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The role of complement factor H-like and factor H-related proteins in age-related macular degeneration

Dr. Simon J. Clark

MRC Career Development Fellow

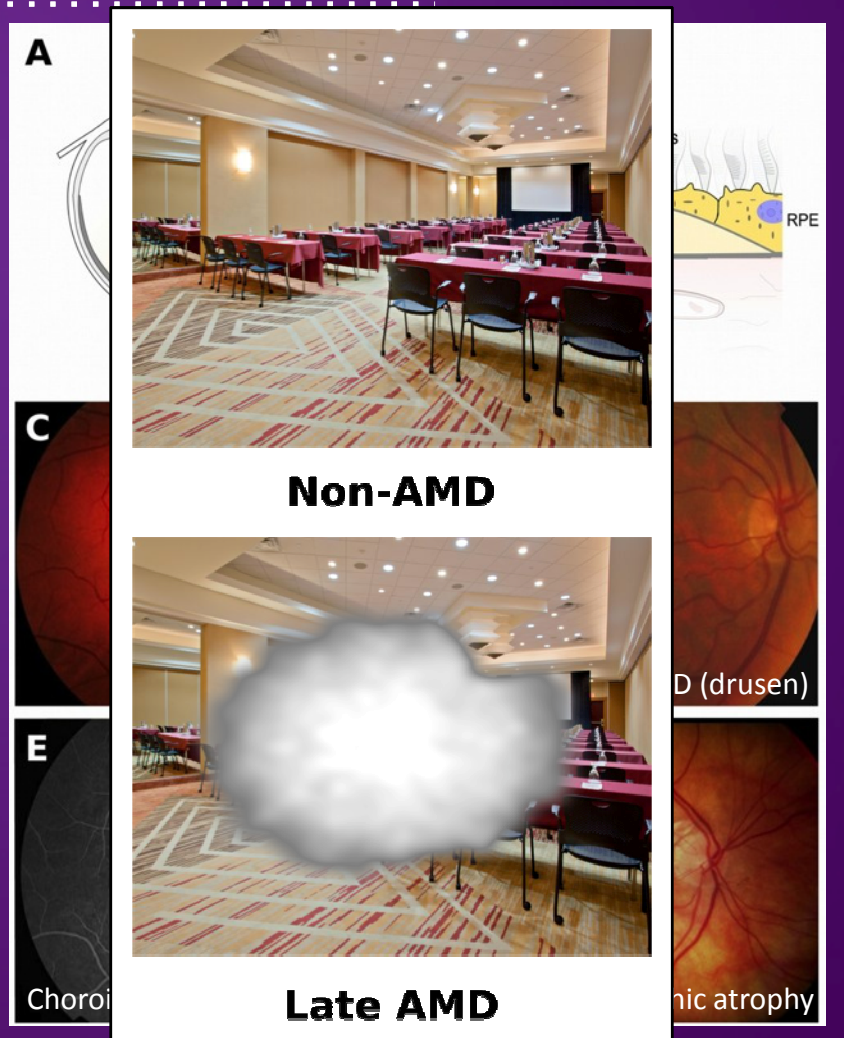
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Age-related macular degeneration (AMD)

- Largest cause of blindness in developed world. ~50 million people affected worldwide
- Occurs around 50 y.o. - Incidence will only ever increase with burgeoning elderly population
- Progressive destruction of the retina, preceded by hallmark lesions (drusen), leading to central vision loss
- Anti-VEGF treatment for ‘wet’ AMD, only halts damage already suffered
- No effective treatments for ‘dry’ AMD



Black and Clark (2015) *Genet. Med.* online 28th May

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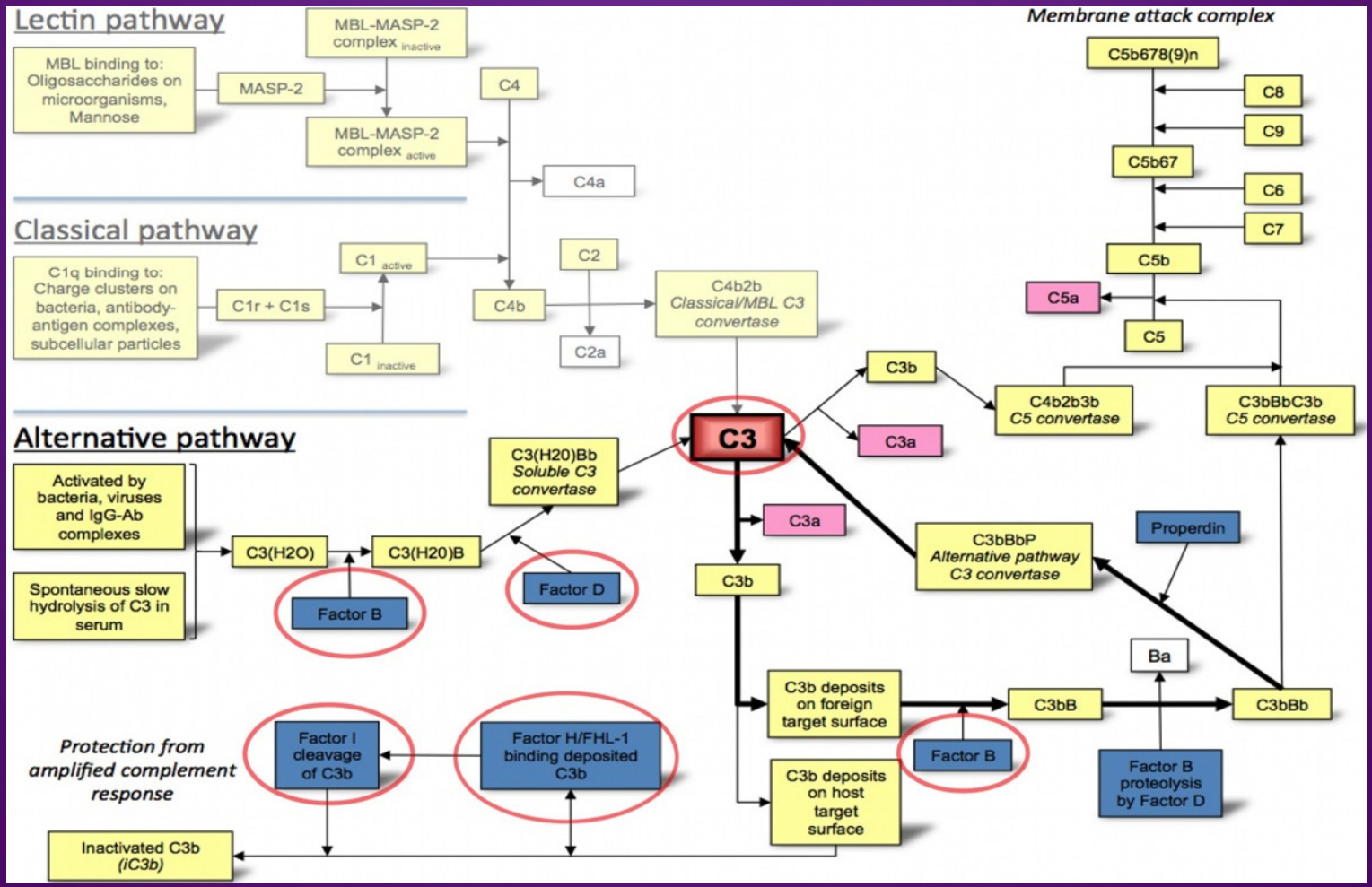
AMD – risk factors

- Risk factors include environment (*i.e.* diet, smoking) but is essentially a genetically-driven disease

Protein function	Locus	SNP/variant, effect/other allele	Chrom-osome	Odds ratio*
Uncertain	*ARMS2/HTRA1*	rs10490924, T/G or rs2284665, T/G	10	3.0
		rs11200638, A/G		
Complement components and regulators	*CFH*	rs1061170, C/T+	1	2.3
		rs1410996, C/T		2.5
	CFHR3-1	84 kb deletion		0.3
	C2-CFB	rs641153, C/T	6	0.5
		rs9332739, C/G		0.7
	C3	rs2230199, G/C	19	1.3
	CFI	rs7690921, T/C	4	1.2
		rs10033900, T/C		1.1
Regulation of lipid metabolism, matrix turnover and angiogenesis	*VEGFA*	rs4711751, T/C	6	1.2
		rs833069, T/C		0.9
	LIPC	rs493258, T/C	15	0.9
		rs10468017, T/C		0.9
	TIMP3	rs9621532, C/A	22	0.9

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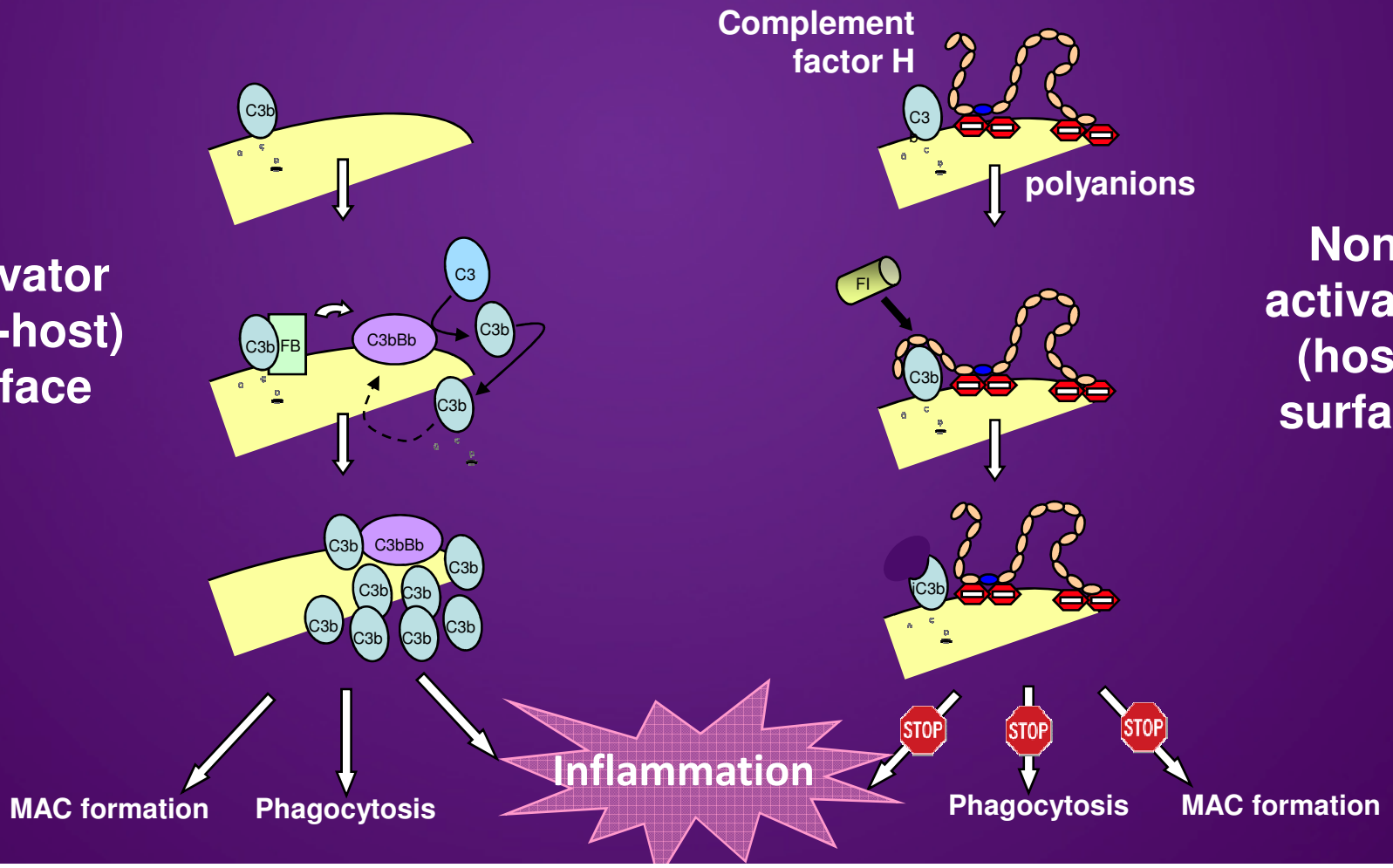
Alternative pathway of complement activation



McHarg S *et al.* (2015) *Mol. Immunol.* **67**, 43-50.

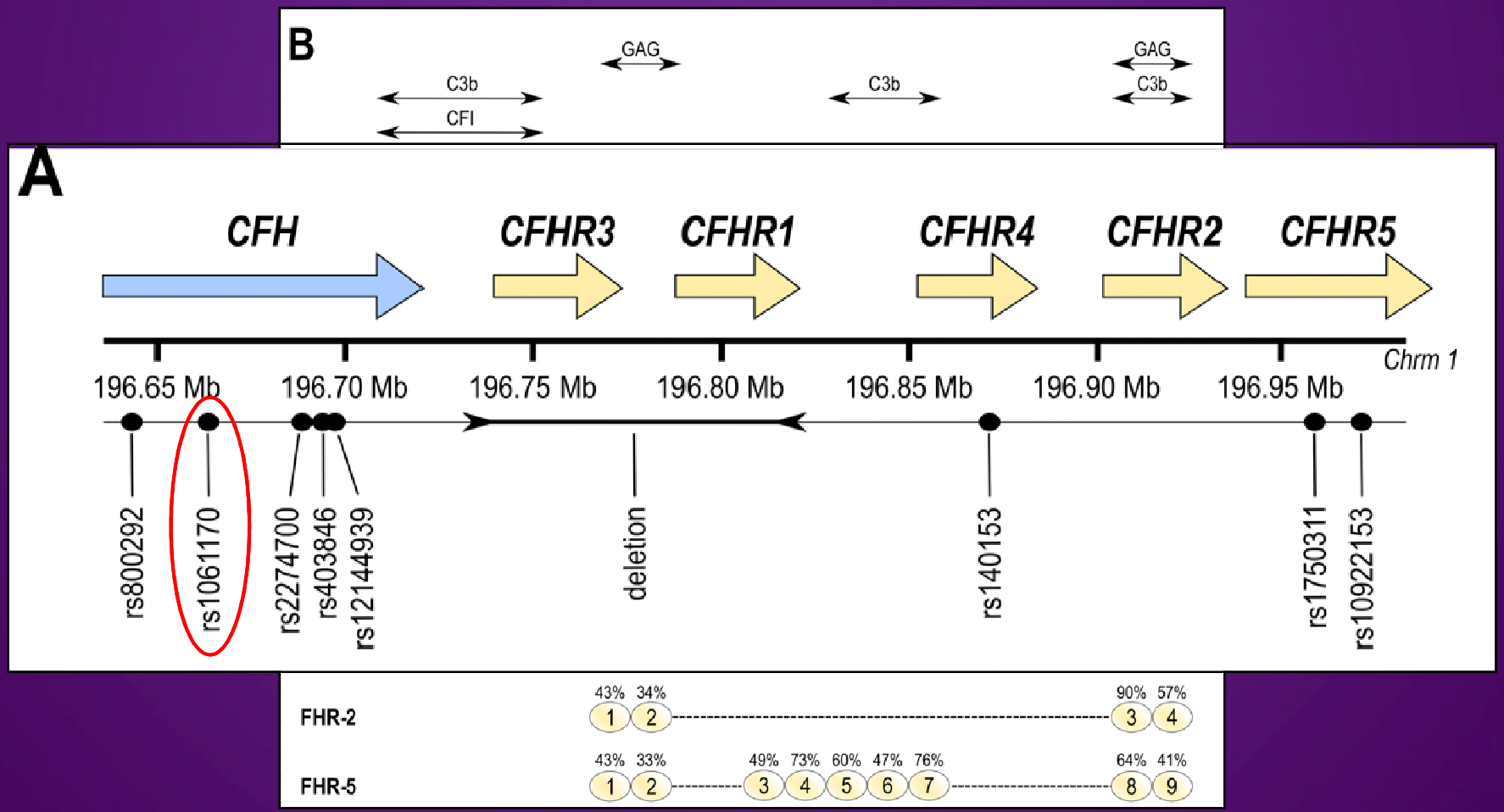
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The alternative pathway of complement



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AMD – risk factors

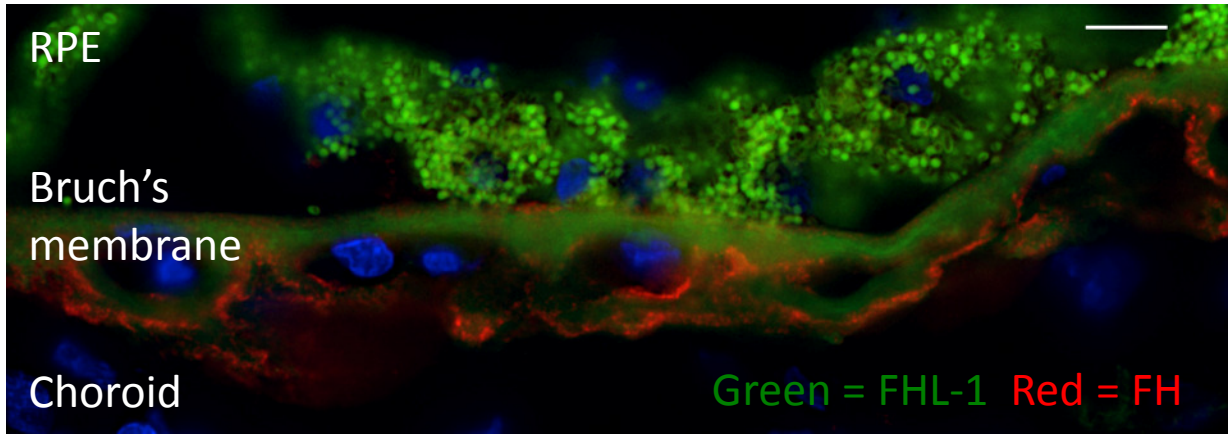


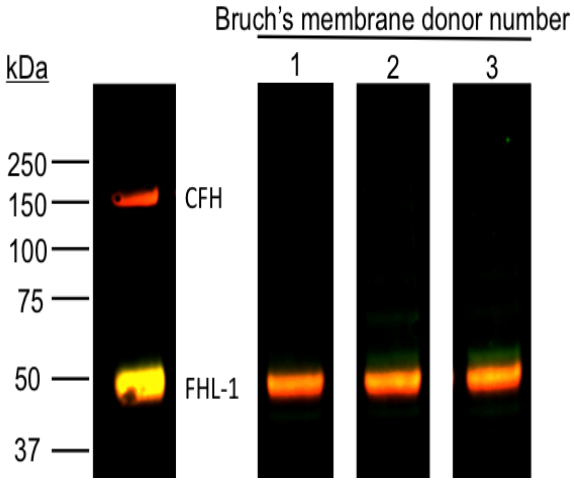
Clark S.J. and Bishop P.N. (2015) *J. Clin. Med.* **3**

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Recent discovery about the role of FHL-1

- Recently identified that Factor H-like protein 1 (FHL-1) concentrates on Bruch's membrane
- Still able to regulate complement but to date no one has worried about elucidating the level of involvement in immune regulation



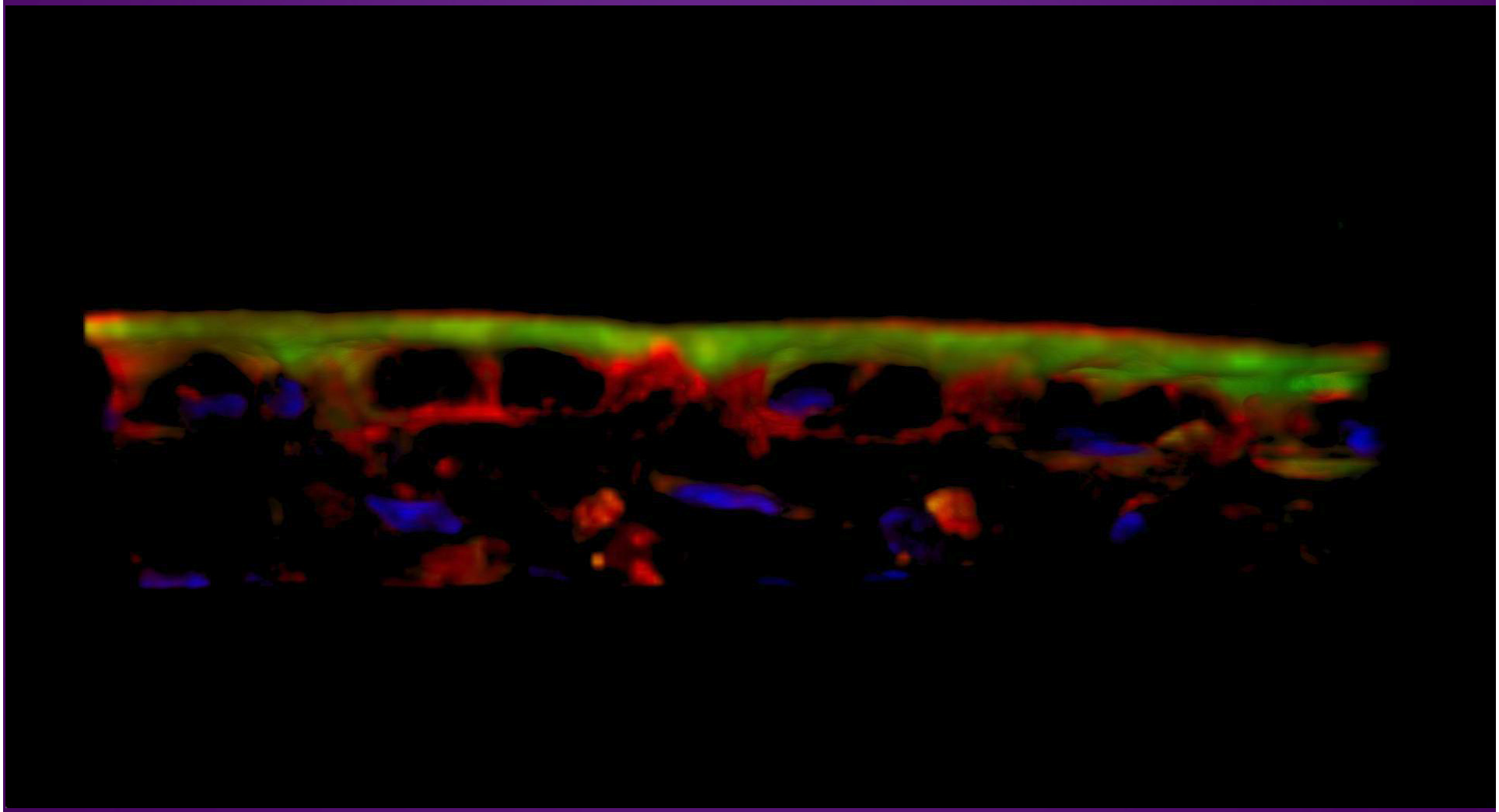


Clark S.J. *et al.* (2014) *J. Immunol.* **193**, 4962-4970.

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The role of FHL-1

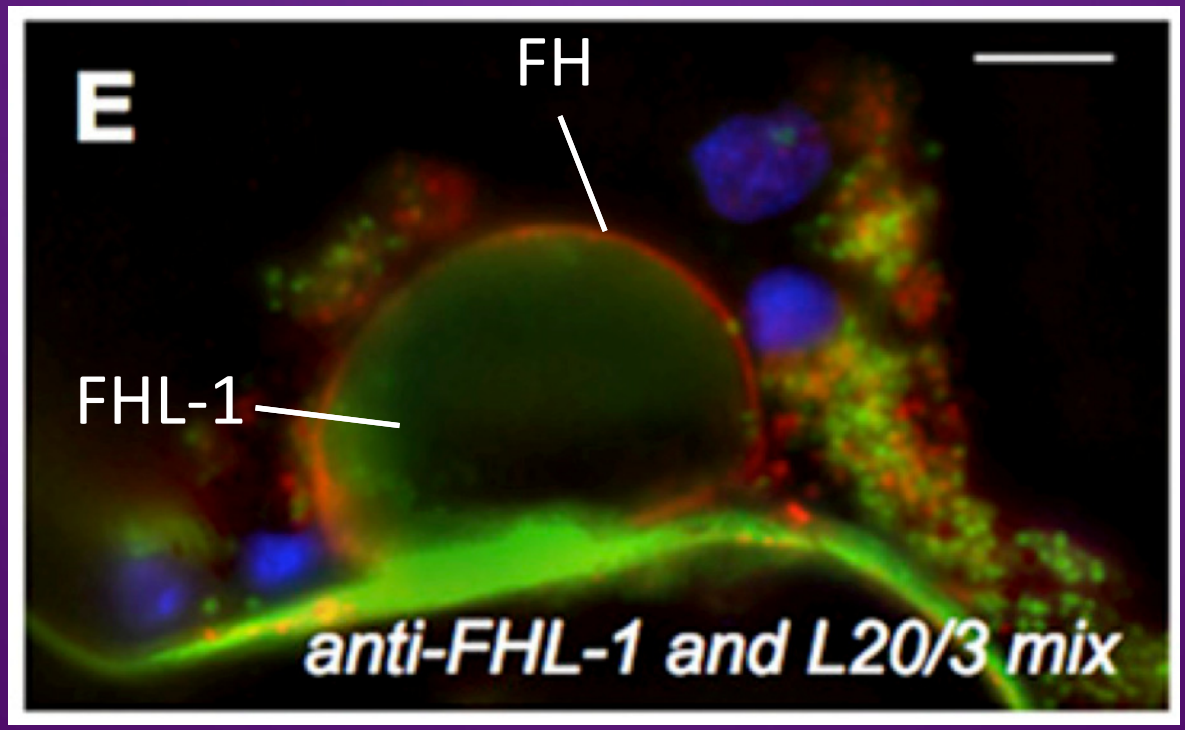
Green = FHL-1 Red = FH



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Curiously, FH seems to coat drusen

- The FH that is secreted by the RPE cells 'coats' drusen that form in Bruch's membrane

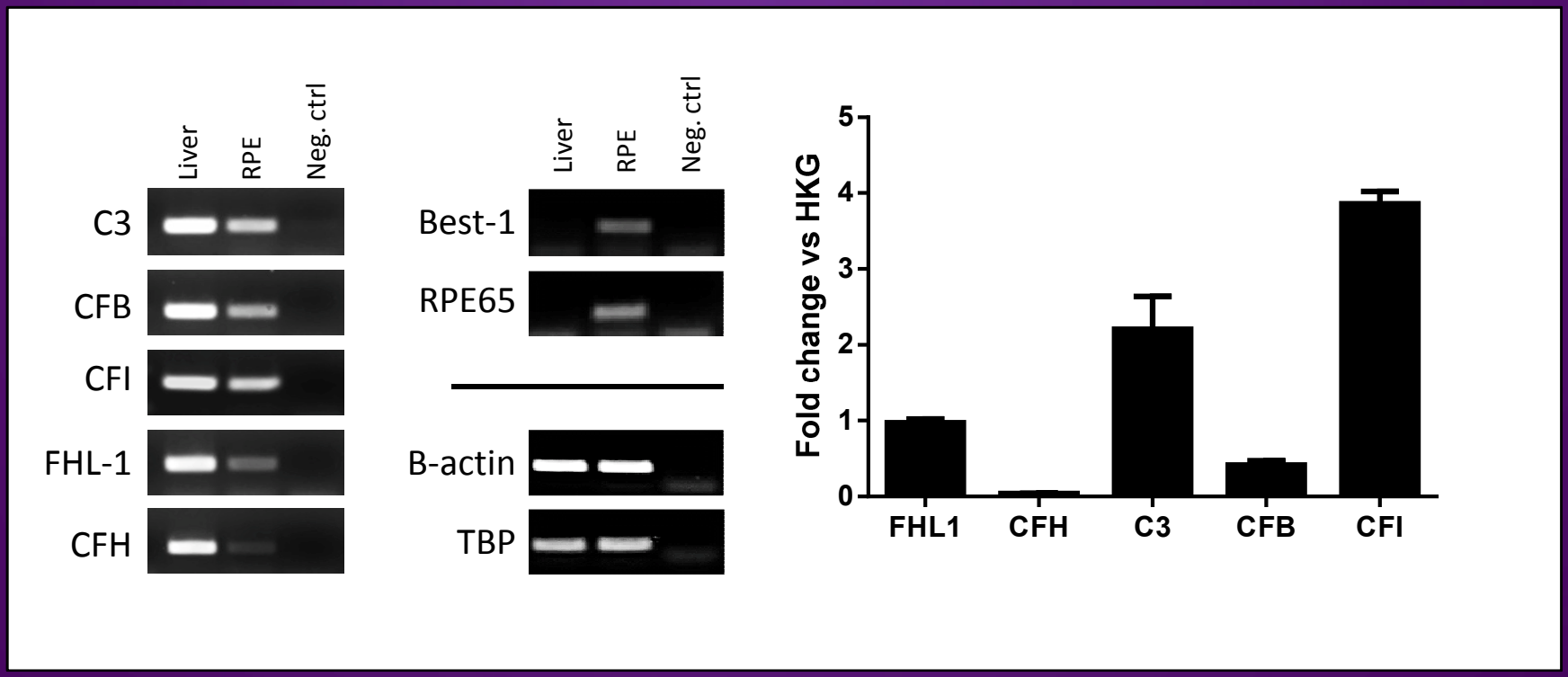


Clark S.J. *et al.* (2014) *J. Immunol.* **193**, 4962-4970.

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FHL-1 is locally expressed by RPE cells

- First to demonstrate that FHL-1 is locally expressed by the RPE cells in human eyes



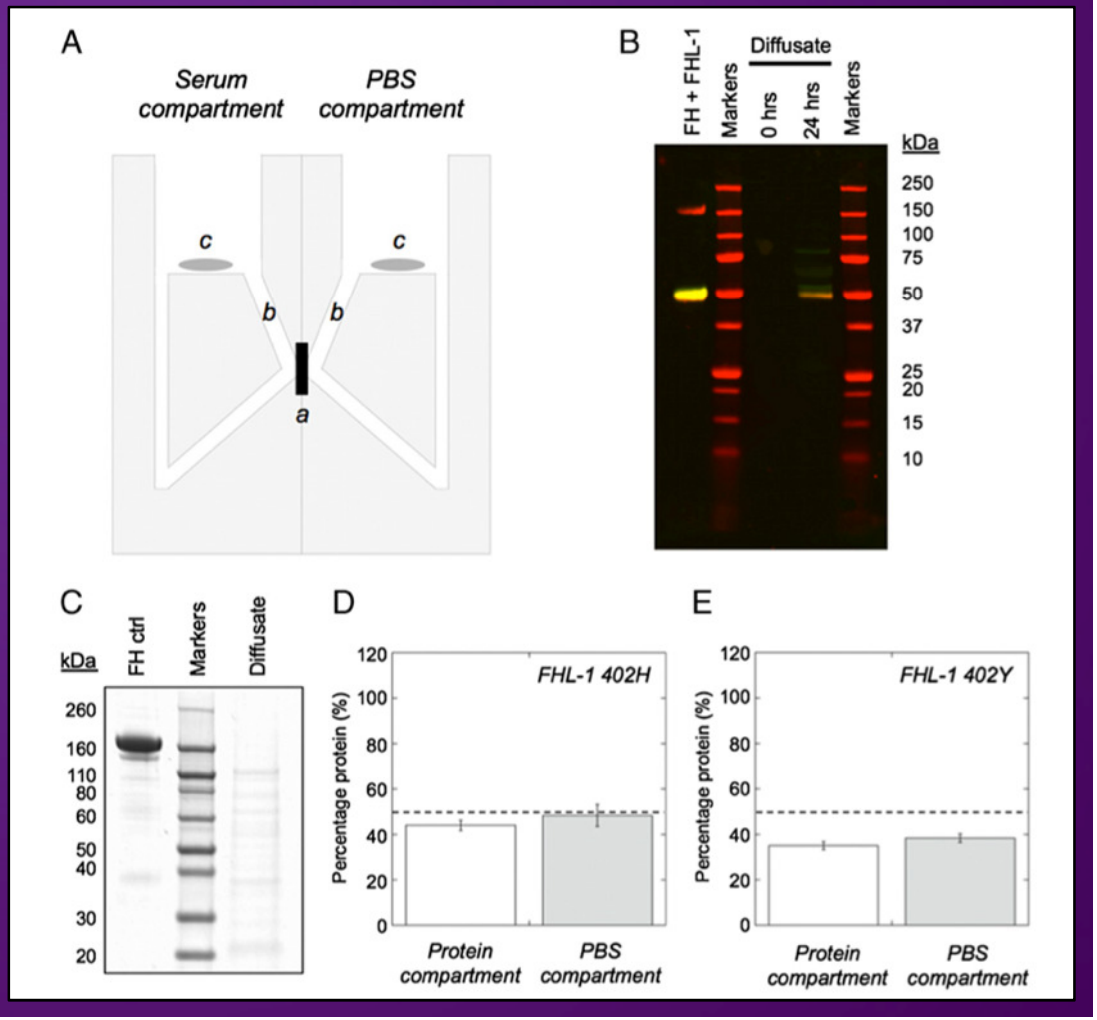
Clark S.J. *et al.* (2014) *J. Immunol.* **193**, 4962-4970.

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FHL-1 can diffuse through Bruch's (FH can not)

- First to demonstrate that FHL-1 can passively diffuse through Bruch's membrane, and that FH can not

- Noted that virtually no AMD-associated form of FHL-1 (402H) was retained by the Bruch's membrane
  - i.e. not being recruited, therefore not able to protect against complement attack

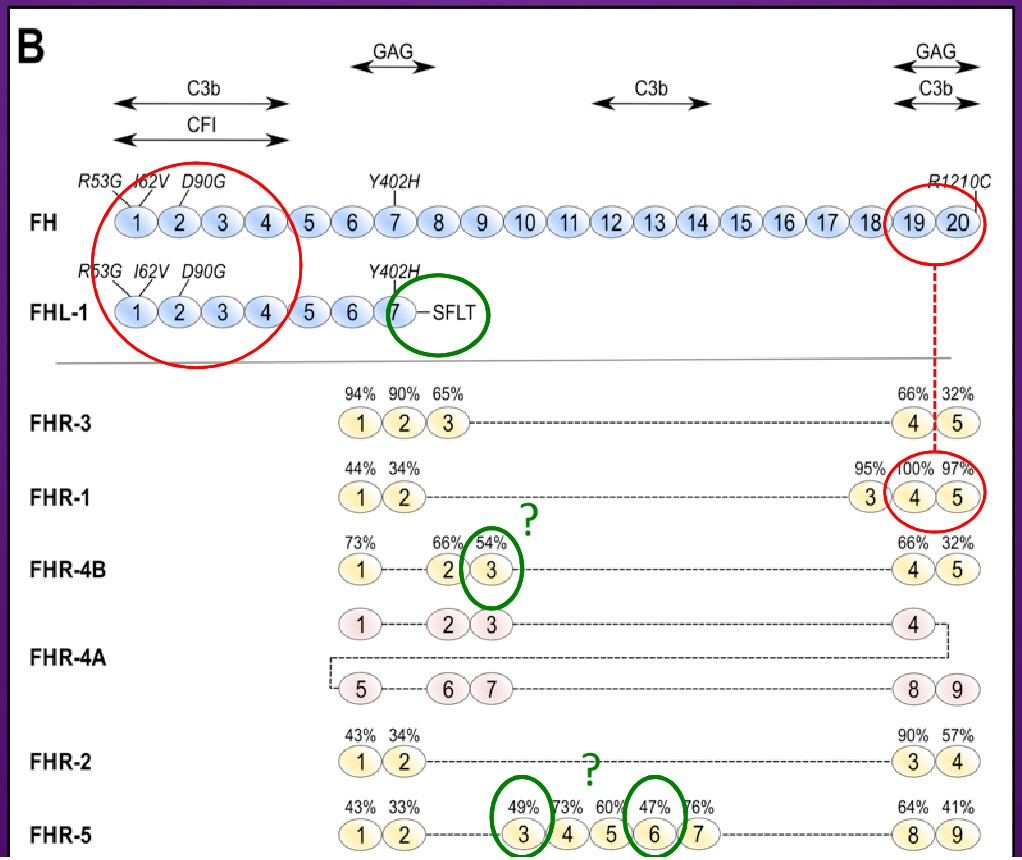


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What about the factor H-related (FHR) proteins?

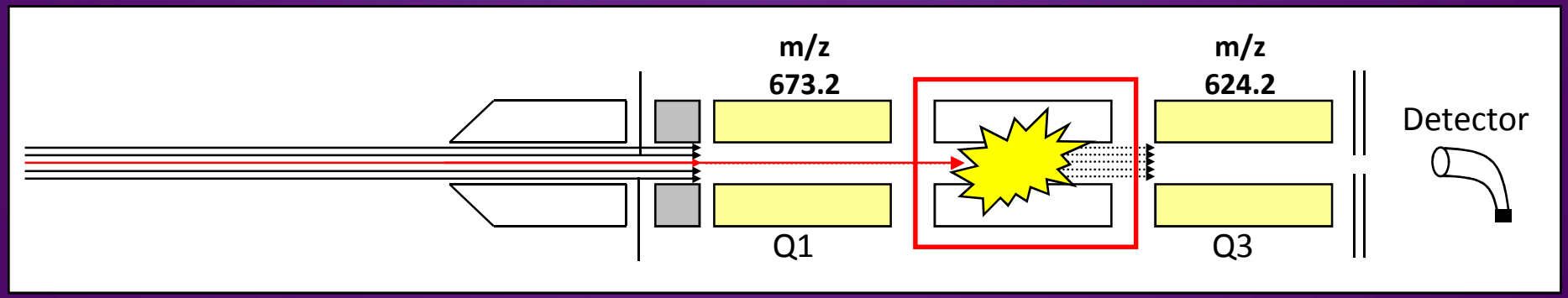
- One major problem with investigating complement in AMD – it was still virtually impossible to distinguish between FH, FHL-1 and the 5 FHR proteins in tissue



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Targeted mass spectrometry

- We have identified an enzyme that will cleave FH, FHL-1 and FHR1-5 and produce (at least one) unique peptide(s) identifiable by mass spectrometry.
- Selected Reaction Monitoring MS (SRM-MS) detects peptides using a defined (peptide specific) mass pair i.e. mass of peptide plus one of its fragments (calculated from peptide sequence).



- We have had each of thee synthesized and used them to develop targeted mass spectrometry methodology that can detect all 7 proteins in tissue
  - Begun work on Bruch's membrane
  - Radiolabeling our standard peptides will make technique quantifiable

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Therapeutic potential

- Complement is very much a focus for novel therapeutic strategies to address AMD

- A number of complement modulating therapies currently in various stages of clinical trial with a range of results

Therapeutic (alternate name)[a]	Treatment type	Complement target	Company	Targeted AMD form	Clinical trials
POT-4	Protease inhibitor	C3	Potentia	Wet	NCT00473928; phase I
Eculizumab[b]	Monoclonal antibody	C5	Alexion	Dry	NCT00935883; phase II
LFG316	Monoclonal antibody	C5	Novartis	Dry	NCT01527500; phase II
				Wet	NCT01535950; phase II
Zimura (ARC1905)	Aptamer-based inhibitor	C5	Ophthotech	Dry	NCT00950638; phase I
				Wet	NCT00709527; phase I
Lampalizumab (FCD4514S)	Antibody Fab fragment	Factor D	Genentech/ Roche	Dry	NCT02247479; phase III NCT02247531; phase III

[a]In some instances, therapeutics have previously been known by a different name. [b]Eculizumab was originally a treatment for other complement-mediated diseases and was in clinical use before genome-wide association studies' association of complement with AMD.

Black and Clark (2015) *Genet. Med.* online 28th May

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Manchester Eye Tissue Repository (ETR)

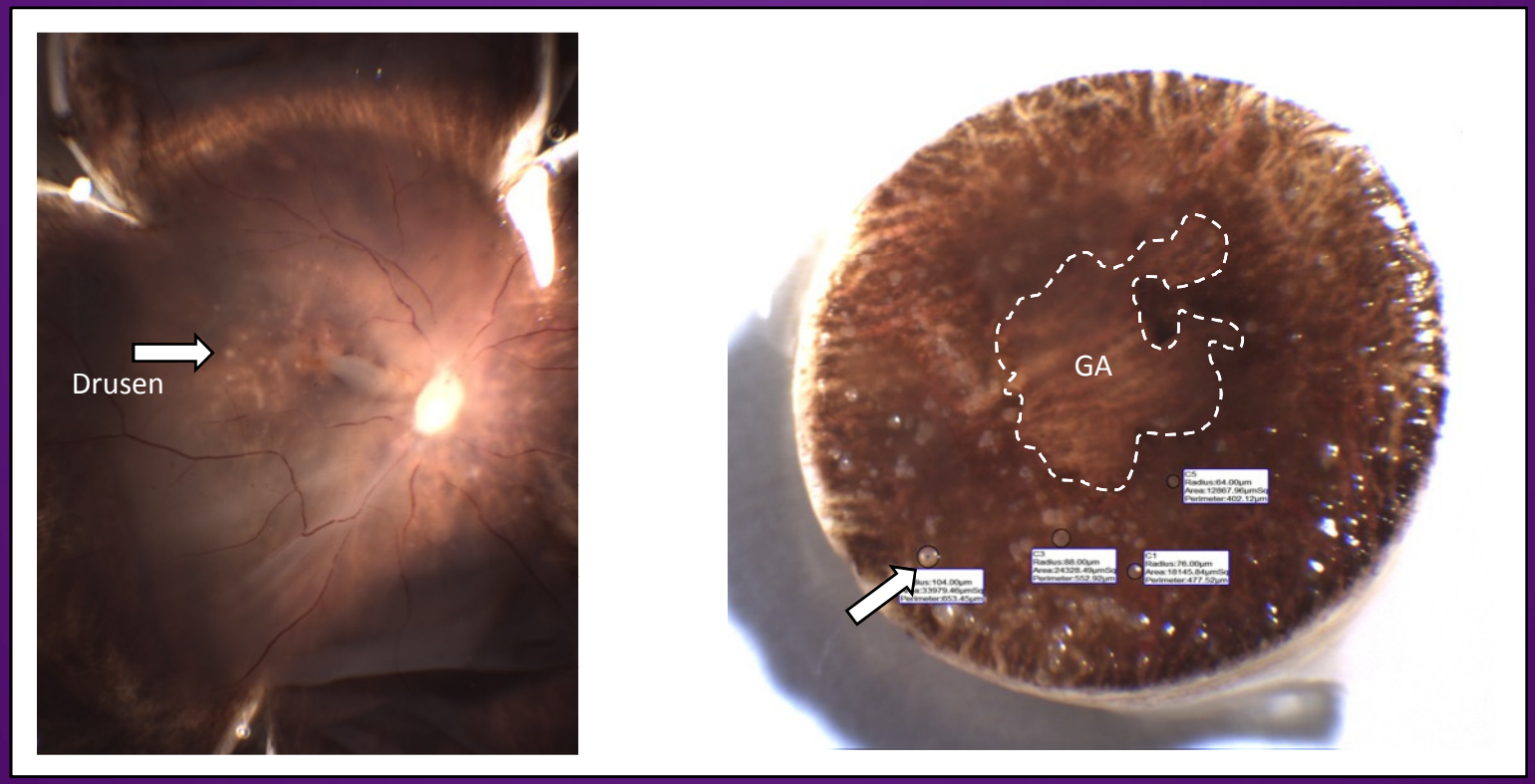
- To support eye research in Manchester we generated a unique resource of human eye tissues

- Manchester Royal Eye Hospital Eye Bank
  - 2000 pairs of eyes per year for corneal transplantation service
  - Remaining globes now given to ETR

- Collect medical record data, phenotype for retinal dystrophies, genotyped (AMD only), dissect and store virtually all aspects of the eye:
  - Sclera; optic nerve; vitreous; neurosensory retina; Bruch's membrane; choroid; remaining DNA from genotyping, culturing RPE cells
  - Also macular and peripheral biopsy punches for IHC

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Manchester Eye Tissue Repository (ETR)



- Hoping to open the facility to other researchers who are working on ocular disease

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- Paul Morgan (Cardiff, UK)

MRC Medical Research Council

Macular Society

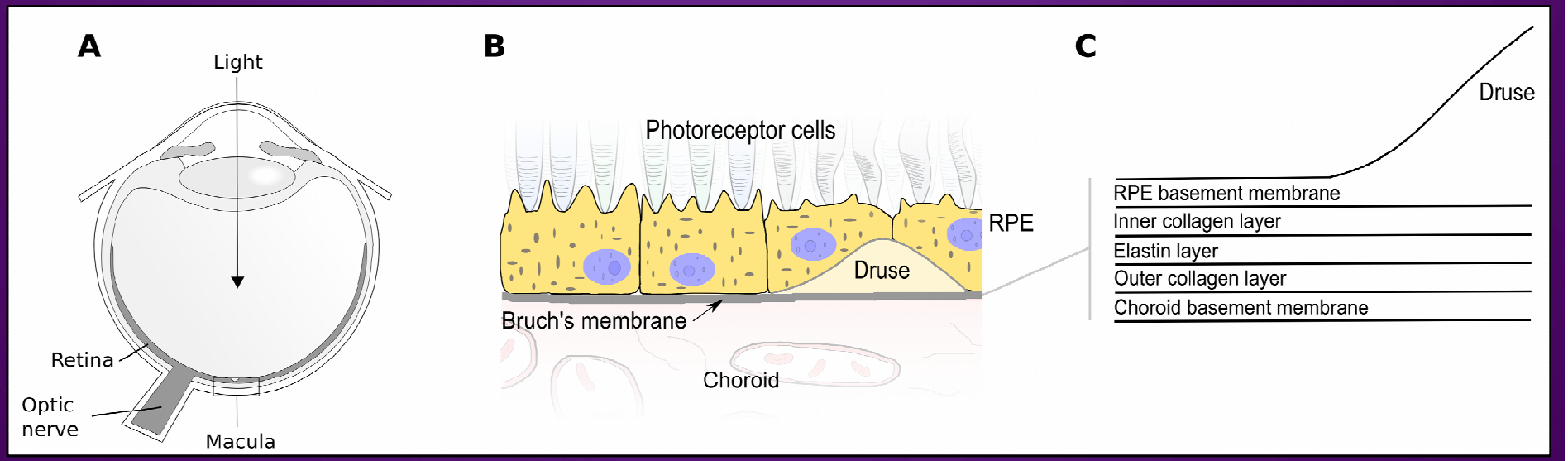
FIGHT FOR SIGHT
The Eye Research Charity



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Bruch's membrane

- Bruch's membrane is an acellular extracellular matrix (ECM) that supports the retinal pigment epithelium (RPE) cells, and subsequently the neurosensory retina.
- Bruch's membrane helps filter nutrients from the choroid that help nourish the RPC cells, but also helps keep out unwanted material/cells.

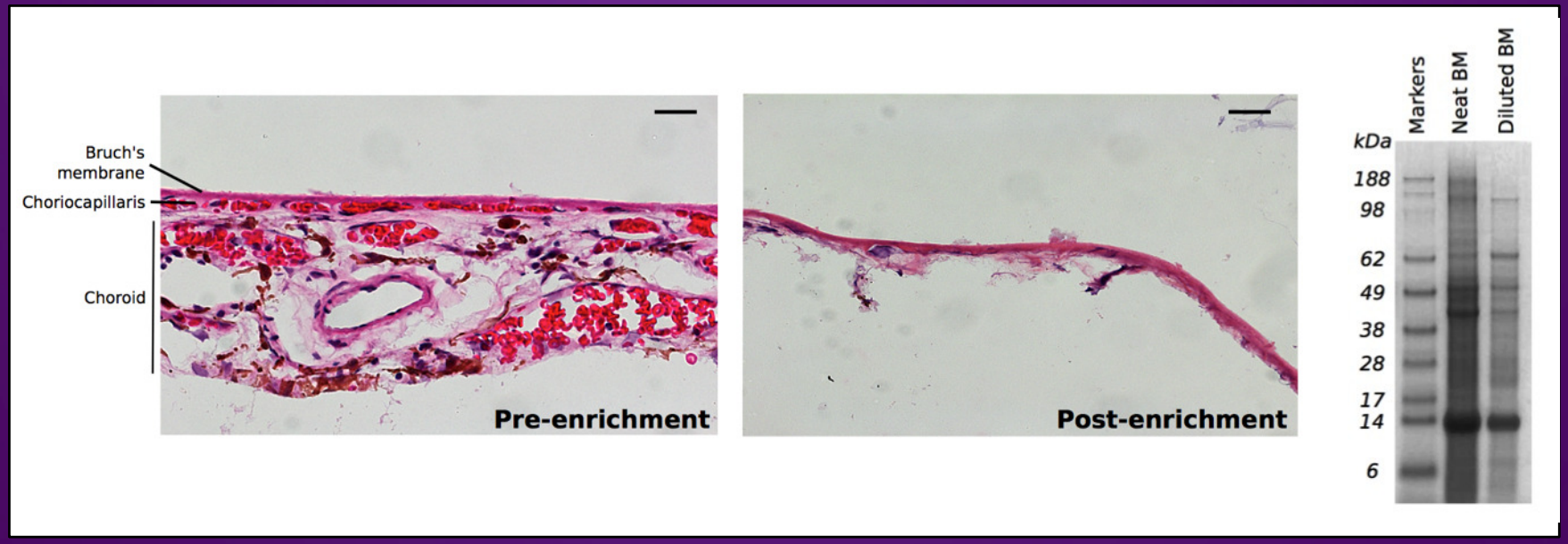


Clark and Bishop (2015) *J. Clin. Med.* **4**, 18-31

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Enrichment of Bruch's membrane

- Publishing detailed method of how to enrich Bruch's membrane
- Can remove RPE, choroid and contaminating blood proteins



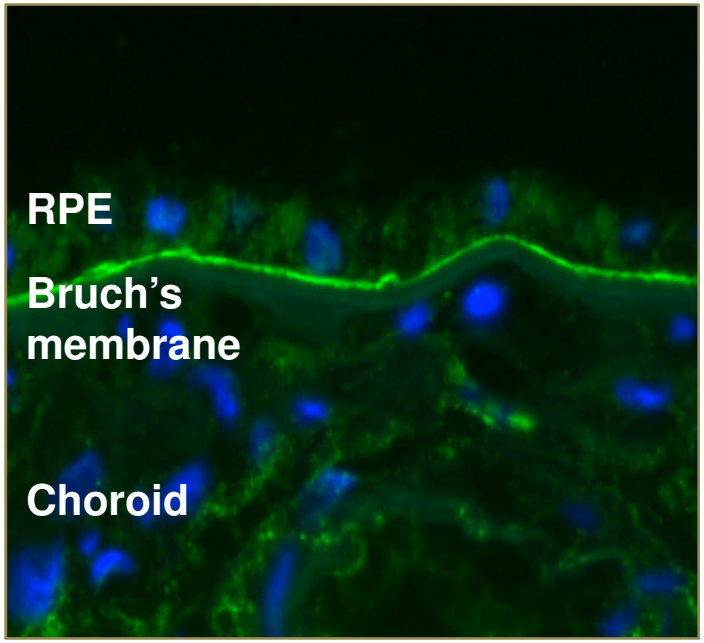
McHarg S *et al. J. Vis. Exp.* In press

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Complement and Bruch's membrane

- Bruch's membrane is one of only two 'basement membrane' ECM directly in contact with the body's blood flow, without prior contact with cell barriers or tight junctions
  - The other is the glomeruli basement membrane (GBM) in the kidney

Eye - macula



Kidney - glomeruli

