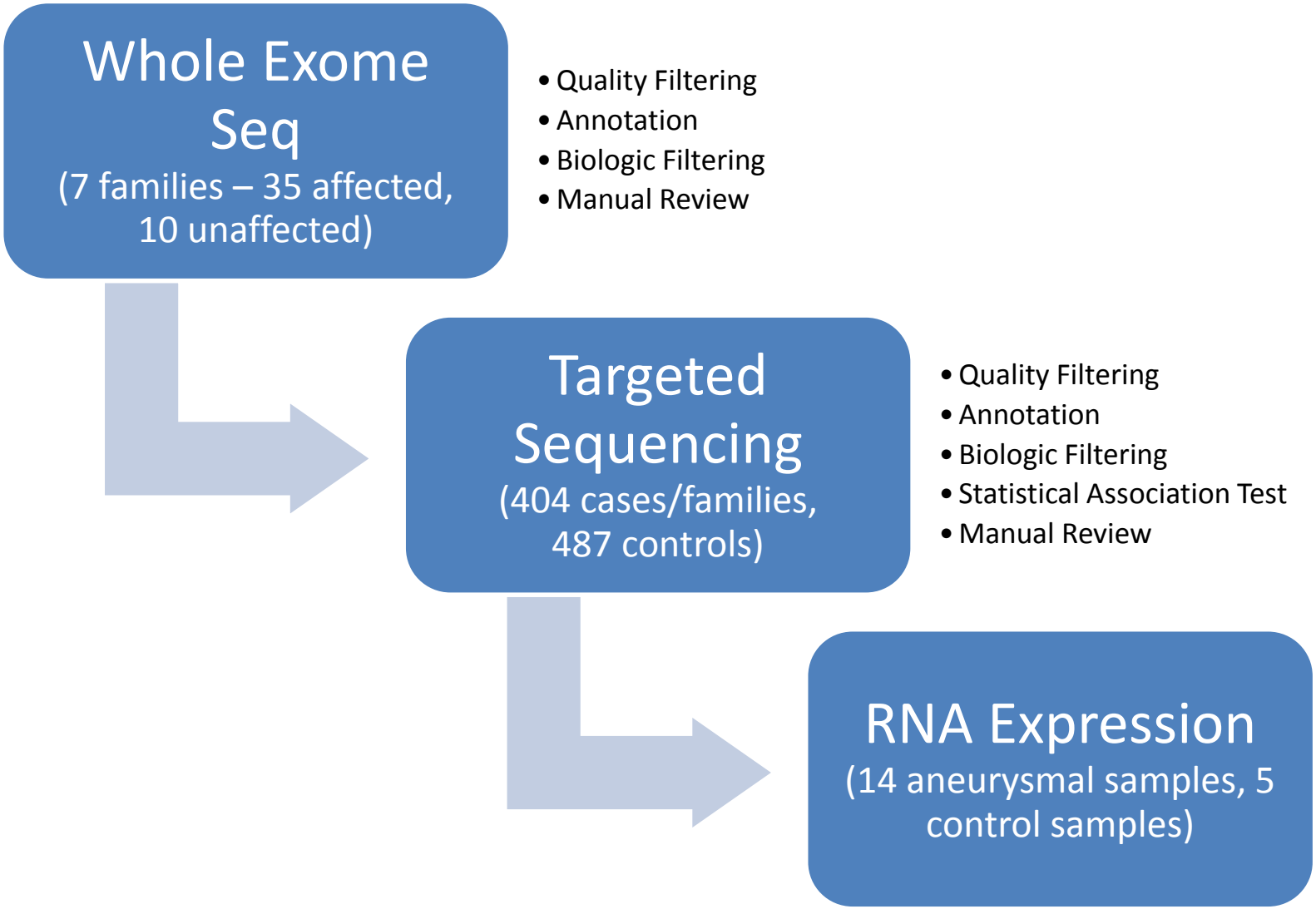
March 27, 2014

Hypothesis: Rare variants with relatively large effects on disease susceptibility contribute to risk of intracranial aneurysms

Such variants are more likely to segregate in families with multiple affected members

High throughput sequencing is now capable of identifying these variants

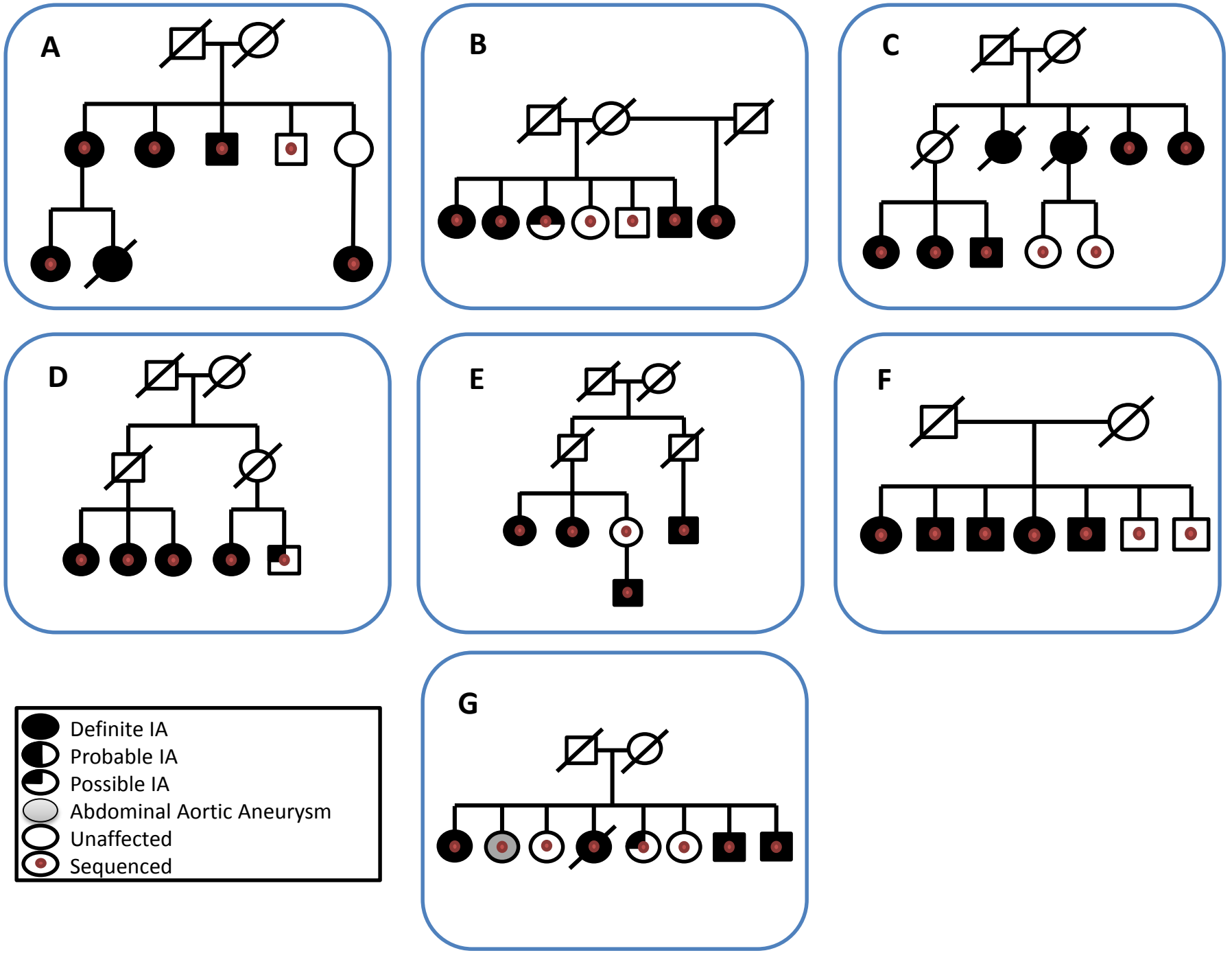
Study Design



Whole Exome Sequencing

- 35 affecteds, 10 unaffecteds
- Sequenced unaffecteds had an MRA confirming IA absence at 45y or older

- Avg study duplicate reproducibility – 99.13%
- Avg concordance with genotyping array – 99.57%
- Transition-Transversion ratio (on exon) – 3.3


A
B
C
D
E
F
G
Definite IA
Probable IA
Possible IA
Abdominal Aortic Aneurysm
Unaffected
Sequenced

Biologic Filtering Criteria

- Variants were kept if:
  - Rare (<1% Eur Amer MAF in 1000G, dbSNP)
  - Predicted to be functional (nonsynonymous variants or indels)
  - Located in an exonic or splicing region
  - Located on an autosome
  - Inherited by all definite affecteds in a family
  - Not observed in any unaffected individual
  - Predicted damaging by at least one protein prediction program (Polyphen, SIFT)
- 28 candidate genes

Targeted Sequencing

- 404 unrelated FIA cases,
  487 controls (Exome Sequencing Project)
- 26 candidate genes from the FIA whole exome sequencing study
- VAAST (Variant Annotation, Analysis and Search Tool) performed; scores based on:
  - Phylogenetic conservation of locus
  - Amino acid substitution severity
  - Case/control frequencies
- Suggestive evidence for association found for one gene (*SMEK2*)

Suppressor of mek1, homolog 2 *(SMEK2)*

- 69kb gene, located at 2p16.1
- Regulatory subunit of the protein phosphatase 4 complex
- Implicated in lipid metabolism and gluconeogensis pathways

Suppressor of mek1, homolog 2 *(SMEK2)*

- p value=0.0031
  (Bonferroni-corrected p value=0.0019)
- 4 variants used in analysis

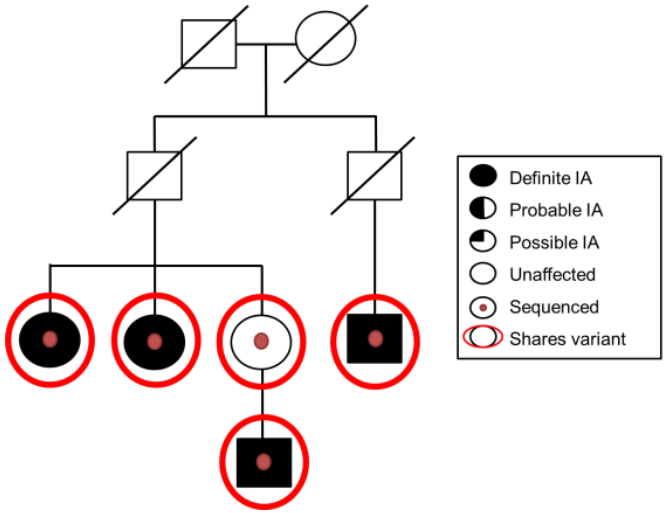
Chr	Position	Ref	Alt	Minor Allele Frequency (MAF)				PolyPhen	SIFT
				Dataset	Cases	Controls	1000 G		
2	55791601	C	T	0.0006	0.0012 (1 case)	0	NA		+
2	55791617	G	A	0.0006	0.0012 (1 case)	0	NA	+	+
2	55825844	A	G	0.0028	0.0061 (5 cases)	0	0.0026	+	+
2	55826059	T	G	0.0011	0	0.0021 (2 ctrls)	NA		+

Suppressor of mek1, homolog 2 *(SMEK2)*

- 4 *SMEK2* variants in the targeted sequencing study
  - Two variants are found in one family each
    - Located 16bp from one another (suggests region might be functionally important)
  - One variant is found in 5 families (+the original WES family)
    - Results in a severe Phe to Ser amino acid substitution in a conserved region
    - Located in a protein domain thought to bind substrates

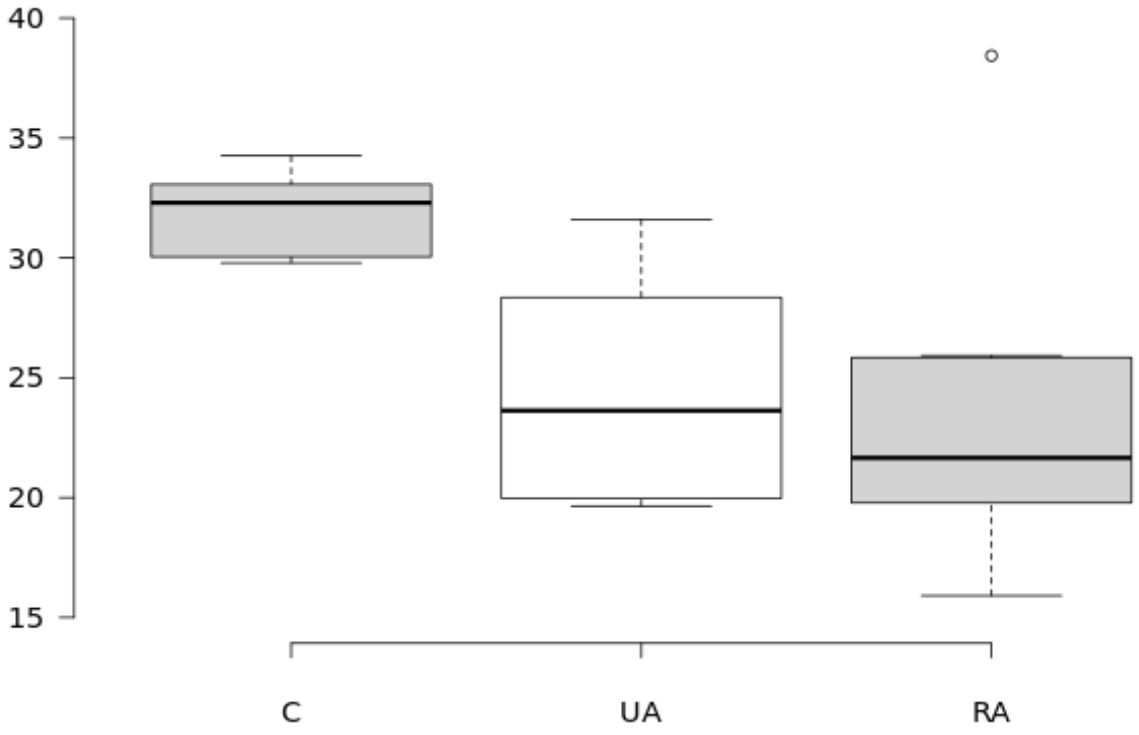
SMEK2 Variant in Original Whole Exome Seq Family

- *SMEK2* was nominated from one WES family to be used in the targeted sequencing experiment
- Variant was observed in 1000G (Eur Amer MAF = 0.26%)
- Variant was inherited by all affecteds in this family and the putative obligate carrier
  - Obligate carrier had NO history of smoking or hypertension
  - All affected individuals had a smoking history, and two had hypertension

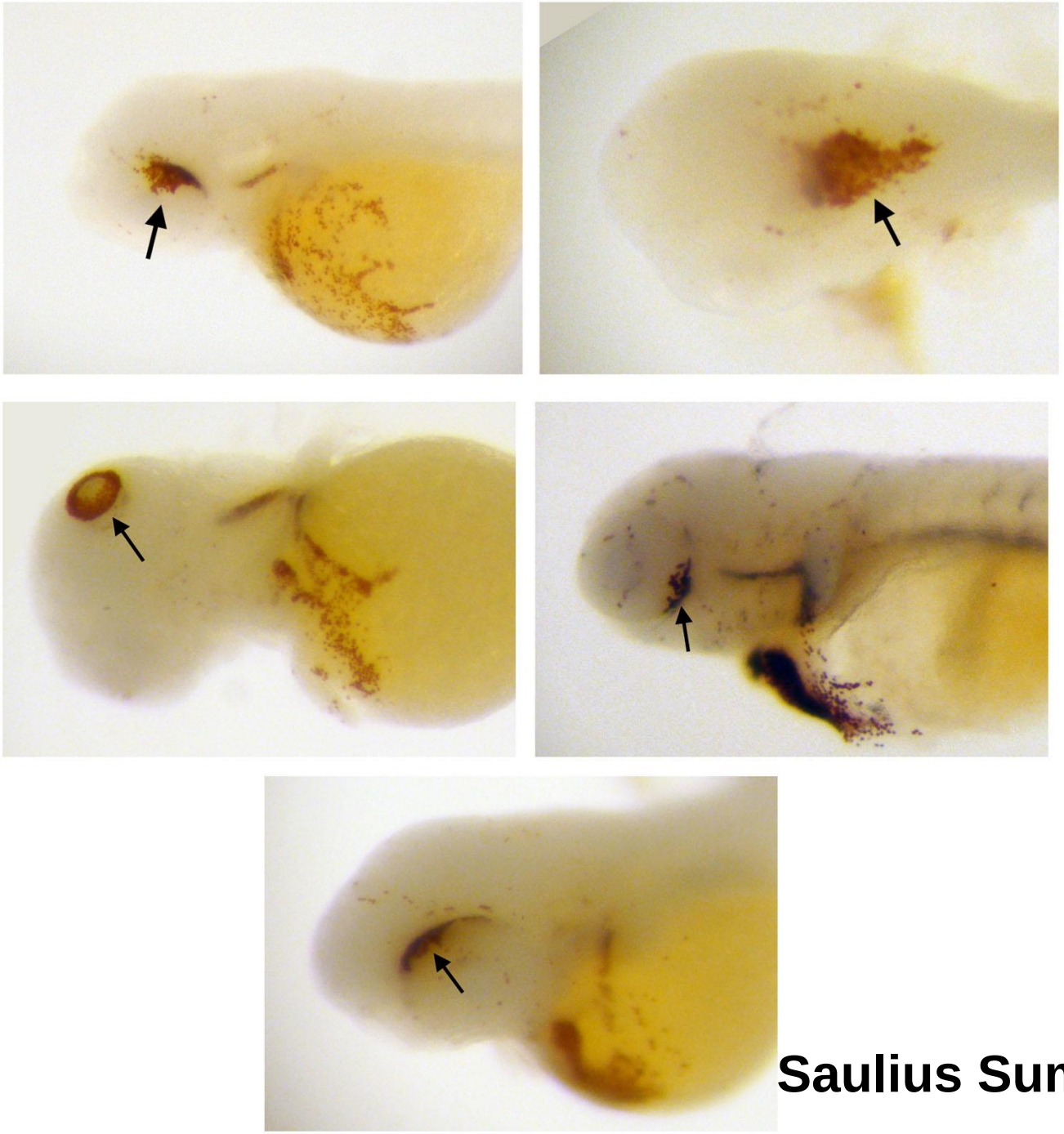


SMEK2 Expression in Intracranial Arterial Tissue

Significantly lower *SMEK2* expression in aneurysmal tissue (p=0.011)



- C – control (middle meningeal artery); n=5
- UA – unruptured aneurysm; n=6
- RA – ruptured aneurysm; n=8



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Summary

- Whole exome sequencing (WES) was performed in 7 families
- WES variants that were rare, functional, and predicted damaging were prioritized
- 26 genes (from 26 WES candidate variants) were sequenced in a targeted sequencing replication study (404 cases, 487 controls)
- Only one gene (*SMEK2*) showed suggestive evidence of association
- *SMEK2* shows significantly lower expression in aneurysmal tissue

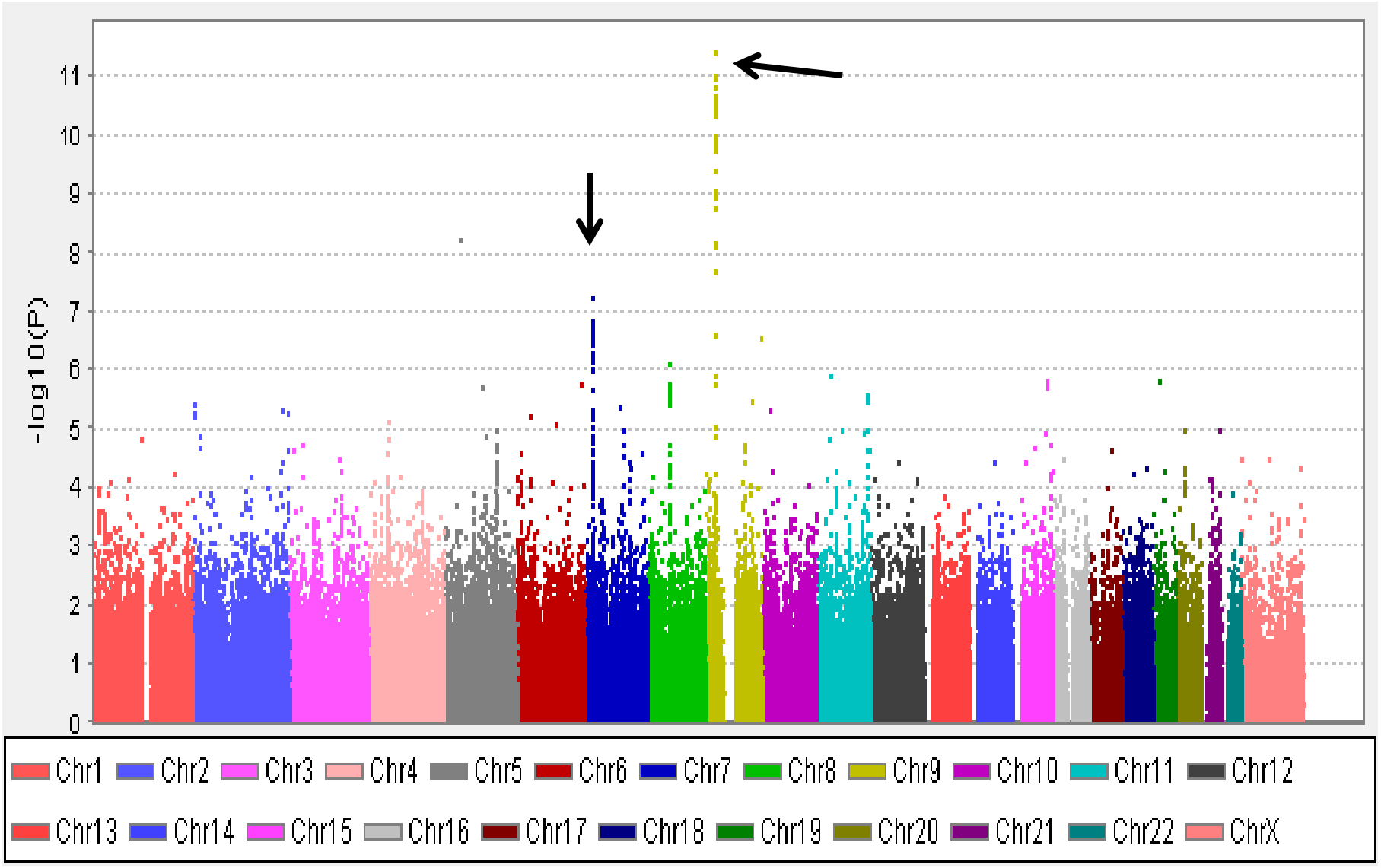
Intracranial Aneurysm Cases: Discovery Sample

Cases	Number	Mean Age of Onset (SD)	% Male
FIA Study multiplex families (1/family)	388	50.7 (11.9)	31
FIA Study (general recruitment)	1443	54.1 (11.7)	20
GERFHS	44	54.7 (12.9)	41
Australia	143	53.7 (16.3)	39
UCSF	128	55.8 (12.0)	33
Poland	498	52.1 (12.9)	41
TOTAL Cases	2,644	53.3 (12.3)	28

Controls: Discovery Sample

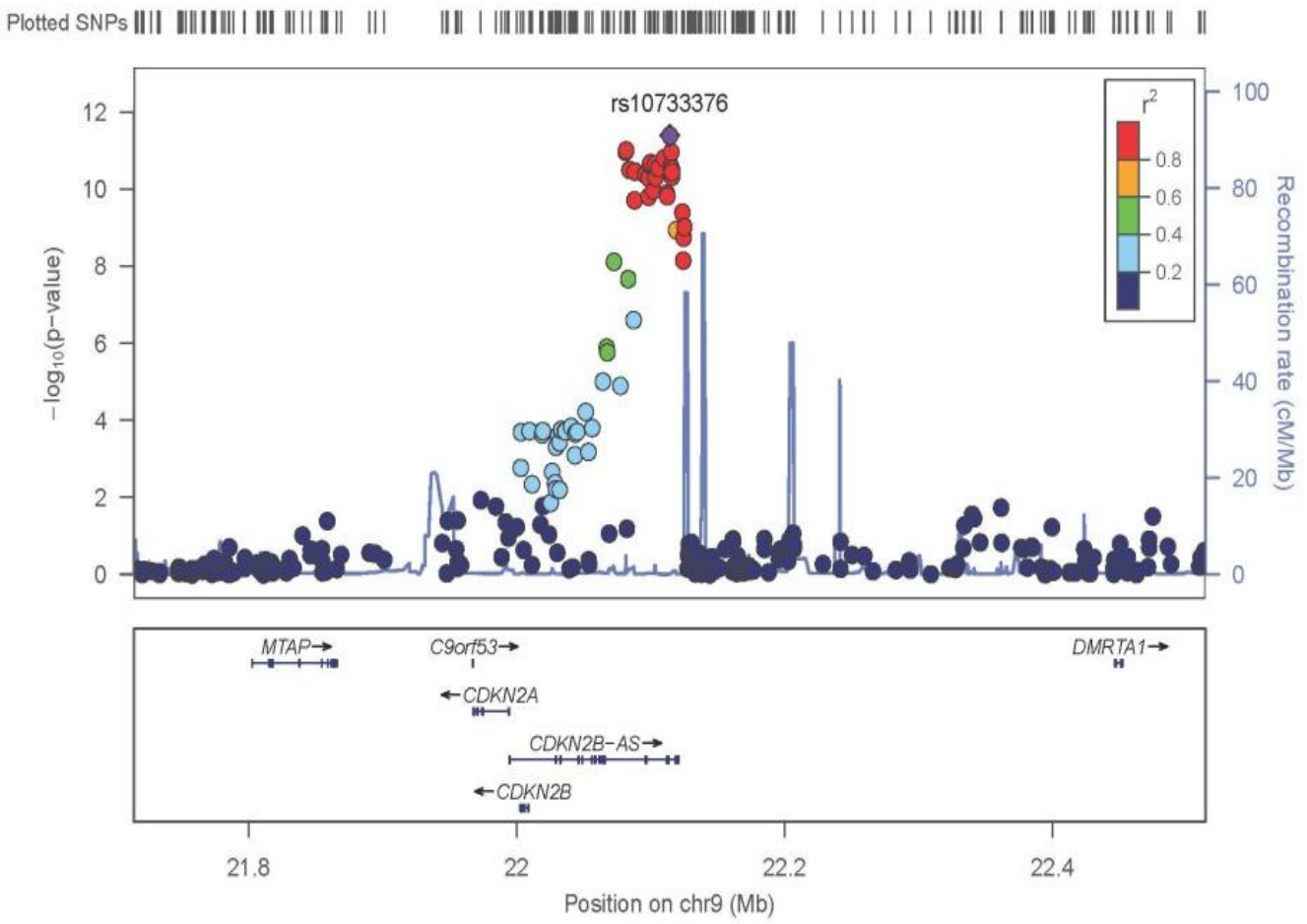
Controls	Number	Mean Age at Recruitment (SD)	Percent Male
CCC	294	64.0 (14.8)	47
GERFHS	484	66.3 (13.0)	53
Australia	154	50.9 (16.1)	39
Poland	484	56.2 (15.8)	40
ARIC	1,132	54.3 (7.5)	28
TOTAL Controls	2,548	57.9 (13.1)	38

GWAS: Regional Association Results in the Discovery Sample

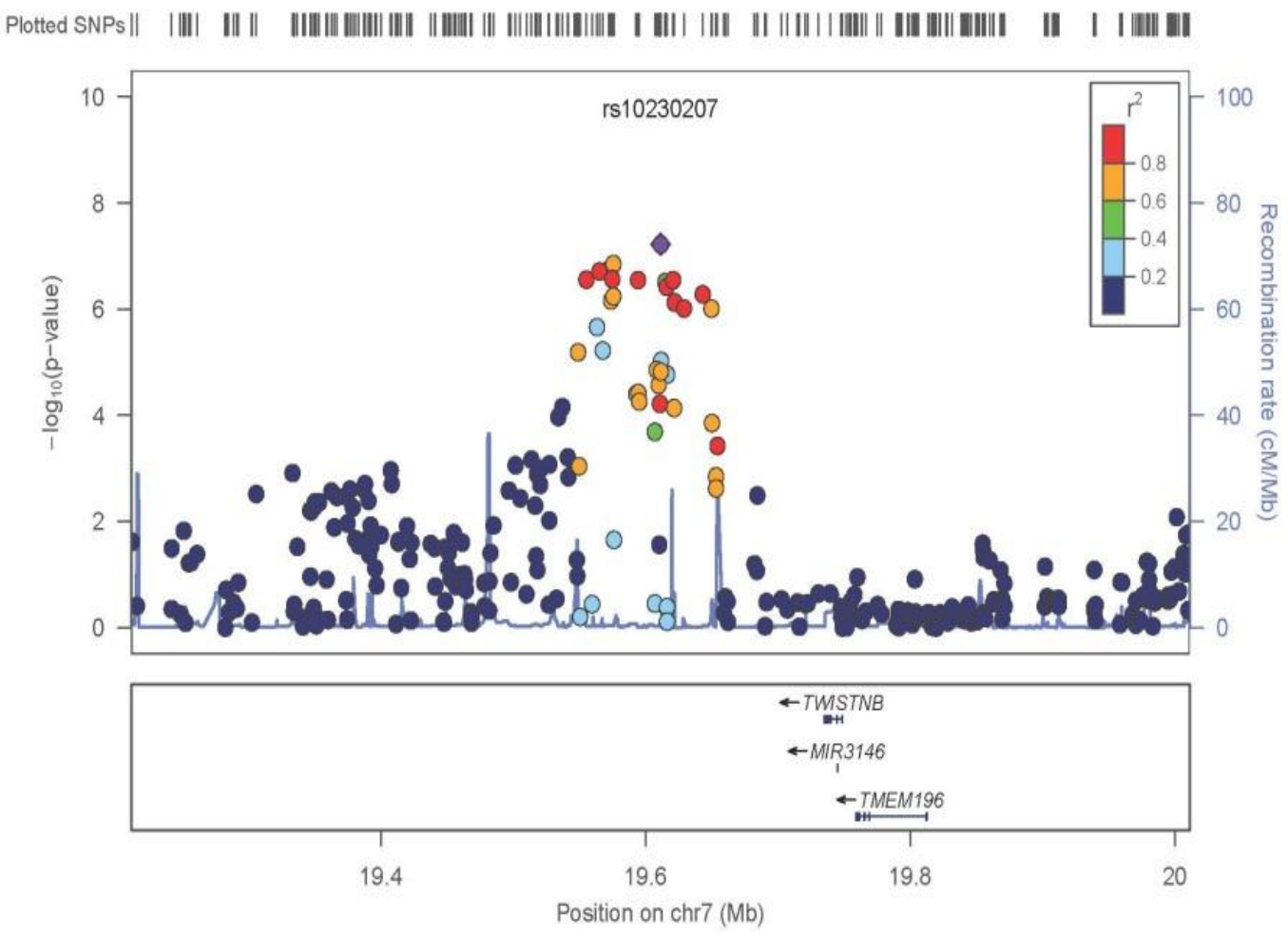


Chromosome 9

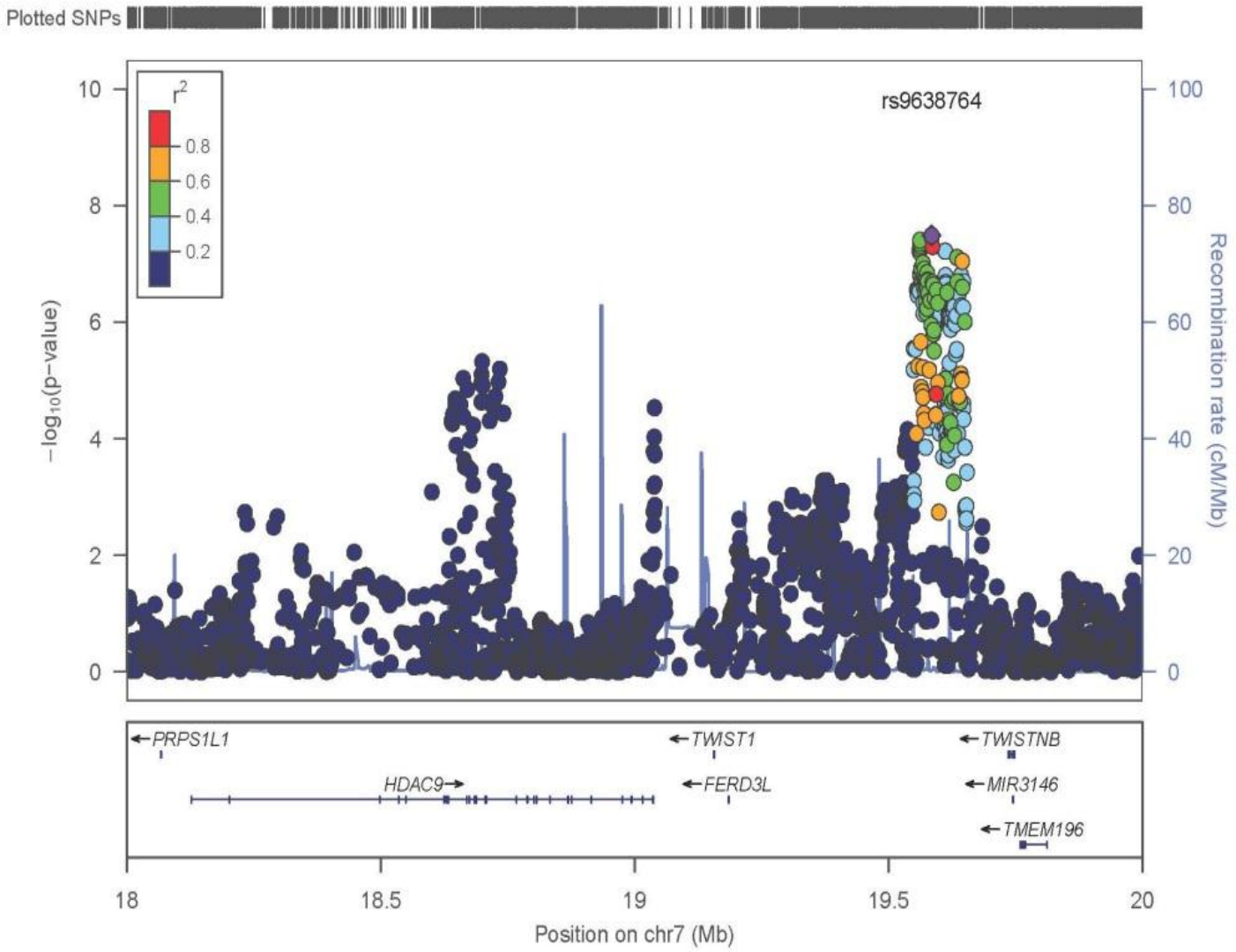
CDKN2BAS/ANRIL



Chromosome 7



Chromosome 7: Expanded View



Replication Sample: Demographics

Cases	Number	Mean Age of Onset (years)	% Male
Dutch	717	54.4	31
Finnish	799	49.6	42
Controls		Mean Age at Recruitment	
Dutch	3,004	61.7	63
Finnish	2,317	60.9	54

Results of Replication Analysis in the Chromosome 7 region

			Discovery Sample		Dutch		Finnish		Meta-analysis	
SNP	Position	Allele	p-value	OR 95% CI	p-value	OR 95% CI	p-value	OR 95% CI	**p-value**	**OR 95% CI**
rs10230207*	19,611,307	T	5.99×10^{-8}	1.26 (1.16-1.38)	0.011	1.18 (1.04-1.33)	0.25	1.08 (0.95-1.23)	**8.50×10^{-9}**	**1.20 (1.13-1.28)**
rs2192476	19,612,305	T	2.09×10^{-7}	1.25 (1.15-1.36)	0.010	1.18 (1.04-1.34)	0.26	1.08 (0.95-1.23)	**2.29×10^{-8}**	**1.19 (1.12-1.27)**

* rs10230207 achieved genome-wide significance in the discovery sample and the SNP tested initially for replication.
OR – odds ratio
95% CI – 95% confidence interval for the odds ratio

Conclusions

- Our primary association overlaps region initially reported by Matarin for ischemic stroke
  - Note: That finding was not genomewide significant in initial report
- Our tertiary association is not in high LD with the SNPs associated with large vessel ischemic disease reported by ISCG et al in 2012
  - The large vessel reports were genomewide significant

Zebrafish Models of Genes

Morpholino	Dose	Hemorrhage	No. of Embryos
PKD1a and PKD1b	5 ng	6%	47
PKD1a and PKD1b	10 ng	34%	74
PKD1a and PKD1b	15 ng	41%	22
HSPG2 MO1	5 ng	23%	161
HSPG2 MO2	2 ng	10%	92
HSPG2 MO2	4 ng	19%	74
Control MO	5 ng	0%	30
Fox-M-1	8-10 ng	0%	67
Uninjected control		1.6%	508