

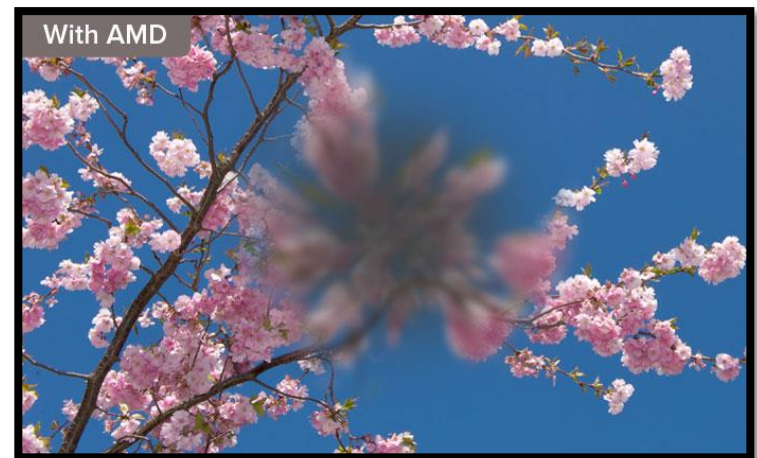
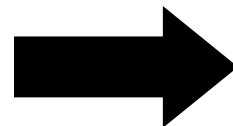
MIXED-MODEL ANALYSIS OF COMMON VARIATION REVEALS PATHWAYS EXPLAINING VARIANCE IN AMD RISK



AMD

Age-related Macular Degeneration

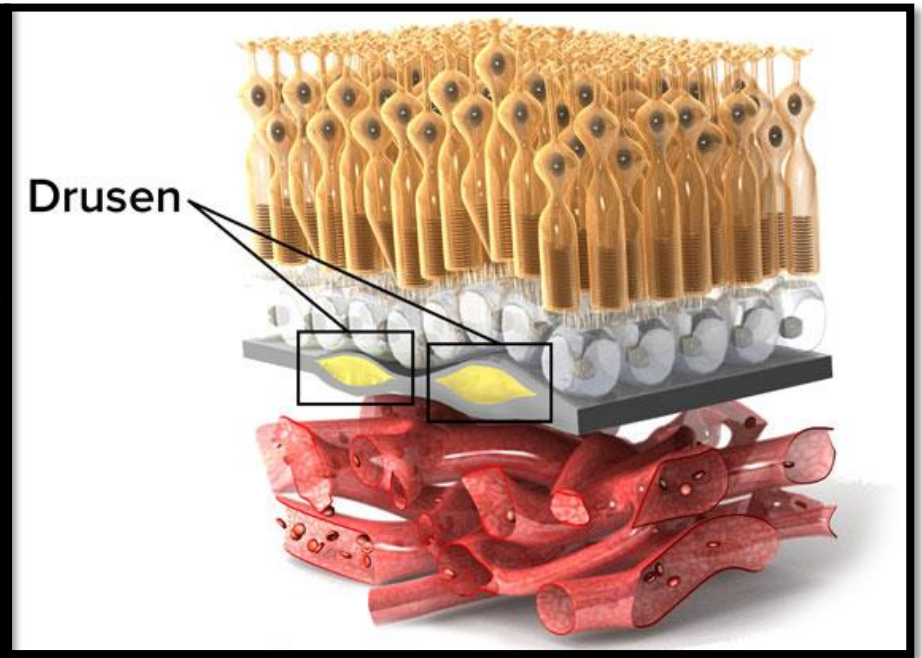
- Progressive, neurodegenerative disease
- Loss of central vision
- A leading cause of blindness
 - **30+ million** affected worldwide



GENETICS OF AMD

- Heritability 45 - 70%*
- Most strongly associated genes:
 - **CFH** [*Complement Factor H*]
 - **C2** [*Complement Component 2*]
 - **C3** [*Complement Component 3*]
 - **CFB** [*Complement Factor B*]
 - **ARMS2/HTRA1** [*Age-Related Maculopathy Susceptibility 2 / High-Temperature Requirement A Serine Peptidase 1*]
- 10 - 30% of heritability explained by 19 known risk SNPs*

SIGNS OF AMD



AMD Pathogenesis...?

AMD PATHOGENESIS IN LITERATURE



Exp. Eye Res. (2001) 73, 887-896
doi:10.1006/exer.2001.1094, available online at <http://www.idealibrary.com>

Complement Activation and Inflammation Formation and Age Related M

LINCOLN J. SIMPSON*, WILLIAM P. LEWIS, and DON H. ANDERSON

Center for the Study of Age-Related Macular Degeneration

(Received 15 October 2001)

Recent findings suggest that complement activation plays a role in the pathogenesis of AMD.

50

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Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne

Correspondence to: T Y Wong, wong@unimelb.edu.au

ISSN 0360-5310/02/0000-0000

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CURRENT RESEARCH

ROBERT WEINREB AND EDWARD COTLIER, EDITORS

The Role of Oxidative Stress in the Pathogenesis of Age Related Macular Degeneration

son, PhD,¹

United Kingdom, ²Department of Ophthalmology, University of Manchester, United Kingdom, and ³Department of Ophthalmology, University of Cardiff, United Kingdom

oxidative stress is a key factor in the development of AMD, which refers to cellular damage caused by reactive oxygen species, singlet oxygen, and they

we RPE and photoreceptors, correlating with the presence of Fas ligand system in the retina. This

with 280 controls. The presence of

BMJ

RESEARCH

Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis

Elaine W-T Chong, PhD candidate,¹ Tien Y Wong, professor of ophthalmology,¹ Andreas J Kreis, ophthalmology fellow,¹ Julie A Simpson, senior lecturer,² Robyn H Guymer, associate professor of ophthalmology¹

ABSTRACT

Objective To evaluate the effectiveness of dietary antioxidants in the primary prevention of age related macular degeneration (AMD).

Design Systematic review and meta-analysis.

Data sources Search of seven databases without limits on year or language of publication, and retrieval of references in pertinent reviews and articles.

Methods Two reviewers independently searched the

lead to severe loss of central vision. The pathogenesis of AMD is unclear^{8,9}; older age, genetic markers,^{10,11} and cigarette smoking are the only risk factors consistently reported.¹²⁻¹⁵ Although new treatments have emerged, they are suitable only for the small proportion of people with "wet" AMD.¹⁶⁻¹⁹ No treatments are available for the "dry" form, and there is little to offer for the primary prevention of AMD in older people. Dietary antioxidants have long been suggested as pos-

AMD PATHOGENESIS IN LITERATURE

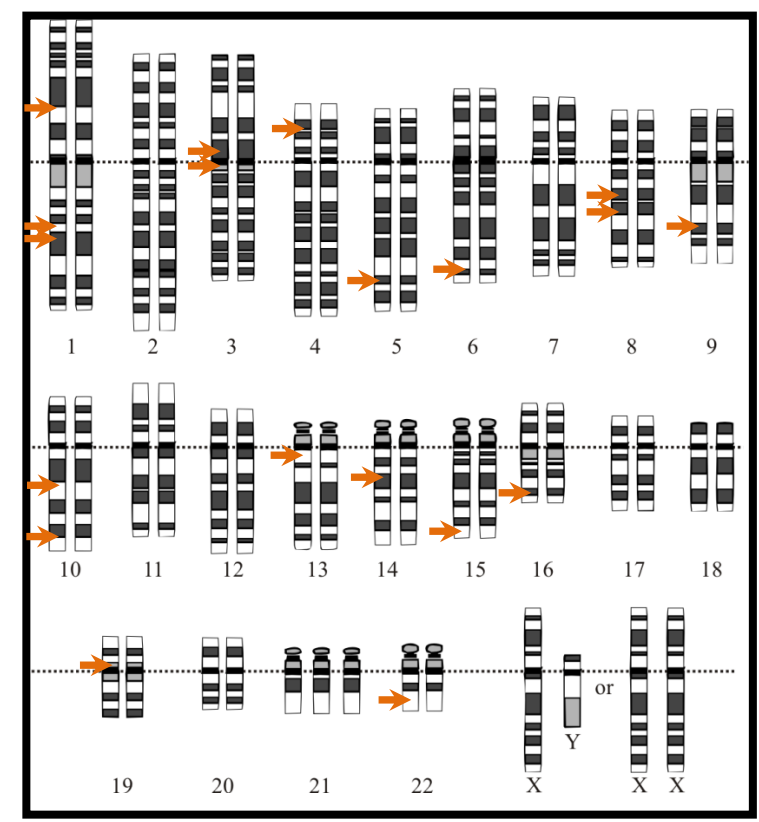
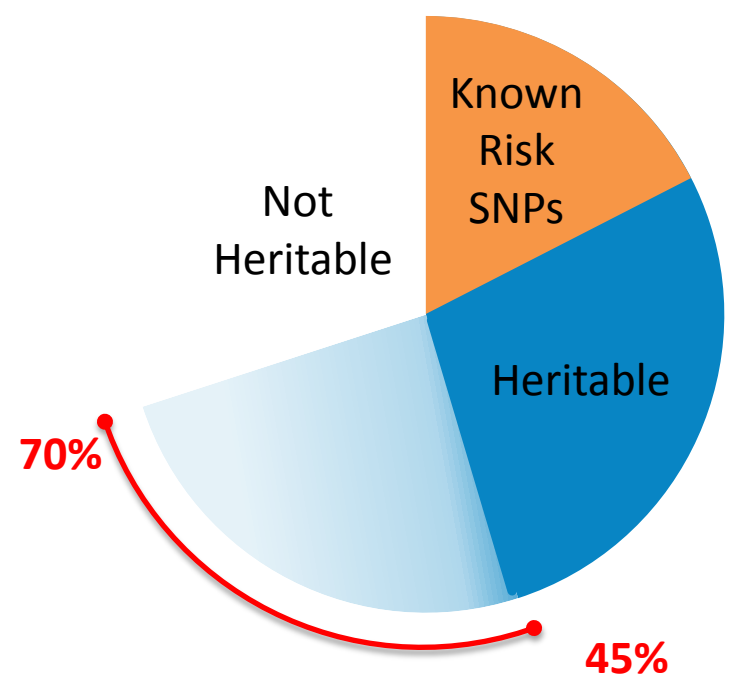
8 POTENTIAL MECHANISMS

Angiogenesis	Inflammation
Antioxidants	Nicotine/Smoking
Apoptosis	Oxidative Phosphorylation
Complement Activation	Tricarboxylic Acid Cycle

MOTIVATION



8 POTENTIAL MECHANISMS	
Angiogenesis	Inflammation
Antioxidants	Nicotine/Smoking
Apoptosis	Oxidative Phosphorylation
Complement Activation	Tricarboxylic Acid Cycle



QUESTION

How much disease risk is explained by SNPs in potentially AMD-related pathways?

DATASET

Case/Control Design

- Subjects ascertained in clinics at:
 - Vanderbilt University
 - Duke University
 - University of Miami Health System
- All European descent
- Fundus photography used to confirm case status
- Primary genotyping: Affymetrix 6.0
- Secondary genotyping: Sequenom & TaqMan



QC STEPS

SNPs Excluded

- Non-autosomal
- Genotyping efficiency < 95%
- Sample efficiency < 90%
- Minor allele frequency < 2%
- HWE p-value < 1×10^{-6}

Covariates Required

- Age (Years)
- Sex (M/F)

Post-QC

Total SNPs: **659,183**

Affymetrix 6.0: **659,108**

Custom Sequenom: **71**

Custom TaqMan: **4**

Cases: **1,145**

Controls: **668**

DEFINING PATHWAYS

8 POTENTIAL MECHANISMS	
Angiogenesis	Inflammation
Antioxidants	Nicotine/Smoking
Apoptosis	Oxidative Phosphorylation
Complement Activation	Tricarboxylic Acid Cycle

DEFINING PATHWAYS

The Gene Ontology [GO]

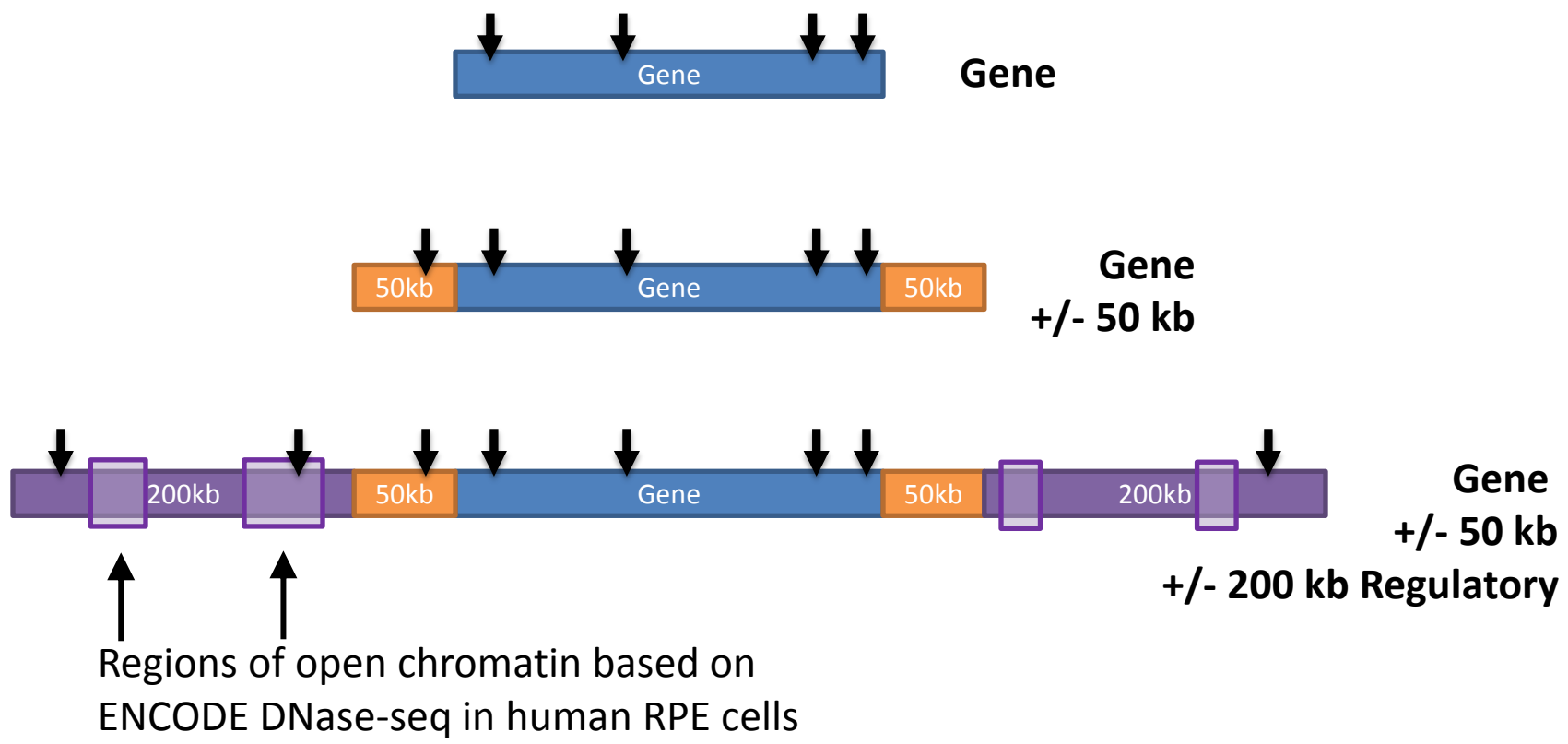
AmiGO browser or download database

Searched for GO Term closes to our mechanism of interest

- I GO:0008150 biological_process
 - I GO:0050896 response to stimulus
 - I GO:0042221 response to chemical
 - I GO:0009719 response to endogenous stimulus
 - I GO:1901698 response to nitrogen compound
 - I GO:0010033 response to organic substance
 - I GO:0010243 response to organonitrogen compound
 - I GO:0043279 response to alkaloid
 - I GO:0014070 response to organic compound
 - ▼ **GO:0035094 response to nicotine**
 - I GO:0035095 behavioral response to nicotine
 - I GO:0071316 cellular response to nicotine

GO Term	GO ID	# Genes
Angiogenesis	GO:0001525	379
Antioxidant Activity	GO:0016209	69
Apoptotic Signaling	GO:0097190	1,635
Complement Activation	GO:0006956	184
Inflammatory Response	GO:0006954	534
Response to Nicotine	GO:0035094	31
Oxidative Phosphorylation	GO:0006119	78
Tricarboxylic Acid Cycle	GO:0006099	33

PATHWAY REGIONS



MIXED MODEL ANALYSIS

- GCTA* - Genetic Relationship Matrices [GRMs] estimate genetic sharing among individuals in dataset using variance-covariance matrix
- Restricted Maximum Likelihood [REML] estimates the genetic contribution of each pathway (GRM) on AMD risk → Proportion of AMD Risk Explained

Weight all SNPs equally

Total number of SNPs

Number of reference alleles person j has at SNP i, MINUS 2 × ref. allele freq.

Same thing, for person k

$$A_{jk} = \frac{1}{N} \sum_{i=1}^N \frac{(x_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1 - p_i)}$$

Genetic relationship value for persons j & k

Normalize using SNP variance

MODEL SIGNIFICANCE

Likelihood Ratio Test (LRT)

Full: pathway GRM + rest GRM + Covariates = Likelihood_{Full}

Reduced: rest GRM + Covariates = Likelihood_{Reduced}

$$D = -2\ln \left(\frac{\text{Likelihood}_{Full}}{\text{Likelihood}_{Reduced}} \right)$$

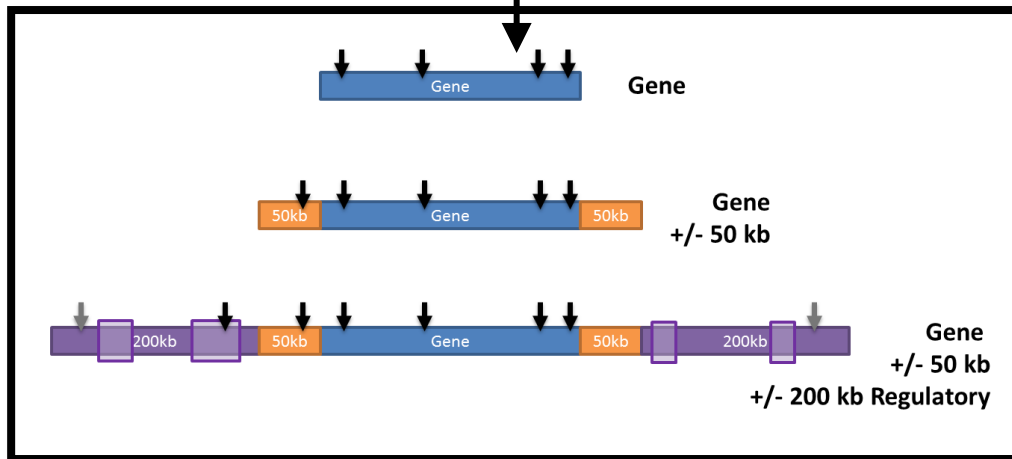
D statistic used to determine the probability that the Pathway/GRM impacts risk for AMD

(All analyses include Age, Sex, & 2 Principal Components)

OVERVIEW

8 POTENTIAL MECHANISMS

Angiogenesis	Inflammation
Antioxidants	Nicotine/Smoking
Apoptosis	Oxidative Phosphorylation
Complement Activation	Tricarboxylic Acid Cycle

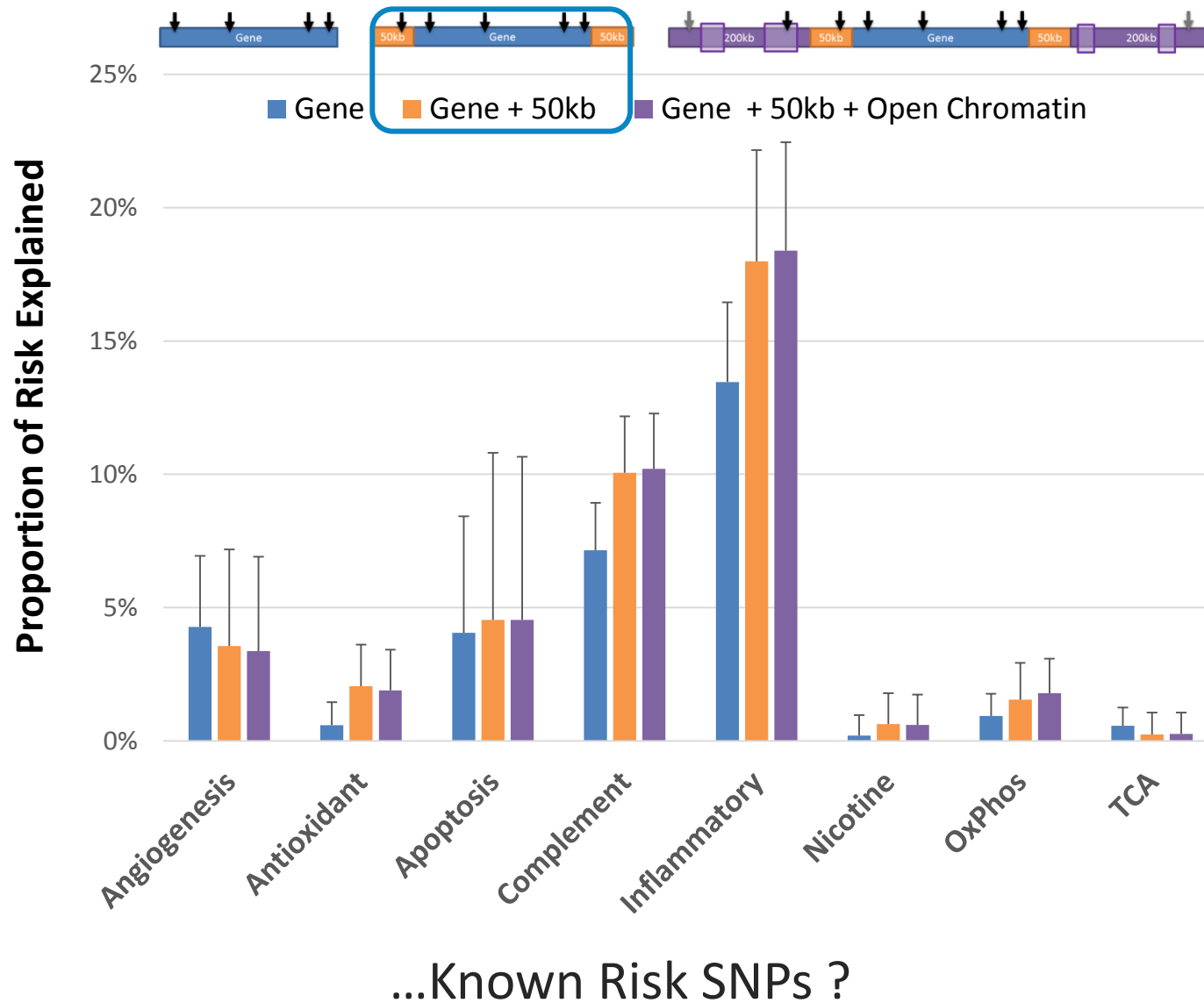


$$A_{jk} = \frac{1}{N} \sum_{i=1}^N \frac{(x_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1 - p_i)}$$

$$D = -2\ln \left(\frac{Likelihood_{Full}}{Likelihood_{Reduced}} \right)$$

**Proportion of AMD Risk
Explained by Pathway**

RESULTS | PATHWAY REGIONS



RESULTS | Risk SNPs

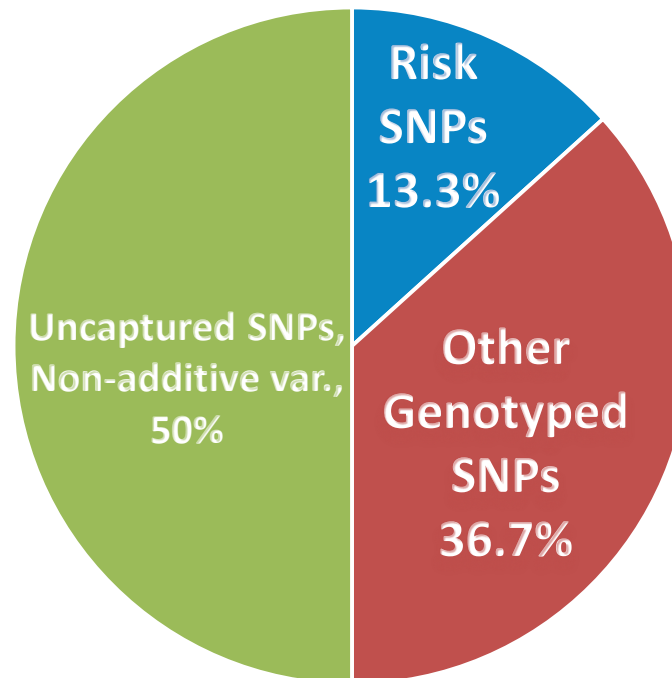
Known Risk GRM + Rest GRM + Covariates = Likelihood_{Full}

Proportion of AMD risk explained

19 Risk SNPs: **13.3%** ($p=1.4^{-61}$)

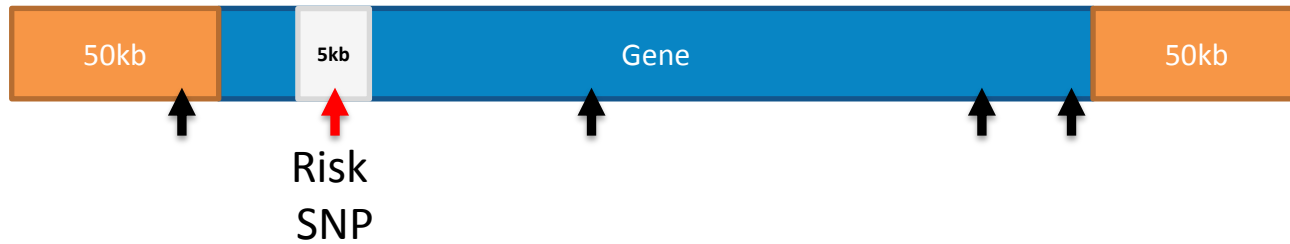
+ 5 kb flanking: **15.4%** ($p=1.6^{-53}$)

HERITABILITY



RESULTS | PRIMARY ANALYSIS PARAMETERS

[Genes \pm 50 kb]

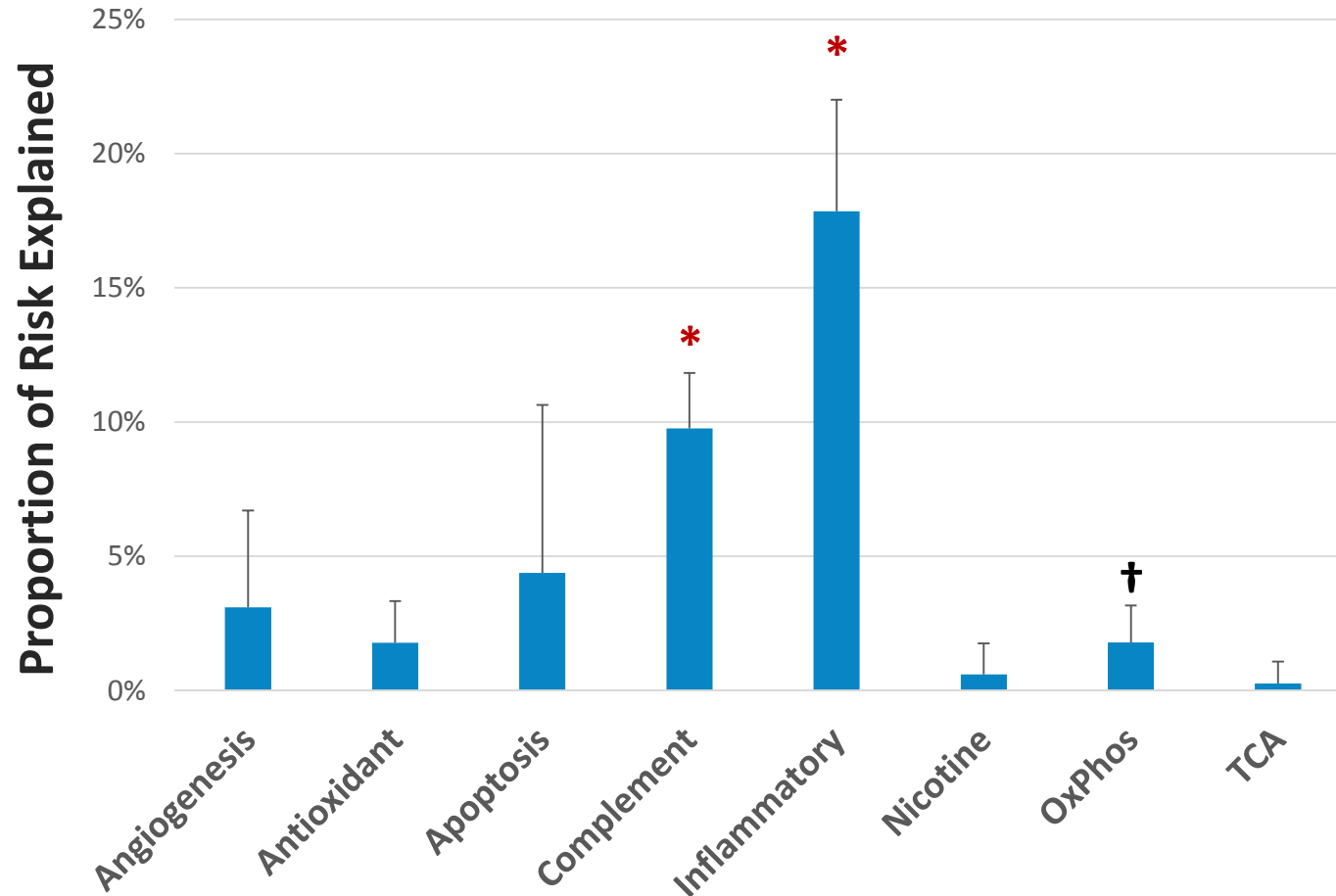


RESULTS

PRIMARY ANALYSIS

[EXCLUDING KNOWN RISK]

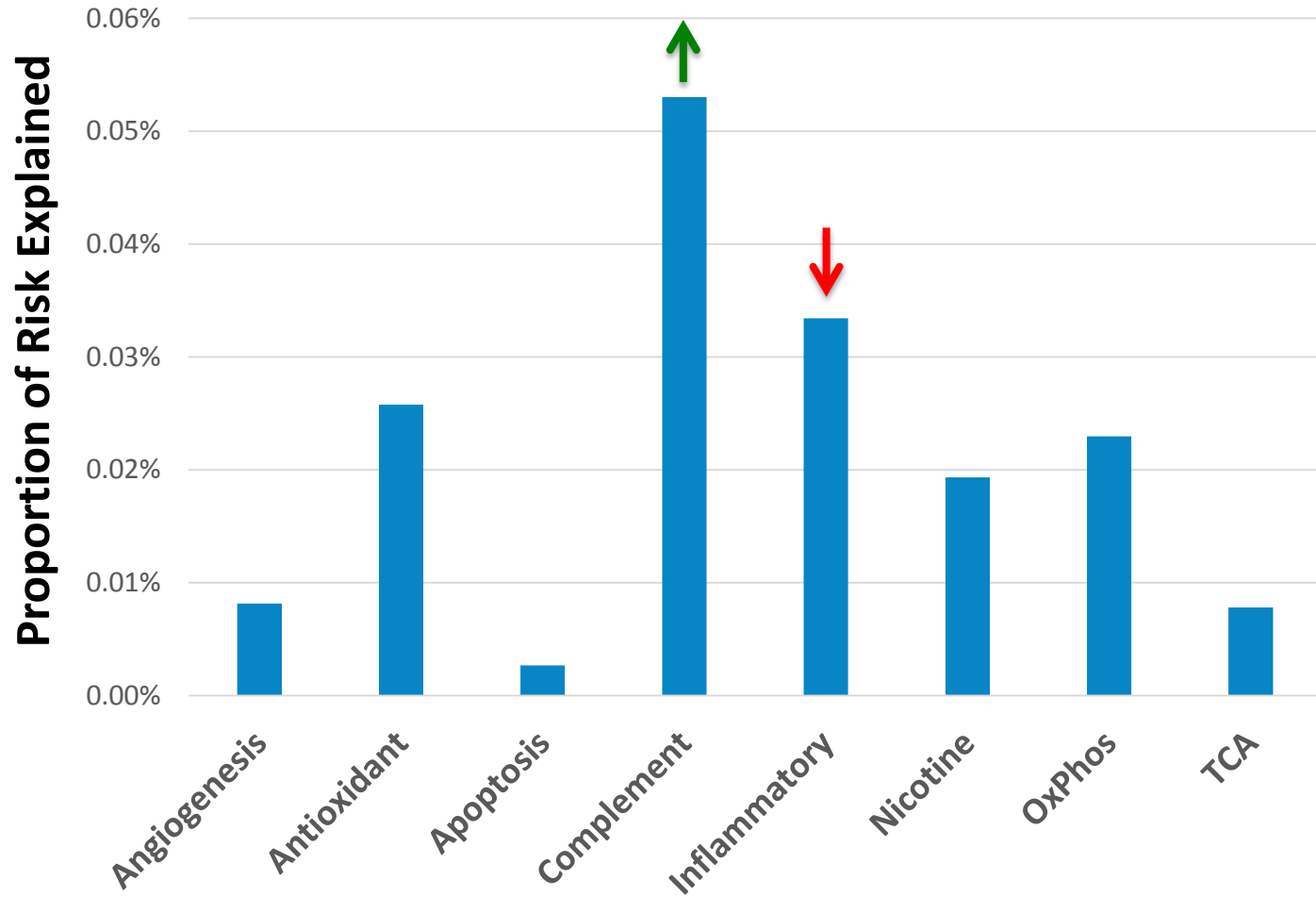
... # Genes / Pathway?



* Significant p-value (Complement: 6.8×10^{-26} / Inflammatory: 9.5×10^{-8})

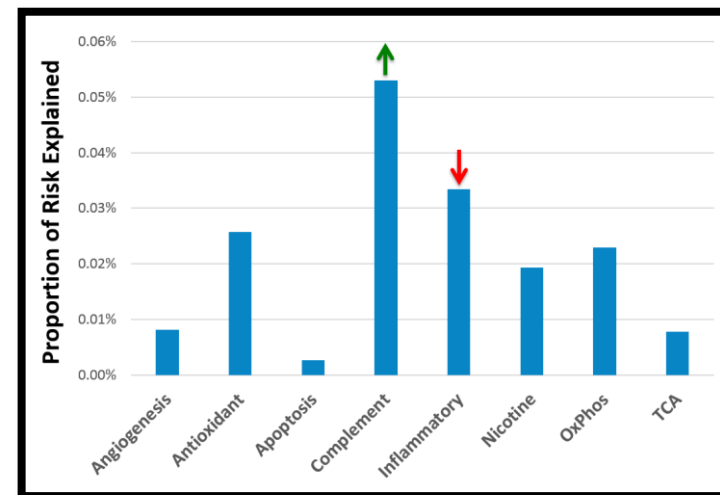
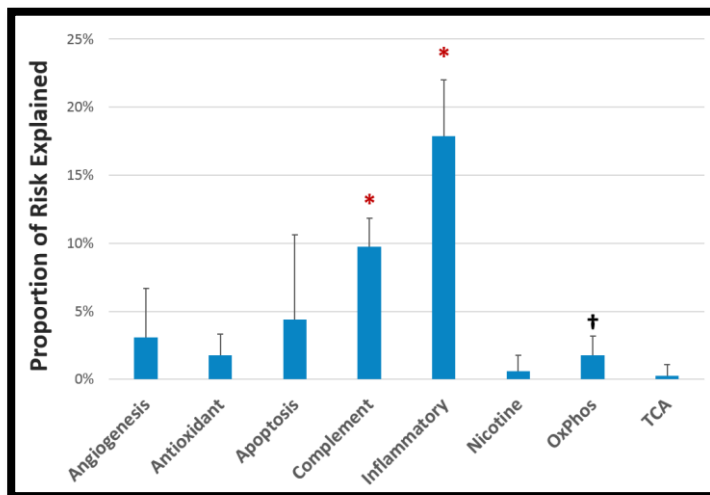
† Oxidative Phosphorylation p-value: **0.08**

RESULTS | RISK EXPLAINED / GENE



CONCLUSIONS

- Accounting for known risk SNPs, **complement** pathway contains fewer additional SNPs with higher average AMD risk explained
- Inflammatory** pathway contains more SNPs with smaller effects, but more overall AMD risk explained
- Variants in **open chromatin** regions 50kb - 250kb away from gene explain *little* AMD risk



ACKNOWLEDGEMENTS

CASE WESTERN RESERVE UNIVERSITY

WILLIAM S. BUSH (ADVISOR)

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JACKLYN L. KOVACH

MARGARET A. PERICAK-VANCE

STEPHEN D. SCHWARTZ

WILLIAM K. SCOTT

- jakehall@case.edu -

PATHWAY SNP OVERLAP

PATHWAY	Angiogenesis	Antioxidant	Apoptotic	Complement	Inflammatory	Nicotine	OxPhos	TCA
Angiogenesis	100.0%	0.0%	8.2%	1.3%	8.2%	0.6%	0.2%	0.0%
Antioxidant	0.0%	100.0%	0.2%	0.0%	0.7%	0.0%	0.4%	0.0%
Apoptotic	8.2%	0.2%	100.0%	0.2%	7.3%	0.5%	0.4%	0.0%
Complement	1.3%	0.0%	0.2%	100.0%	6.1%	0.0%	0.0%	0.0%
Inflammatory	8.2%	0.7%	7.3%	6.1%	100.0%	1.6%	0.2%	0.0%
Nicotine	0.6%	0.0%	0.5%	0.0%	1.6%	100.0%	0.0%	0.0%
OxPhos	0.2%	0.4%	0.4%	0.0%	0.2%	0.0%	100.0%	1.1%
TCA	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	100.0%

Index SNP	Nearby Gene(s)		
	Symbol	Name	Distance to index SNP [kb] / Location
rs10490924	<i>ARMS2</i>	Age-related maculopathy susceptibility 2	0 / coding sequence
	<i>HTRA1</i>	HtrA serine peptidase 1	6.6 / upstream
rs10737680	<i>CFH</i>	Complement factor H	0 / intronic
rs429608	<i>C2</i>	Complement component 2	17 / downstream
	<i>CFB</i>	Complement factor B	10.6 / downstream
	<i>SKIV2L</i>	Superkiller viralicidic activity 2-like (<i>S. cerevisiae</i>)	0 / intronic
rs2230199	<i>C3</i>	Complement component 3	0 / coding sequence
rs5749482	<i>TIMP3</i>	TIMP metallopeptidase inhibitor 3	137.1 / upstream
	<i>SYN3</i>	Synapsin III	0 / intronic
rs4420638	<i>APOE</i>	Apolipoprotein E	10.3 / downstream
	<i>APOC1</i>	Apolipoprotein C-I	5.0 / downstream
rs1864163	<i>CETP</i>	Cholesteryl ester transfer protein, plasma	0 / intronic
rs943080	<i>VEGFA</i>	Vascular endothelial growth factor A	72.4 / downstream
rs13278062	<i>TNFRSF10A</i>	Tumor necrosis factor receptor superfamily, member 10a	0.3 / upstream
rs920915	<i>LIPC</i>	Lipase, hepatic	35.7 / upstream
rs4698775	<i>CFI</i>	Complement factor I	71.4 / downstream
	<i>CCDC109B</i>	Coiled-coil domain containing 109B	0 / intronic
rs3812111	<i>COL10A1</i>	Collagen, type X, alpha 1	0 / intronic
rs13081855	<i>COL8A1</i>	Collagen, type VIII, alpha 1	0 / intronic
rs3130783	<i>IER3</i>	Immediate early response 3	62 / upstream
	<i>DDR1</i>	Discoidin domain receptor tyrosine kinase 1	77.5 / upstream
rs8135665	<i>SLC16A8</i>	Solute carrier family 16, member 8 (monocarboxylic acid transporter 3)	0 / intronic
rs334353	<i>TGFB1</i>	Transforming growth factor, beta receptor 1	0 / intronic
rs8017304	<i>RAD51B</i>	RAD51 homolog B (<i>S. cerevisiae</i>)	0 / intronic
rs6795735	<i>ADAMTS9</i>	ADAM metallopeptidase with thrombospondin type 1 motif, 9	32 / upstream
	<i>ADAMTS9-AS2</i>	ADAMTS9 antisense RNA 2 (non-protein coding)	0 / intronic
	<i>MIR548A2</i>	microRNA 548a-2	0.3 / upstream
rs9542236	<i>B3GALTL</i>	Beta 1,3-galactosyltransferase-like	0 / intronic

LIMITATIONS

- Pathway definitions
- Same platform used in meta
- Europeans only
- SNPs overlapping between pathways
- Additional genetic risk for AMD may exist (non-additive variation, SNPs not genotyped, etc.)

DATASET

Pre-QC:

Case / Control study design

- Cases: 1,247
- Controls: 708

Total SNPs: 906,688

- Affymetrix 6.0: 906,600
- Custom Sequenom: 84
- Custom TaqMan: 4

INTRODUCTION

- Heritability -

81%



55%



51%



30%



QUESTION

METHODS

- Genes -

HMGA2

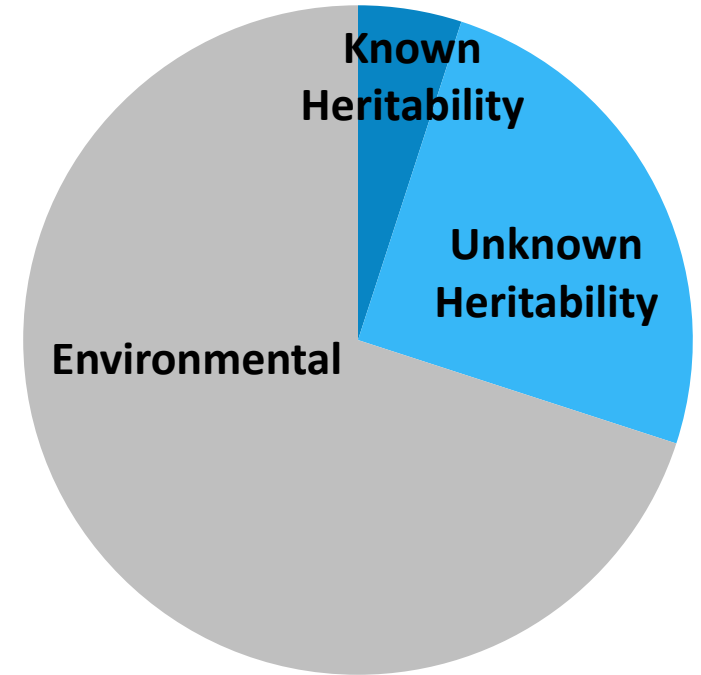
HLA-DRB1

**FTO
TMEM18
TFAP2B
MC4R**

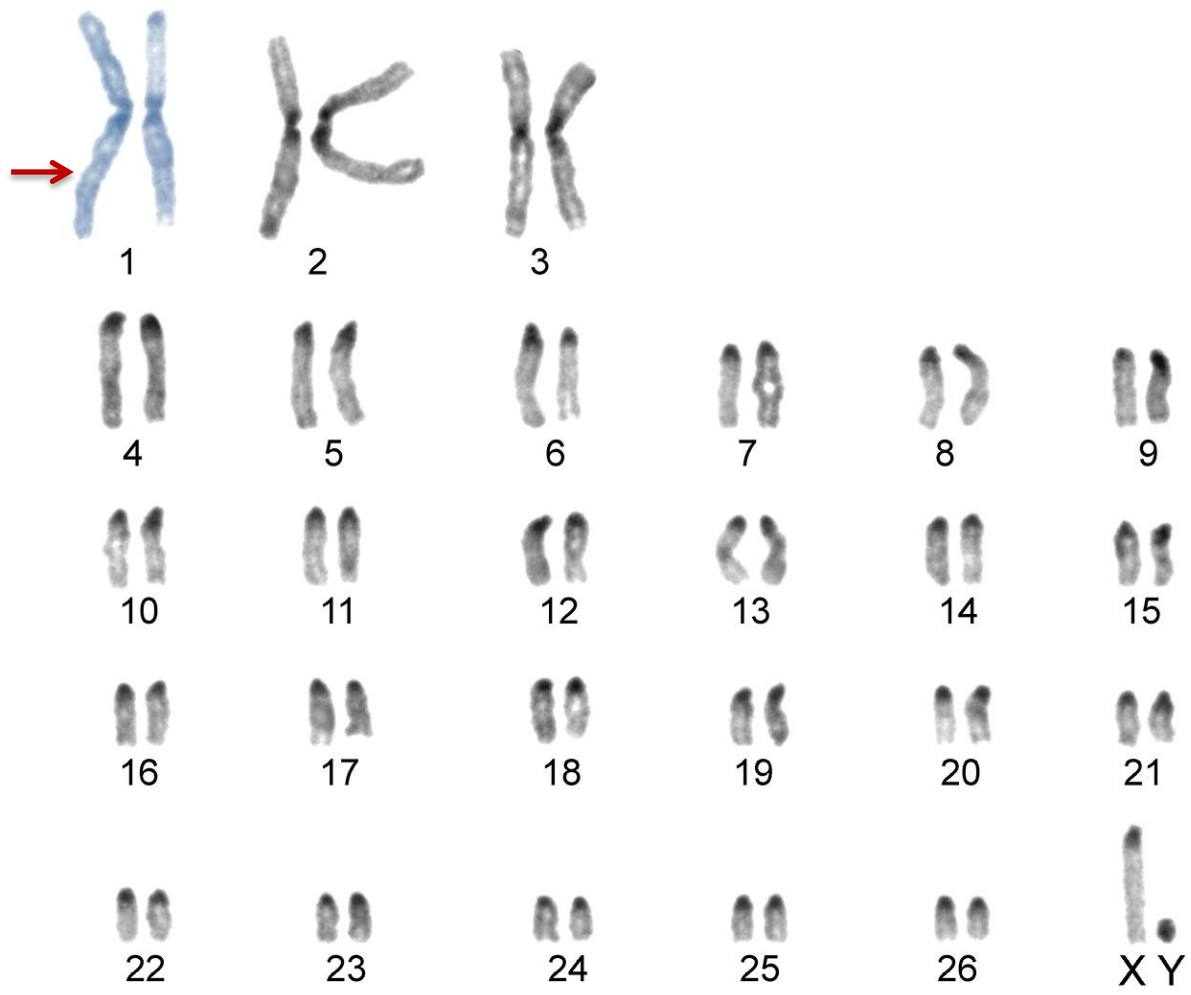
**CSTM1
IL10
LTC4S
> 100 genes**

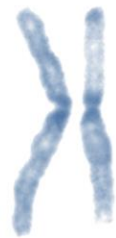
RESULTS

- Trait Variance / Risk -



CONCLUSIONS





1



2



3



4



5



6



7



8



9



10



11



12



13



14



15



16



17



18



19



20



21



22



23



24



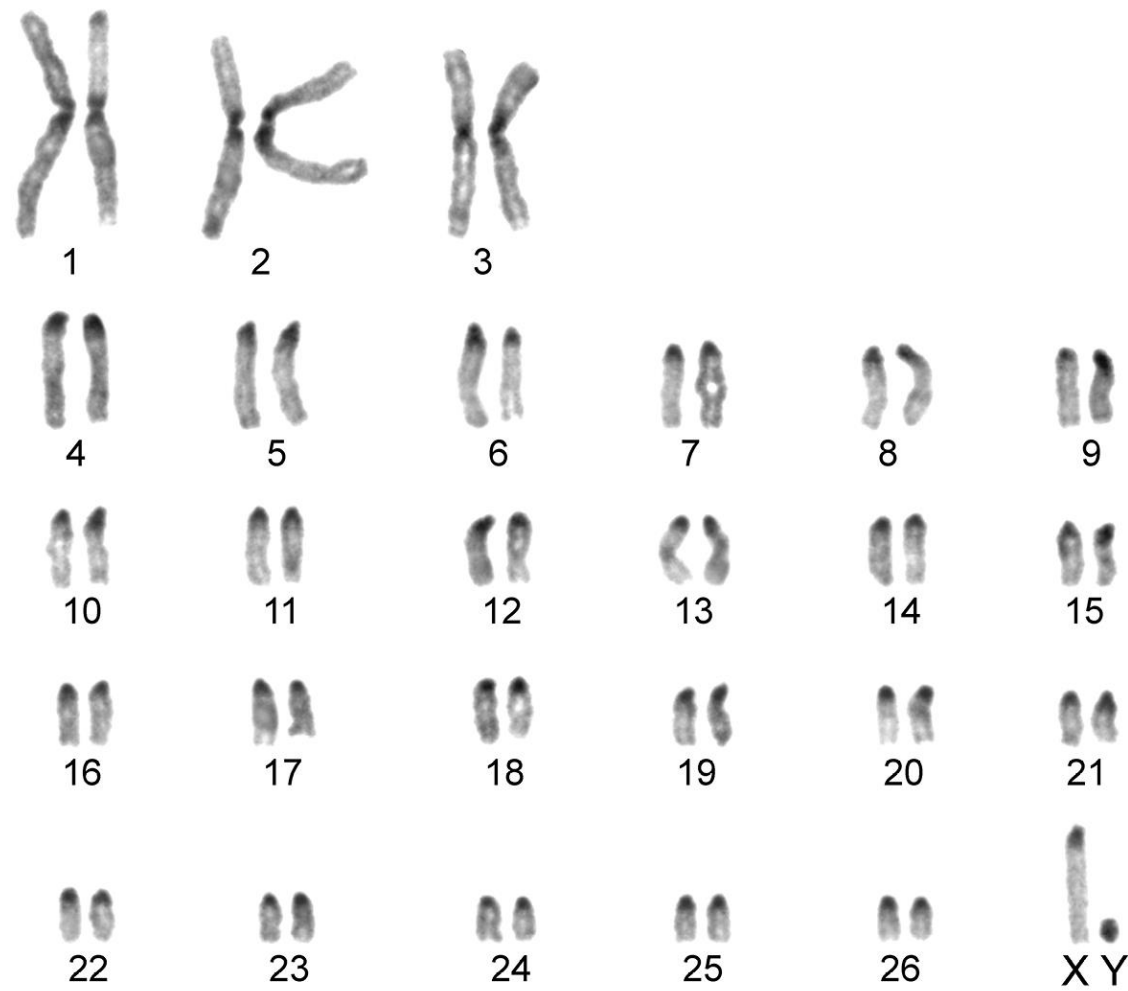
25



26



X Y








Chromosome, Chromosome Region, Pathway, Gene, SNP

16/19 SNPs – Logistic Regression
 639,825 SNPs; 1955 total individuals
 Age, Sex, 2 PCs covariates


Gene Symbol	Chr.	rs#	P-value
<i>CFH</i>	1	rs10737680	1.67 E-25
<i>ARMS2-HTRA</i>	10	rs10490924	9.85 E-24
<i>C2-CFB</i>	6	rs429608	8.69 E-13
<i>C3</i>	19	rs2230199	2.57 E-06
<i>CEPT</i>	16	rs1864163	2.02 E-05
<i>COL8A1</i>	3	rs13081855	0.001
<i>B3GALTL</i>	13	rs9542236	0.001
<i>APOE-APOC1</i>	19	rs4420638	0.002
<i>ADAMTS9-MIR548A2</i>	3	rs6795735	0.018
<i>TNFRSF10A</i>	8	rs13278062	0.057
<i>RAD51B</i>	14	rs8017304	0.095
<i>VEGFA</i>	6	rs943080	0.103
<i>TIMP3-SYN3</i>	22	rs5749482	0.121
<i>TGFBR1</i>	9	rs334353	0.127
<i>CFI</i>	4	rs4698775	0.296
<i>LIPC</i>	15	rs920915	0.449

DEFINING PATHWAYS

← → ↻ 🏠 amigo.geneontology.org/amigo/landing    

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AmiGO 2

More information on quick search 

response to nicotine |

- cellular response to nicotine (GO:0071316)
- behavioral response to nicotine (GO:0035095)
- response to nicotine (GO:0035094)


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DEFINING PATHWAYS

Term Information

Accession GO:0035094

Name response to nicotine

Ontology biological_process

Synonyms None

Definition Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a nicotine stimulus. *Source:* [CHEBI:17688](#), [ISBN:0198506732](#), [ISBN:0582227089](#), GOC:bf, GOC:ef

Comment None

History See term [history for GO:0035094](#) at QuickGO

Subset None

Community [GN Add](#) usage comments for this term on the [GONUTS](#) wiki.

Related [Link](#) to all **genes and gene products** associated to response to nicotine.

[Link](#) to all direct and indirect **annotations** to response to nicotine.

[Link](#) to all direct and indirect **annotations download** (limited to first 10,000) for response to nicotine.

DEFINING PATHWAYS

[Associations](#)[Graph Views](#)[Inferred Tree View](#)[Ancestors and Children](#)[Mappings](#)

- I** GO:0008150 biological_process
 - I** GO:0050896 response to stimulus
 - I** GO:0042221 response to chemical
 - I** GO:0009719 response to endogenous stimulus
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 - I** **GO:0035094 response to nicotine**
 - I** GO:0035095 behavioral response to nicotine
 - I** GO:0071316 cellular response to nicotine

DEFINING PATHWAYS

Associations Graph Views Inferred Tree View Ancestors and Children Mappings

Free-text filtering

Your search is pinned to these filters

- + document_category: annotation
- + regulates_closure: GO:0035095

No current user filters.

- Source
- Assigned by
- Ontology (aspect)
- Evidence type
- PANTHER family
- Qualifier
- Taxon
- Direct annotation
- Inferred annotation
- Annotation extension

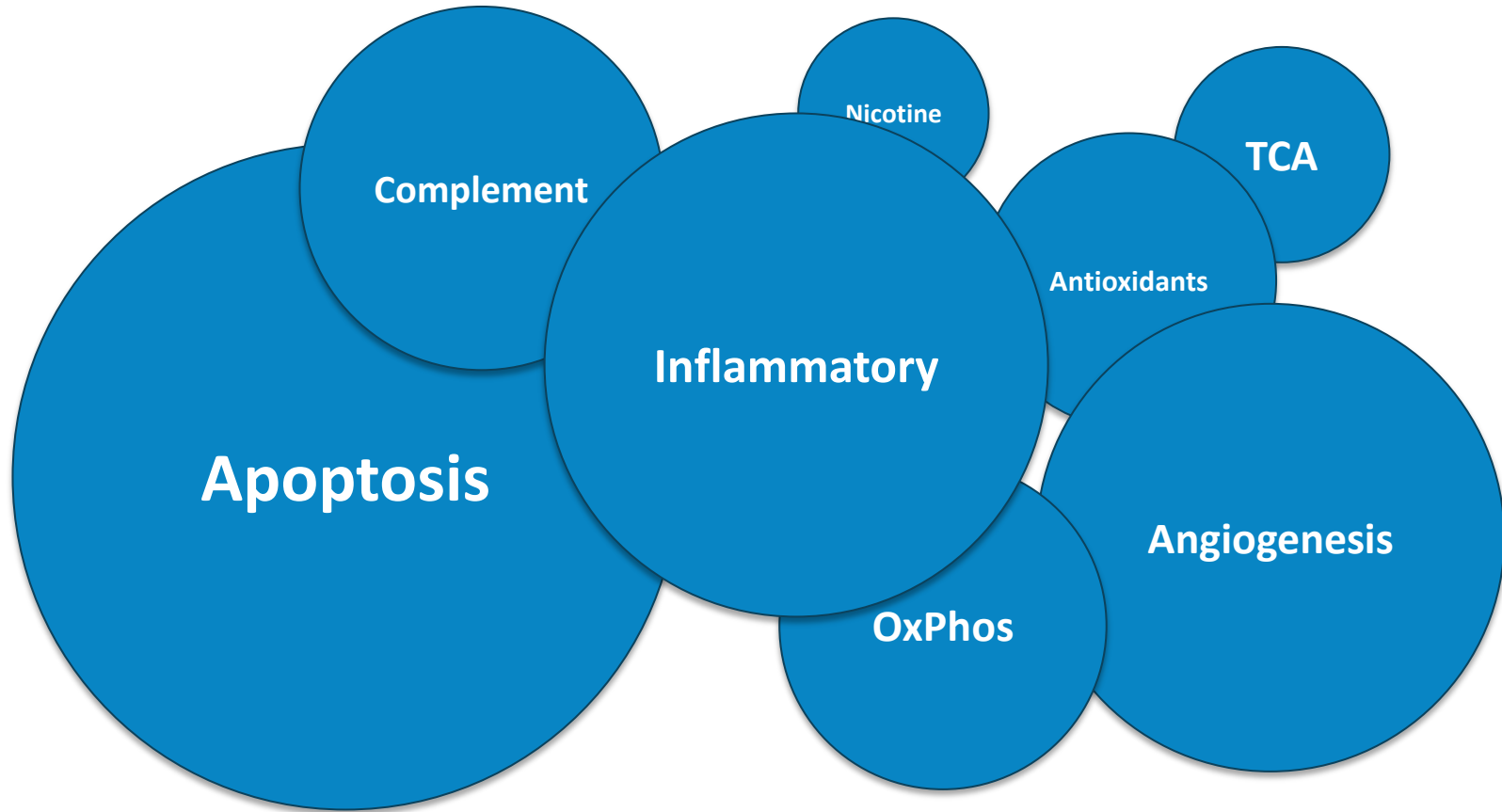
Found entities

Total: 96; showing 1-10 Results count 10

Gene/product	Gene/product name	Qual
<input type="checkbox"/> CHRNB4	Neuronal acetylcholine receptor subunit beta-4	
<input type="checkbox"/> CHRNB2	Uncharacterized protein	
<input type="checkbox"/> CHRNB2	Uncharacterized protein	
<input type="checkbox"/> PPARA	Peroxisome proliferator-activated receptor alpha	
<input type="checkbox"/> CHRNA7	Uncharacterized protein	
<input type="checkbox"/> Chrna4	Neuronal acetylcholine receptor subunit alpha-4	
<input type="checkbox"/> Chrna4	Neuronal acetylcholine receptor	

GO Term	GO ID	# Genes
Angiogenesis	GO:0001525	379
Antioxidant Activity	GO:0016209	69
Apoptotic Signaling	GO:0097190	1,635
Complement Activation	GO:0006956	184
Inflammatory Response	GO:0006954	534
Response to Nicotine	GO:0035094	31
Oxidative Phosphorylation	GO:0006119	78
Tricarboxylic Acid Cycle	GO:0006099	33

AMD PATHOGENESIS IN LITERATURE



Extras

