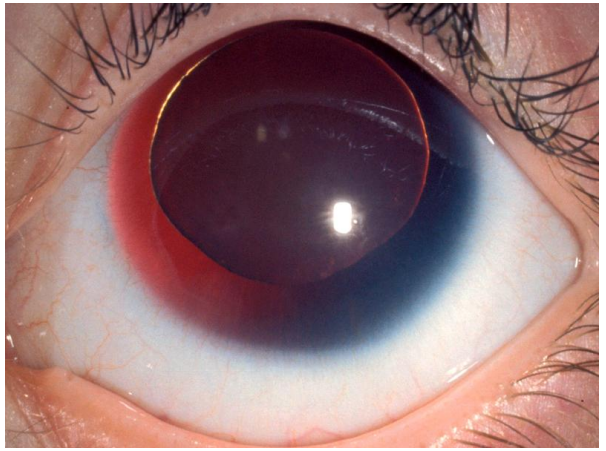
Moorfields Eye Hospital NHS Foundation Trust

THE FRANCIS CRICK INSTITUTE

UCL

WAGR syndrome: Genetic diagnosis, ocular implications and management



Professor Mariya Moosajee MBBS BSc PhD FRCOphth

Professor of Molecular Ophthalmology and Consultant Ophthalmologist

Moorfields Eye Hospital, Great Ormond Street Hospital for Children,
The Francis Crick Institute and UCL Institute of Ophthalmology

Moorfields Eye Hospital NHS Foundation Trust

THE FRANCIS CRICK INSTITUTE

UCL

Clinician Scientist

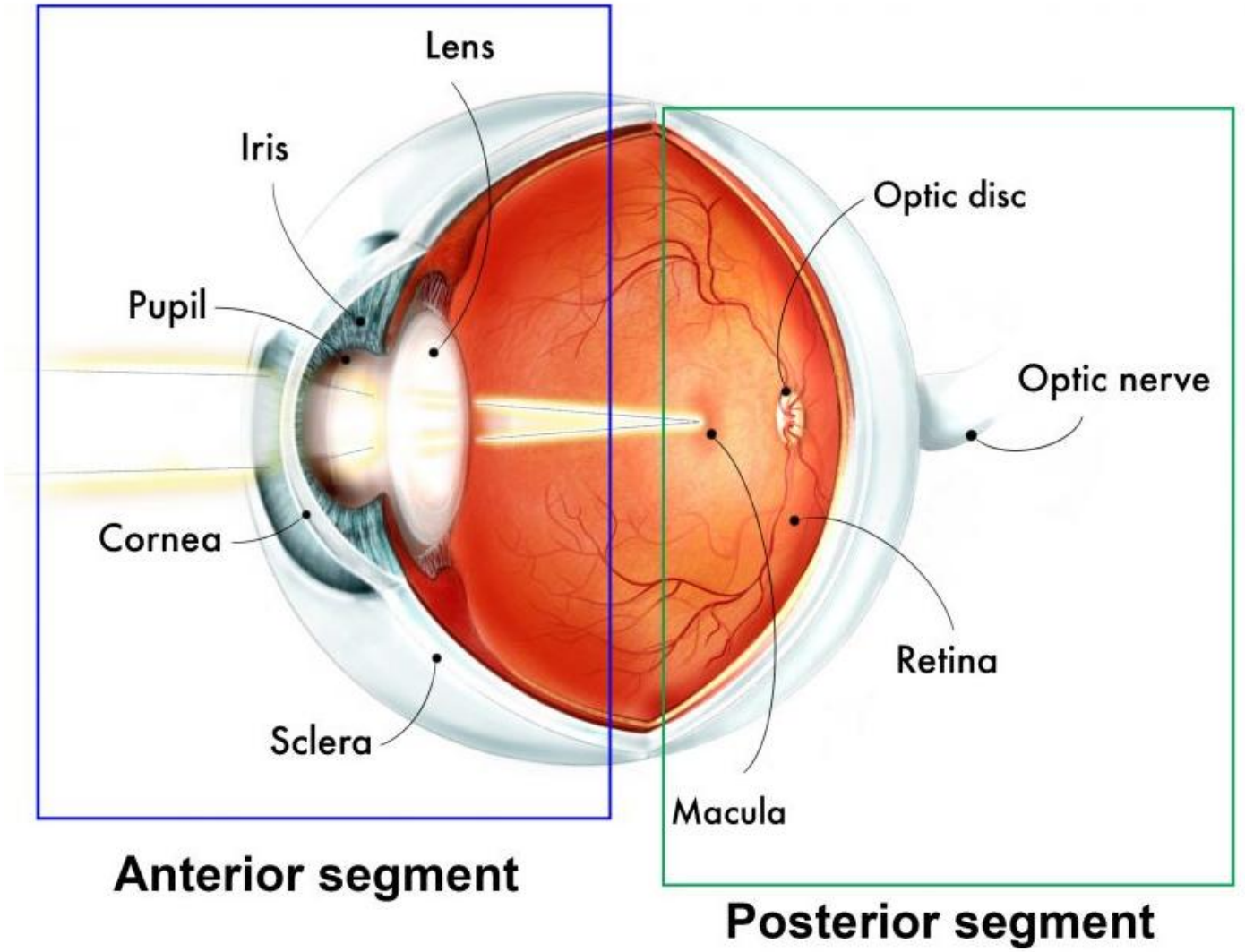
WAGR syndrome- 4 main features

- Wilms tumour (nephroblastoma)- 45-60% risk
- Aniridia (nearly 100%)
- Genitourinary anomalies
  - Males: undescended testes, hypospadias
  - Females: underdeveloped ovaries or malformation of the womb or vagina
- Range of developmental delays
  - >80% developmental delay or learning difficulties
  - Behavioural issues- ADHD, autism spectrum disorder, OCD, anxiety, depression

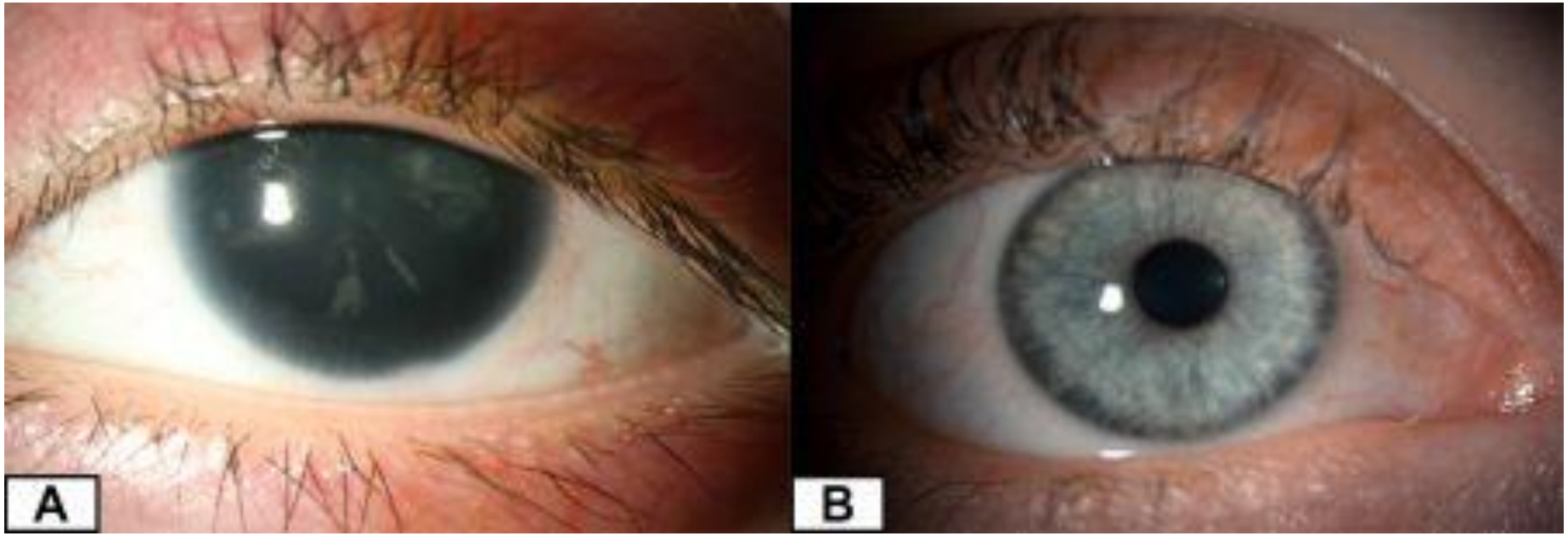
Other associated WAGR features (not all)

- Chronic kidney disease- up to 60%
- Central auditory processing disorder- 90%
- Early onset obesity
- Dyslipidaemia (elevated levels of fat in the blood)
- Sleep disorders and sleep apnoea
- >75% gastrointestinal issues
- Scoliosis- 20%

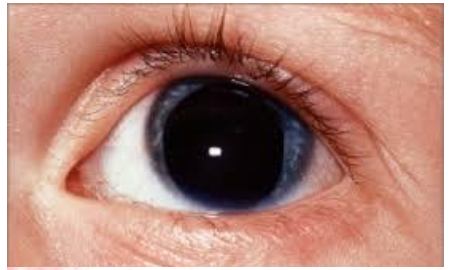
Eye Anatomy

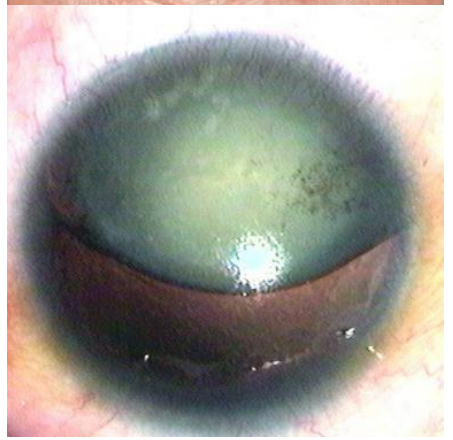

Lens
Iris
Pupil
Cornea
Sclera
Optic disc
Optic nerve
Retina
Macula
Anterior segment
Posterior segment

Main presenting feature- Aniridia



Aniridia- affects all parts of the eye





- Complete or partial loss of the iris
- Early onset nystagmus (usually apparent by six weeks of age)
- Reduced vision in childhood due to:
  - Foveal hypoplasia- perform OCT
  - Optic nerve abnormalities e.g. optic nerve hypoplasia or coloboma
- Cataracts
- Glaucoma
- Limbal stem cell deficiency with abnormal blood vessel growth and opacification of the cornea
- Ptosis (droopy eyelids)
- Retinal detachment

If a baby presents with aniridia…

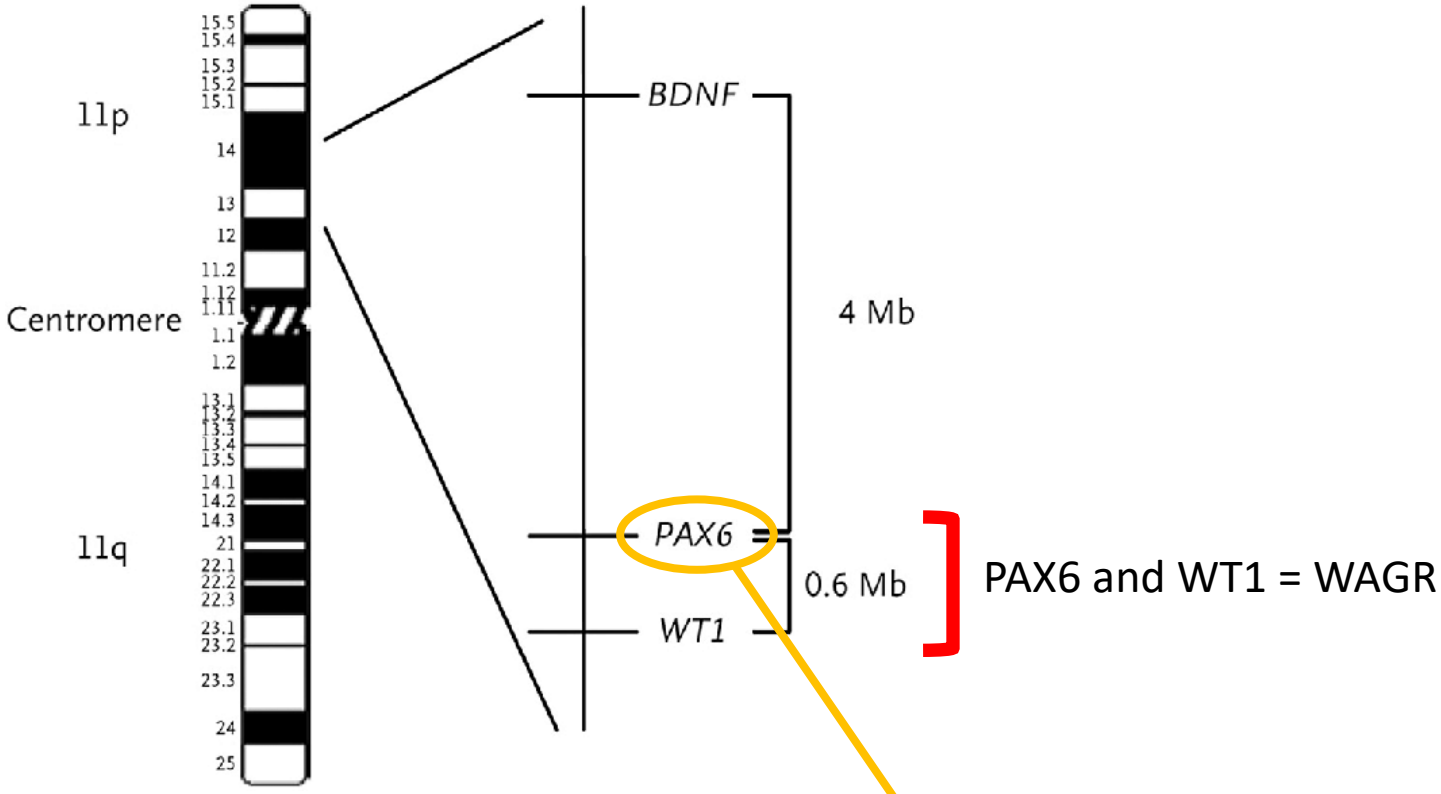
1/3 WAGR syndrome - deletions involving *PAX6* and *WT1* genes

Ultra-rare affecting 1 in 500,000-1,000,000

2/3 aniridia caused by mutations in *PAX6*

Aniridia 1 per 40,000-100,000

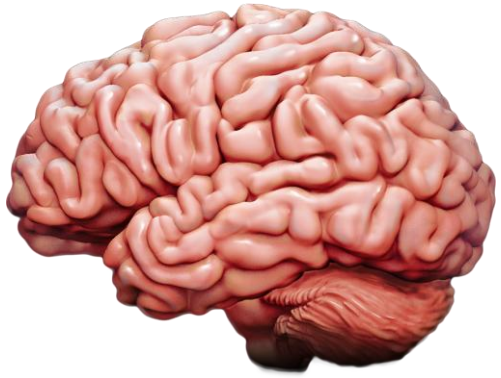
Genetic loci on chromosome 11p

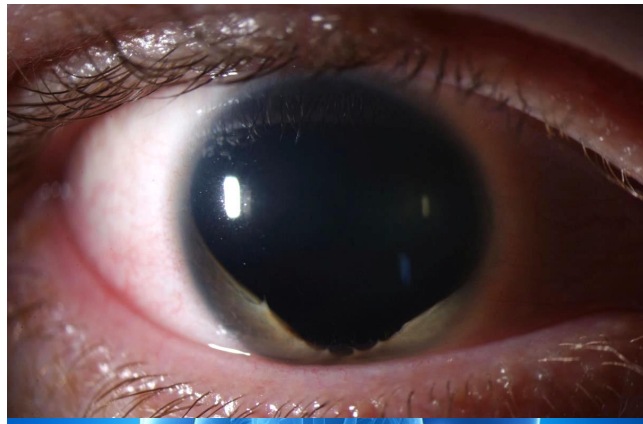


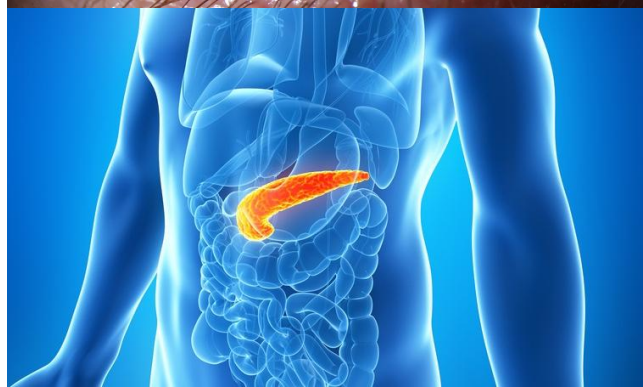
Aniridia is no longer considered an isolated eye disorder, it is a syndrome with significant crossover with WAGR

PAX6 related systemic features

- Obesity
- Hyperglycaemia, insulin resistance and diabetes
- Central auditory processing disorder
- Olfactory dysfunction and anosmia
- Midline brain anomalies
- Sleep disturbances
- Autism or ADHD
- Depression and anxiety







What does that mean for WAGR patients?

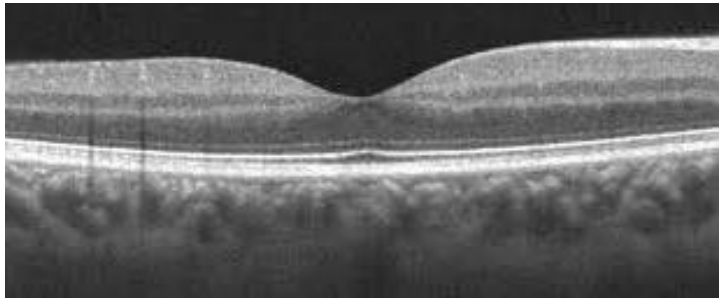
- Management, treatments and research into aniridia may be applicable to WAGR patients
- It may help with understanding aspects of WAGR
- The lack of *PAX6* in the eye is the main cause of the ocular problems in WAGR, hence a potential therapy to boost PAX6 levels would help both aniridia and WAGR patients

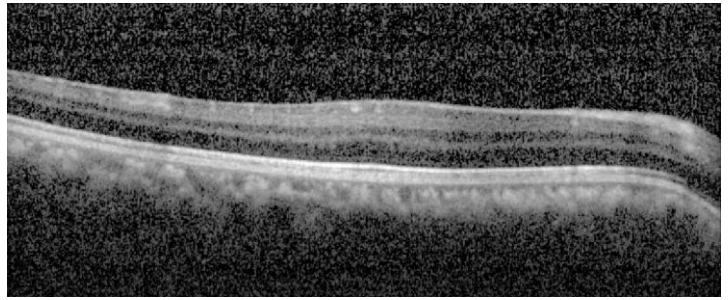
Moorfields Genetics Team

For paediatrics the importance of a MDT approach to provide the best possible care to the family

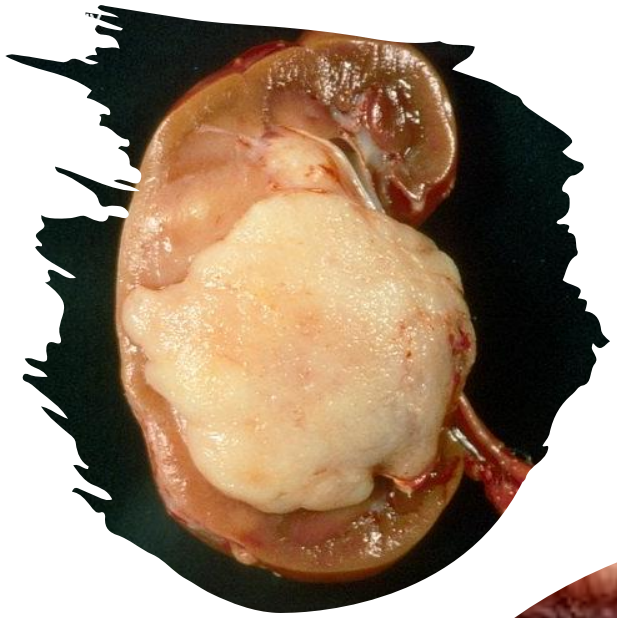
What care should patients receive from Ophthalmology?

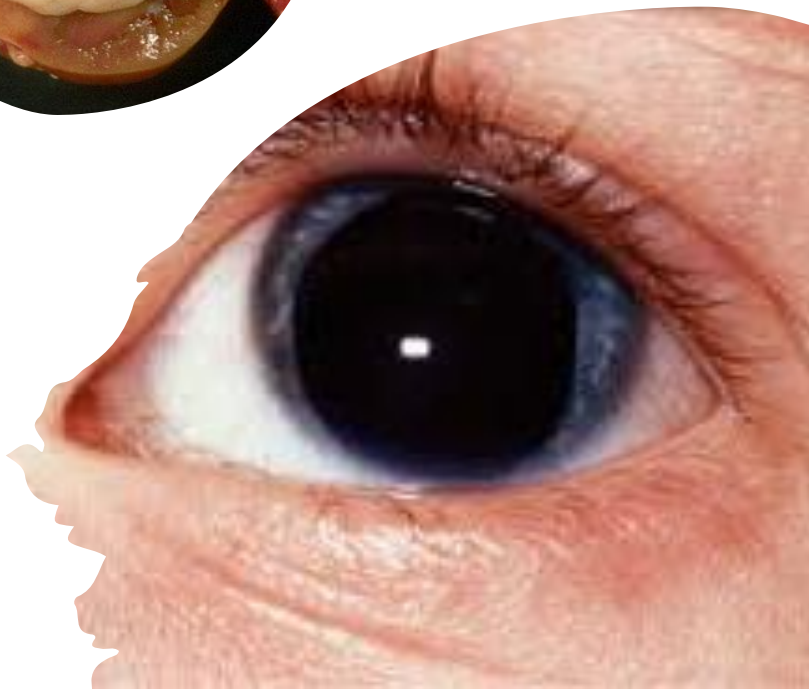
- Initially:
  - Full history and clinical examination
  - Retinal imaging (OCT and autofluorescence scans) to assess for foveal hypoplasia- only needed once if good imaging acquired
  - Genetic testing (through ophthalmology or genetics)





Importance of genetic testing and molecular diagnosis



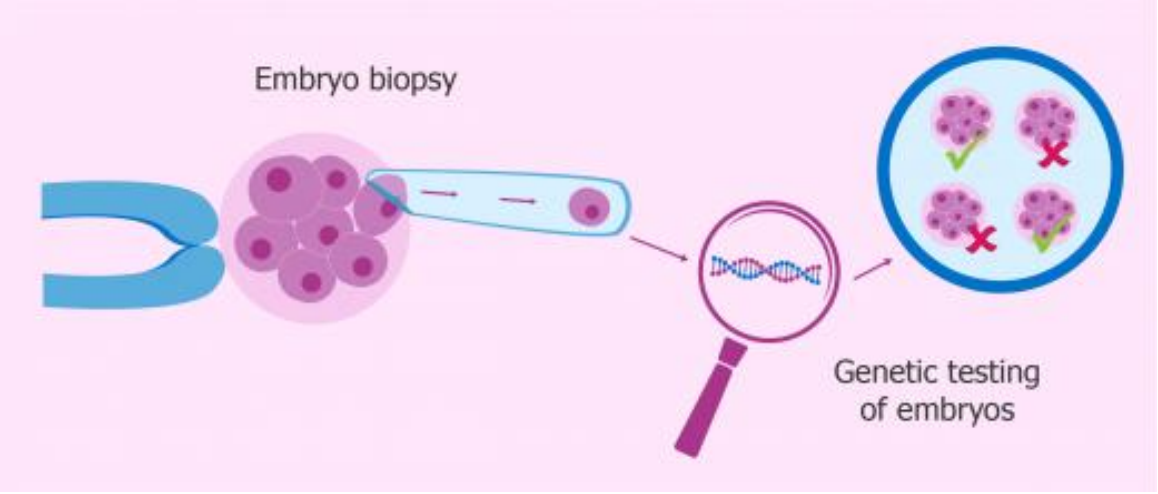


1. Patients and families want to know the cause of their condition
   - Guide prognosis with genotype-phenotype correlations
2. It helps clinicians identify other disease associations e.g. renal disease, learning difficulties
   - Can assemble the correct MDT

Importance of genetic testing and molecular diagnosis

3. Provide informed genetic counselling
    - Inheritance patterns
    - Risk of having affected children

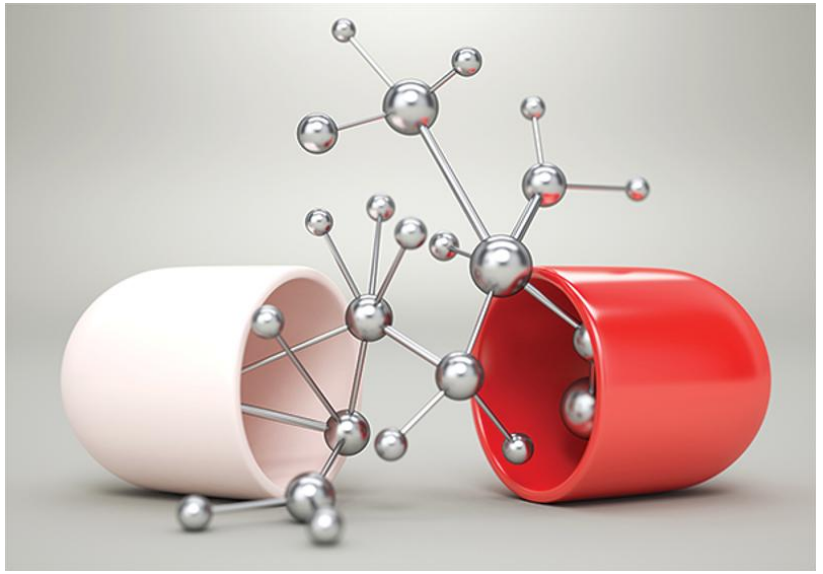
4. Family planning advice
    - Preimplantation genetic diagnosis
    - Non-invasive prenatal testing



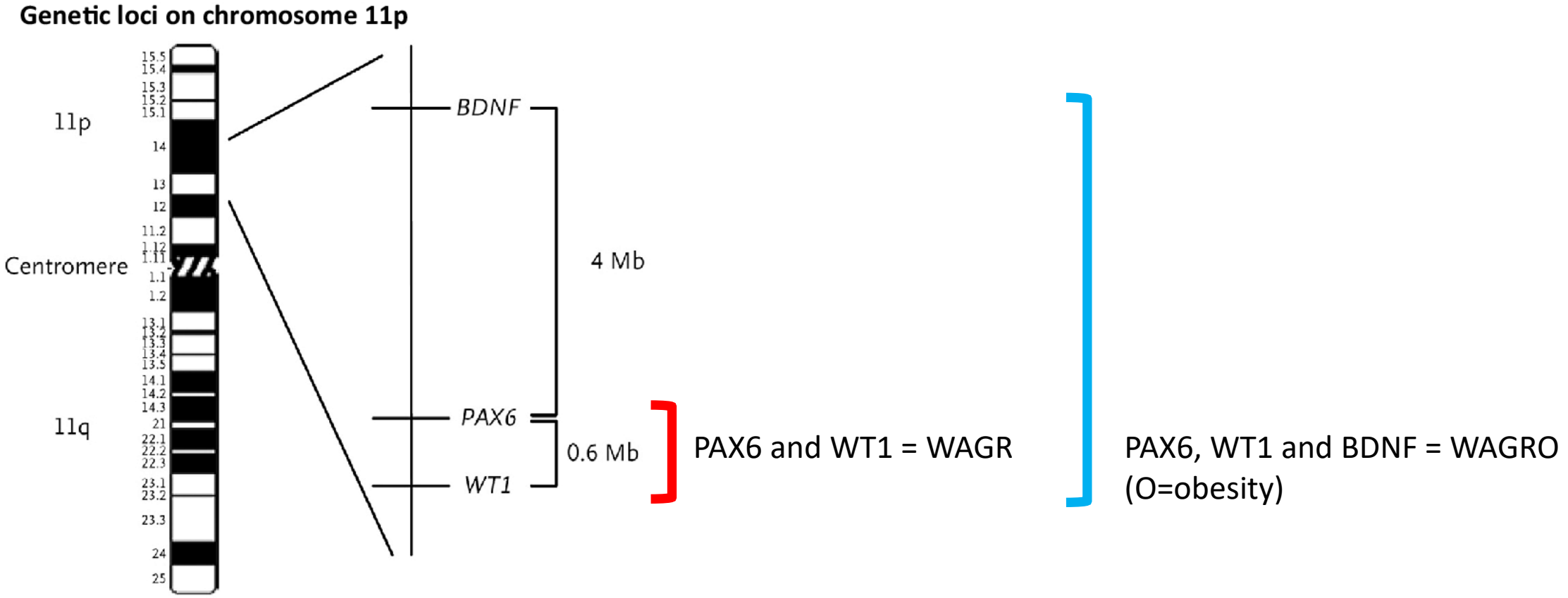
Importance of genetic testing and molecular diagnosis

5. Access to appropriate management and treatments

6. Advances in research
   - Access to clinical trials
   - Natural history studies

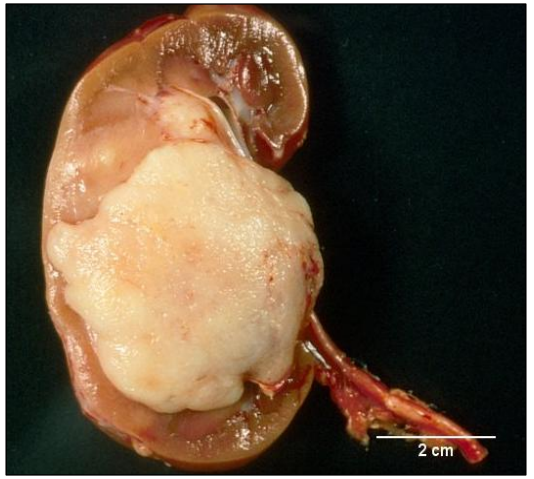


How do we test for WAGR? Array CGH



We need to identify WAGR patients ASAP and get them the correct care

- In the UK- refer to clinical genetics to oversee the systemic care
- Children with WAGR require renal USS every 3 months and follow-up by a paediatric oncologist until they reach age 8 years, then 6 monthly till 18 years, then annually thereafter
- Children need to see a paediatrician for full systems review including endocrine and behavioural assessments



What care should patients receive from Ophthalmology?

Depending on age- under 8 should have 4 monthly eye checks for vision, refraction (annually), orthoptic assessment, and ophthalmology review- follow-up intervals depends on ocular disease

- Monitor intraocular pressure for glaucoma (patients should be under a glaucoma consultant)- may need medical and surgical management
- Monitor for cataracts- refer for surgery only if impacting on vision (challenging surgery, best undertaken later)
- Monitor for corneal keratopathy- use ocular lubricants to protect surface, may need corneal and limbal grafts

What care should patients receive from Ophthalmology?

- UV protected sunglasses glasses for glare/photophobia
- Low vision assessment for aids and assistive digital technology
- Family support- ensure communication with QTVI
- Register patient sight impaired
- Ensure support for parents
- Connect patients with International WAGR syndrome association, Aniridia Network, Aniridia Europe, RNIB and Guide Dogs

What happens if your ophthalmologist is unfamiliar with WAGR/Aniridia?

Ask for a second opinion referral to Moorfields from your consultant or GP

- If for genetics- Name Professor Mariya Moosajee
- If for paediatric cornea- Name Ms. Kirithika Muthusamy
- If for paediatric glaucoma- Name Mr John Brookes or Ms Alessandra Martin
- If for adult cornea- Name Mr Sajjad Ahmad

Moorfields Eye Hospital NHS Foundation Trust

THE FRANCIS CRICK INSTITUTE

UCL

How to ask for a referral without offending!

Say you attended a WAGR weekend and Prof Mariya Moosajee gave a talk, happy to see patients for a clinical review of their aniridia.

Plus the family will be on the Moorfields genetics database for future research opportunities!

What can you do to help maintain visual function?

- Regular follow-up with Ophthalmologist
- Healthy diet- full of fresh fruit and vegetables, fish twice a week
- Regular exercise
- UV protected tinted/sunglasses in bright sunlight
- Blue-light screen protectors on devices
- Try not to compare your child's vision with others/internet
- Ensure you get access to all the support available- sight registration
- Visual interest- Ngozi will expand

Charles Bonnet syndrome (CBS)

- Visual hallucinations in individuals with reduced vision
- Not because of a mental health problem
- Can occur in any age group and from any eye disease
- No known incidence or prevalence but thought to be a common association- up to 30% of those with sight loss
- There is no auditory, smell or touch component

Visual hallucinations

Two main types:

- Simple repeated patterns or shapes, such as grids or brickwork patterns
- Complex hallucinations of people, objects and landscapes

Simple distraction techniques

Box 1. Techniques for minimising or eliminating visual hallucinations secondary to Charles Bonnet syndrome.

When the hallucinations start, look from right to left once every 15 s without moving your head
Try to touch the hallucination
Stare straight at the hallucination
Turn your head to alternative sides, then move the head towards each shoulder in turn
Walk around the room or to another room
Shine a torch from below your chin in front of (not into) your eyes
Change the light level in your room or the activity you are doing

Best et al. Ther Adv Ophthalmol 2019, 11: 1–2

Support groups and resources

- Judith Potts set up Esme's Umbrella
  - Leading support group for CBS

Esme's Umbrella
FOR EVERYONE WORKING FOR THE GREATER AWARENESS OF
Charles Bonnet Syndrome

- Several charity helplines
  - RNIB
  - Retina UK

Moorfields Eye Hospital NHS Foundation Trust

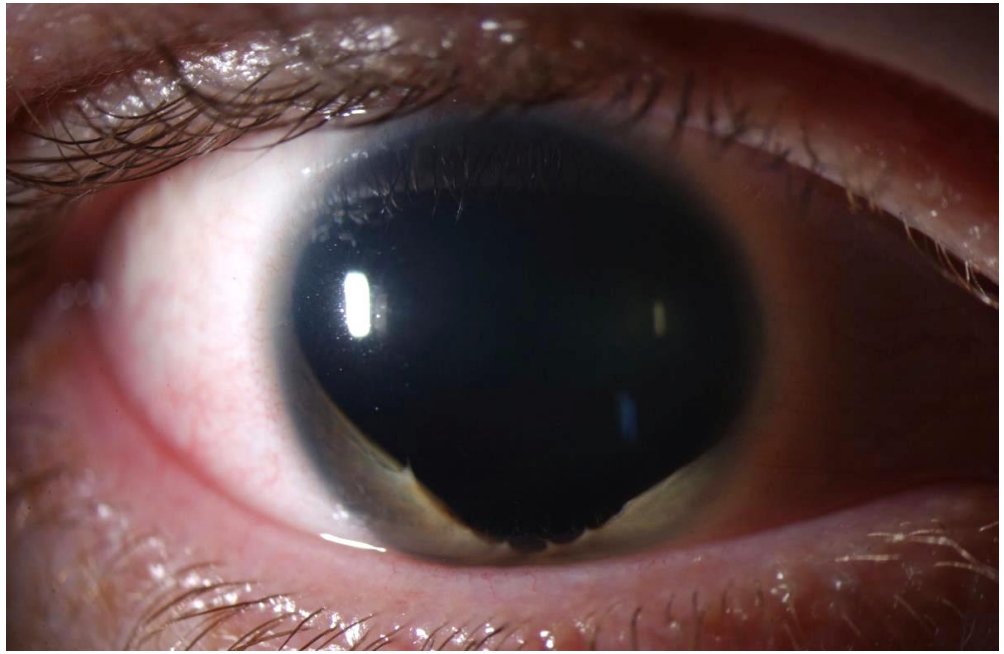
THE FRANCIS CRICK INSTITUTE

UCL

Research that can help WAGR patients

What we can learn from aniridia studies

- Natural history
- Metabolomics
- Therapies



JCI insight

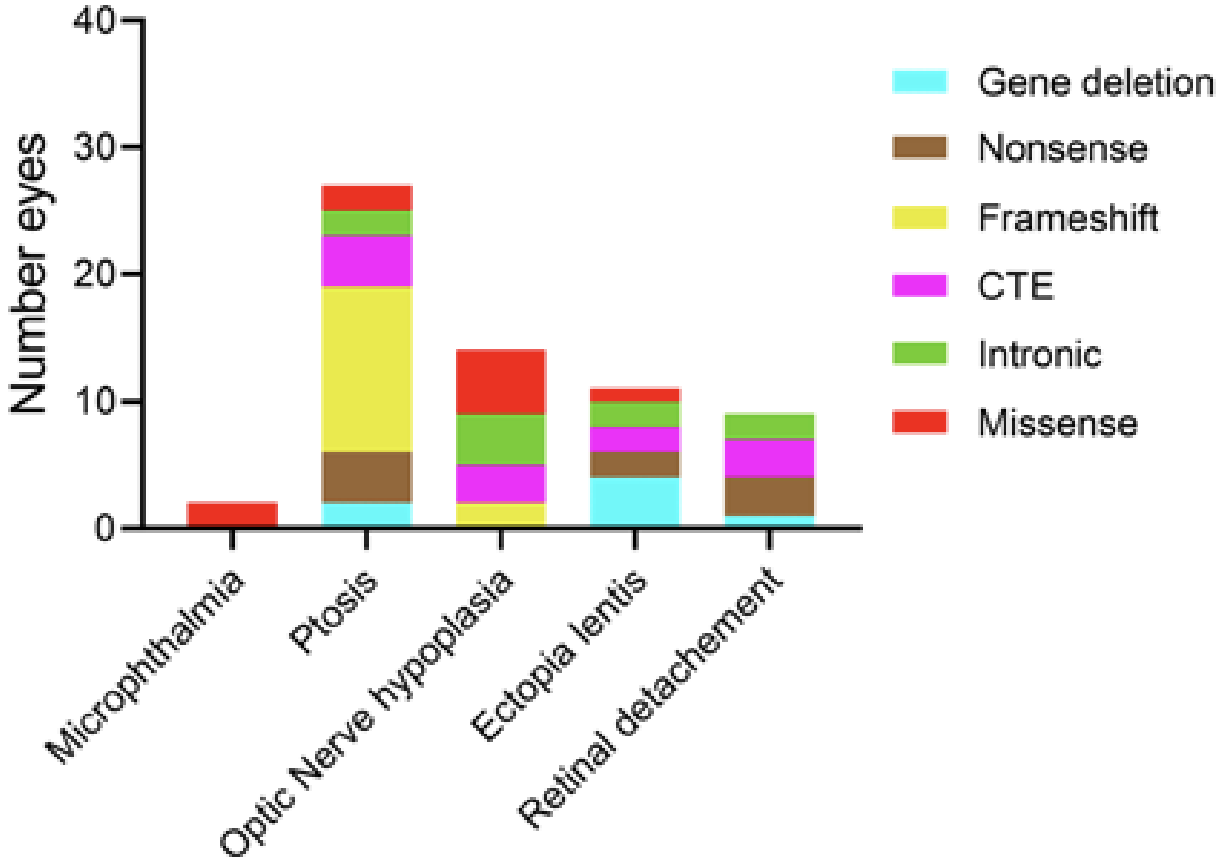
RESEARCH ARTICLE

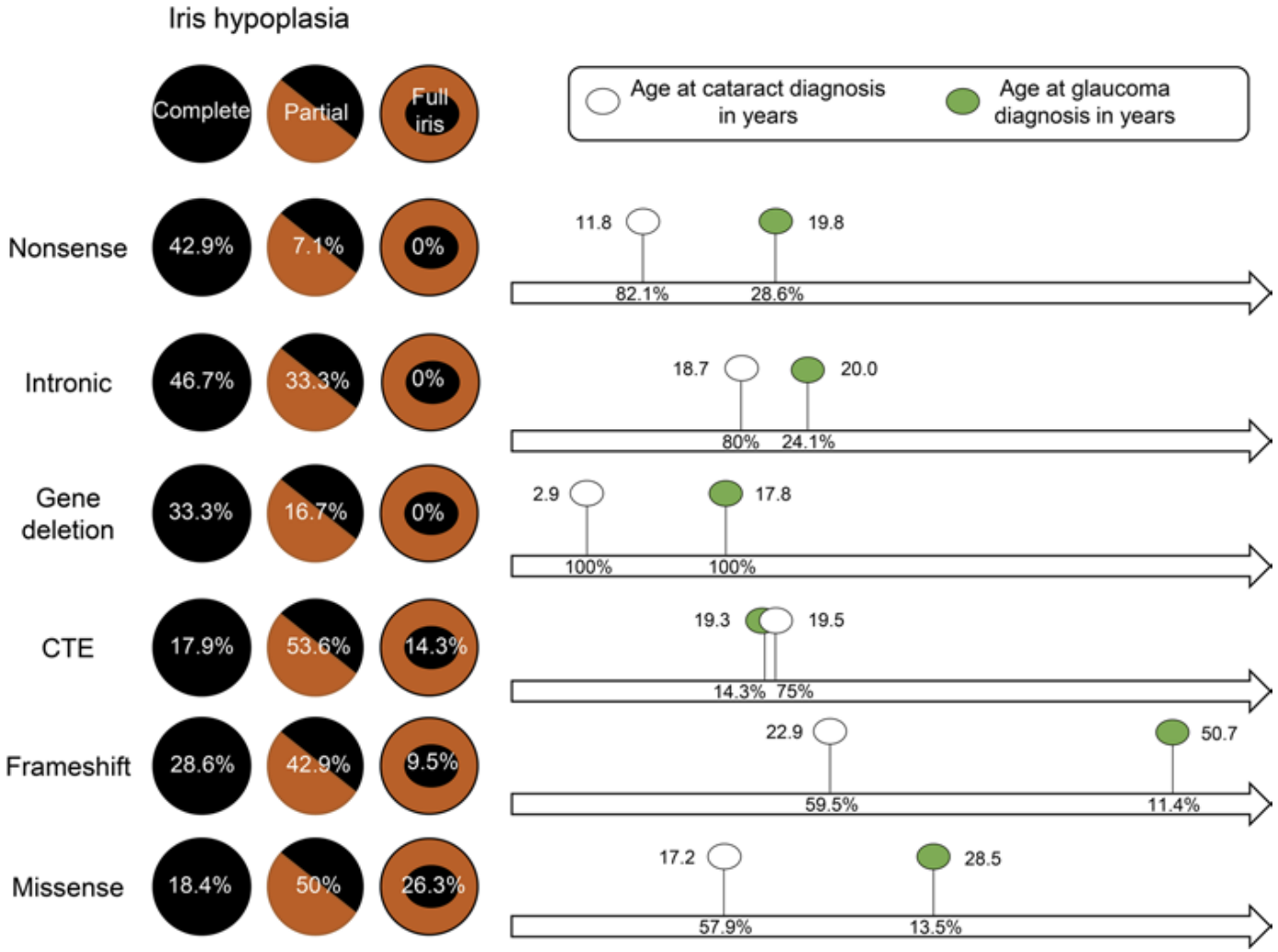
Longitudinal genotype-phenotype analysis in 86 patients with *PAX6*-related aniridia

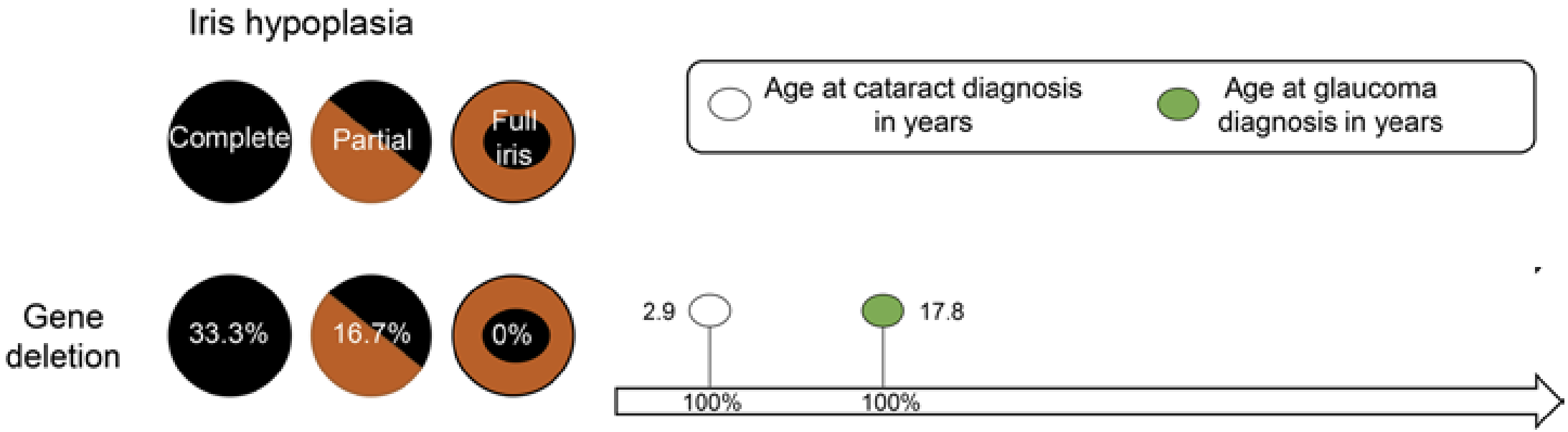
Vivienne Kit,[1,2] Dulce Lima Cunha,[2] Ahmed M. Hagag,[1,2] and Mariya Moosajee[1,2,3,4]

[1]Moorfields Eye Hospital, NHS Foundation Trust, London, United Kingdom. [2]UCL Institute of Ophthalmology, London, United Kingdom. [3]Great Ormond Street Hospital for Children, NHS Foundation Trust, London, United Kingdom. [4]The Francis Crick Institute, London, United Kingdom.

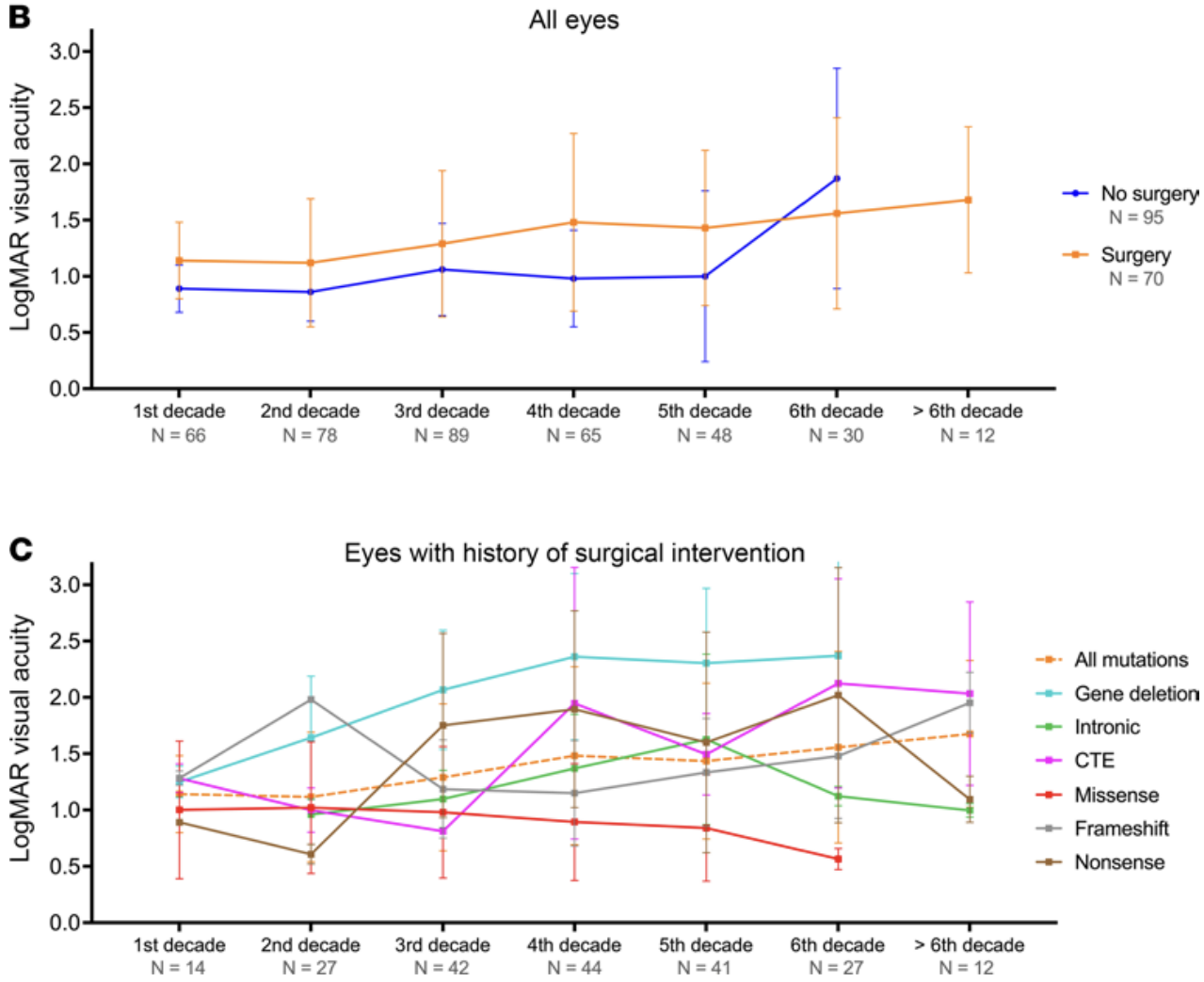
Complex ocular features seen in aniridia patients



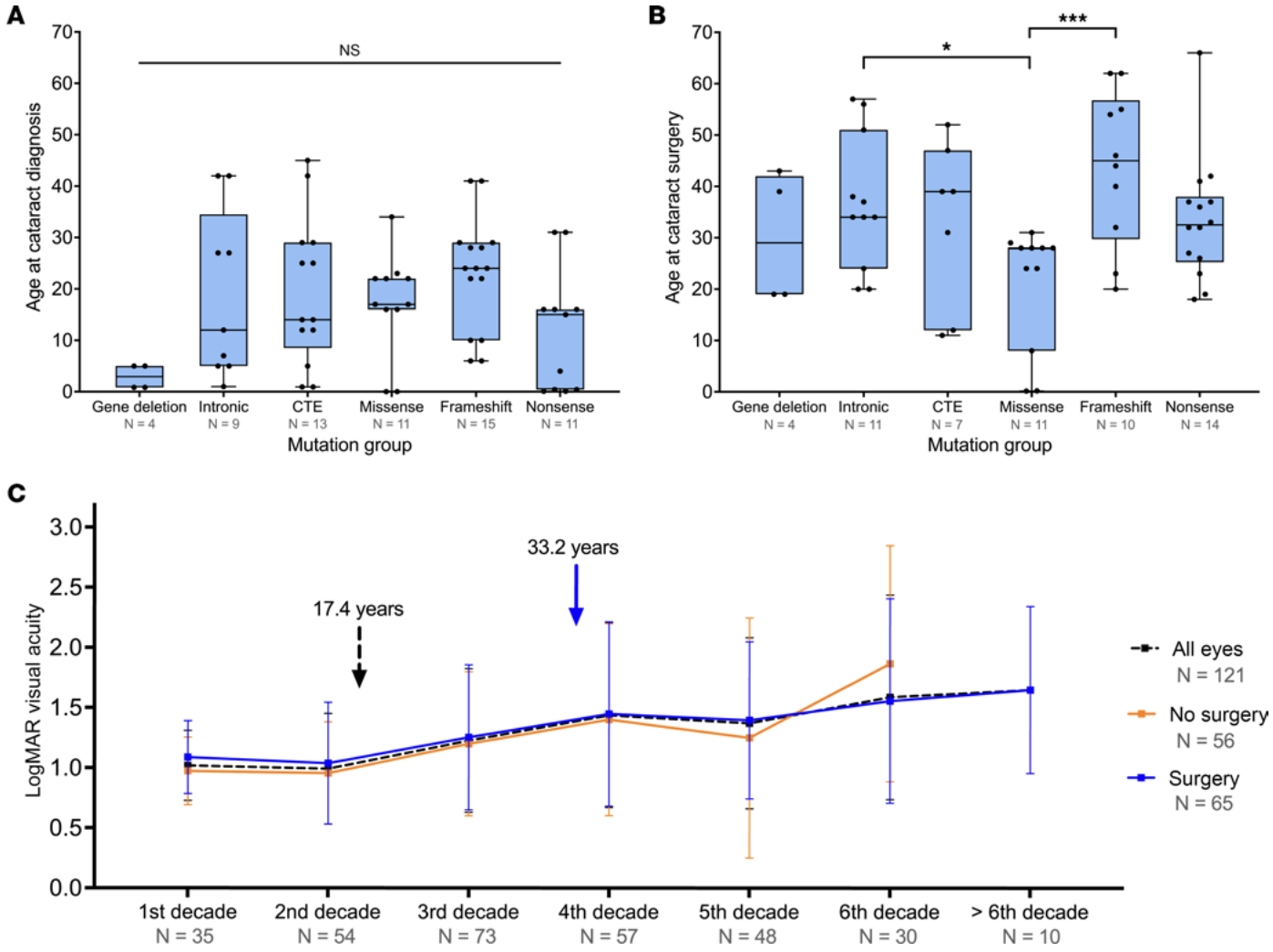

Iris hypoplasia
Complete
Partial
Full iris
Age at cataract diagnosis in years
Age at glaucoma diagnosis in years
Nonsense
42.9%
7.1%
0%
11.8
19.8
82.1%
28.6%
Intronic
46.7%
33.3%
0%
18.7
20.0
80%
24.1%
Gene deletion
33.3%
16.7%
0%
2.9
17.8
100%
100%
CTE
17.9%
53.6%
14.3%
19.3
19.5
14.3%
75%
Frameshift
28.6%
42.9%
9.5%
22.9
50.7
59.5%
11.4%
Missense
18.4%
50%
26.3%
17.2
28.5
57.9%
13.5%



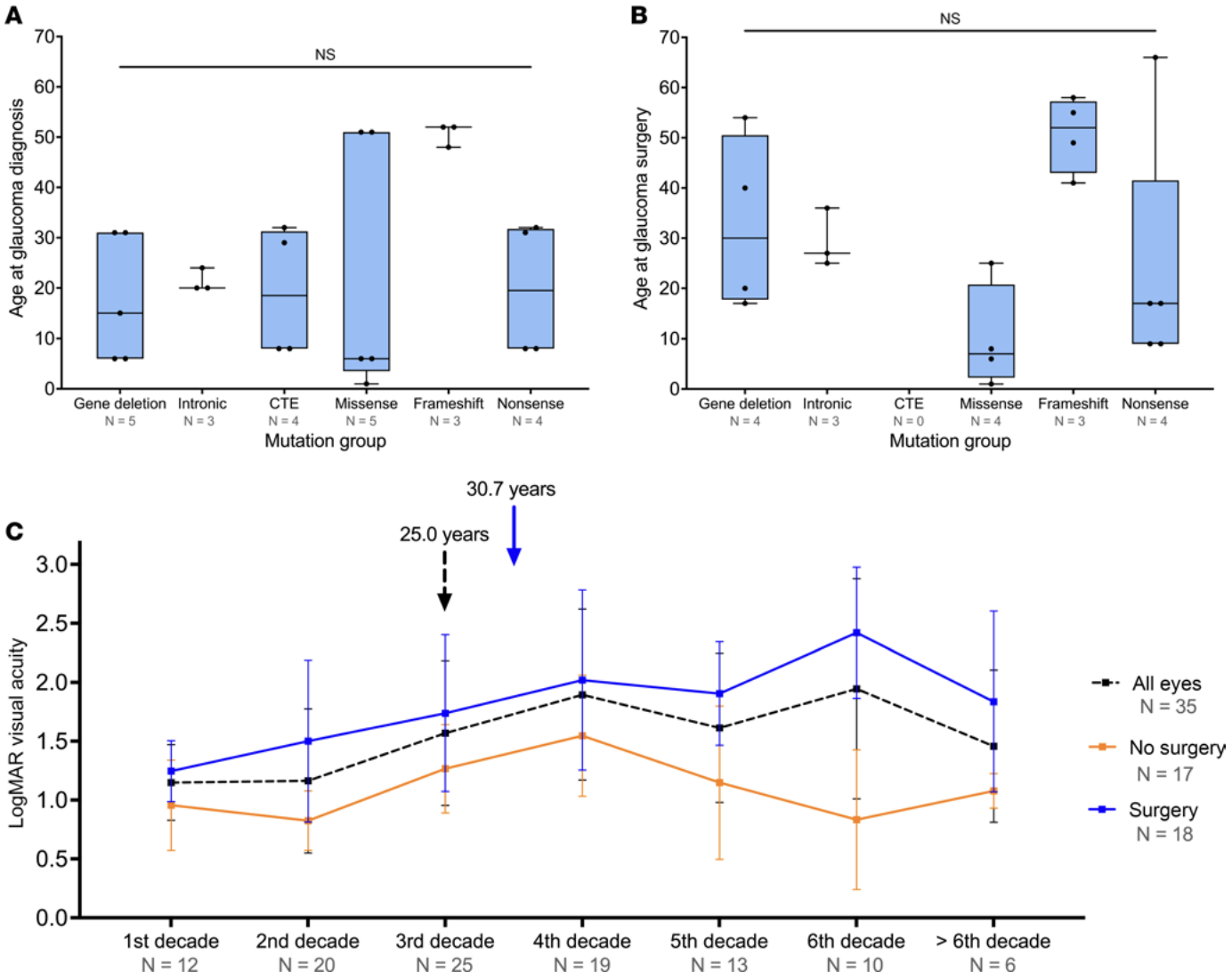
n=4 patients and up to 8 eyes



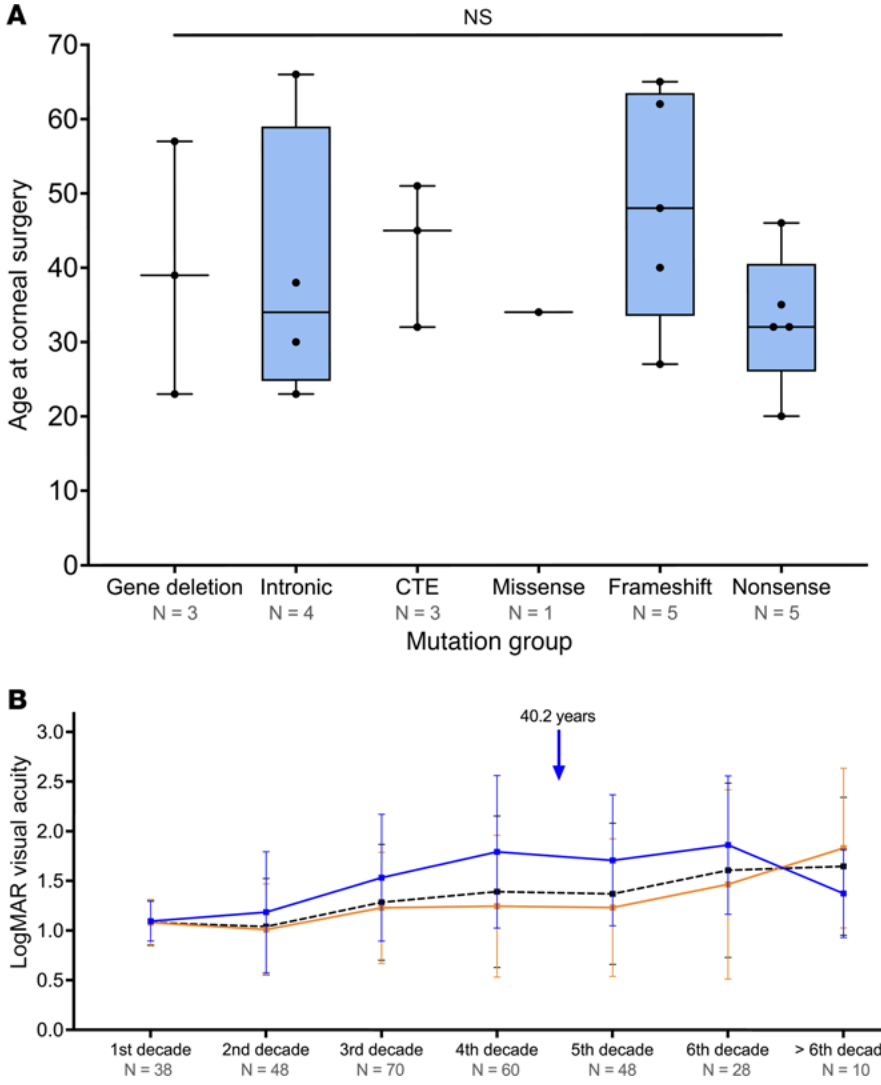
- Eyes with no surgery had better vision
- Patients with whole gene deletions faired worse after surgery
- Closest scenario to WAGR but we need to undertake this study for WAGR patients to compare



- Patients with whole gene deletions appear to develop cataracts earlier but no significant difference found.
- They did not have surgery until late 20s
- No difference in vision after cataract surgery

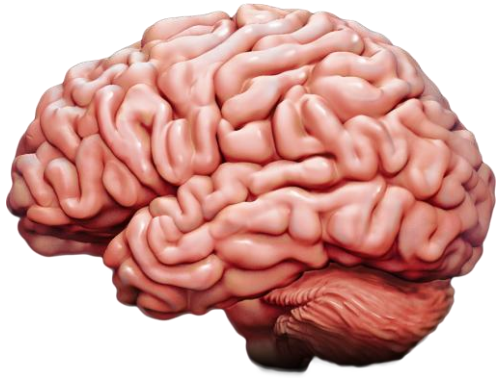


- Patients with whole gene deletions developed glaucoma at a similar age to other mutations
- Patients who had surgery had a worse visual outcome

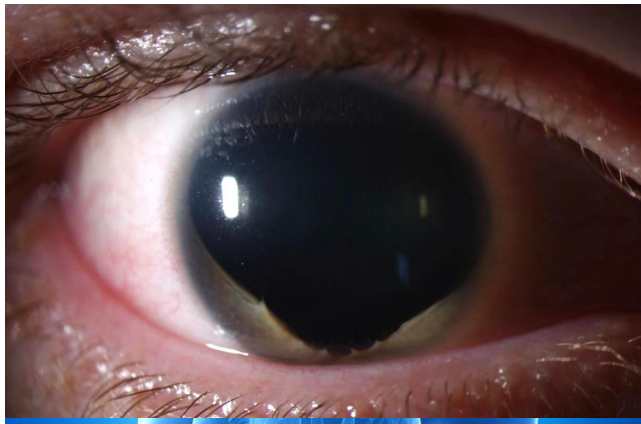


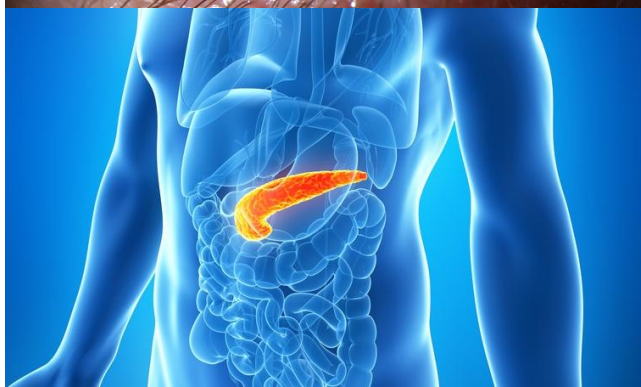
- Patients with whole gene deletions developed corneal issues at a similar age to other mutations
- Patients who had surgery had a worse visual outcome

PAX6 outside the eye

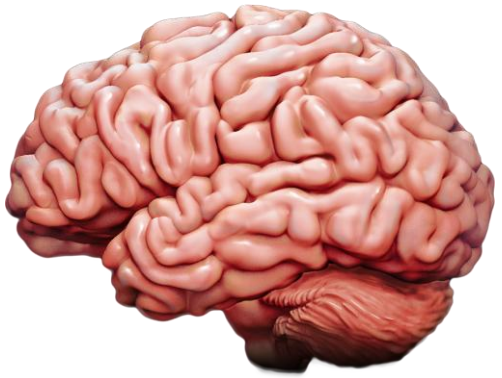


- PAX6 is essential for the development of the eye, pancreas and central nervous system
- Haploinsufficiency is the underlying cause resulting in developmental structural defects with associated secondary disease features arising later in life
- Evidence for neuronal and endocrine abnormalities
  - With obesity, hyperglycaemia, insulin resistance and diabetes
  - Autism or ADHD, depression and anxiety, sleep disturbances





Structural brain anomalies

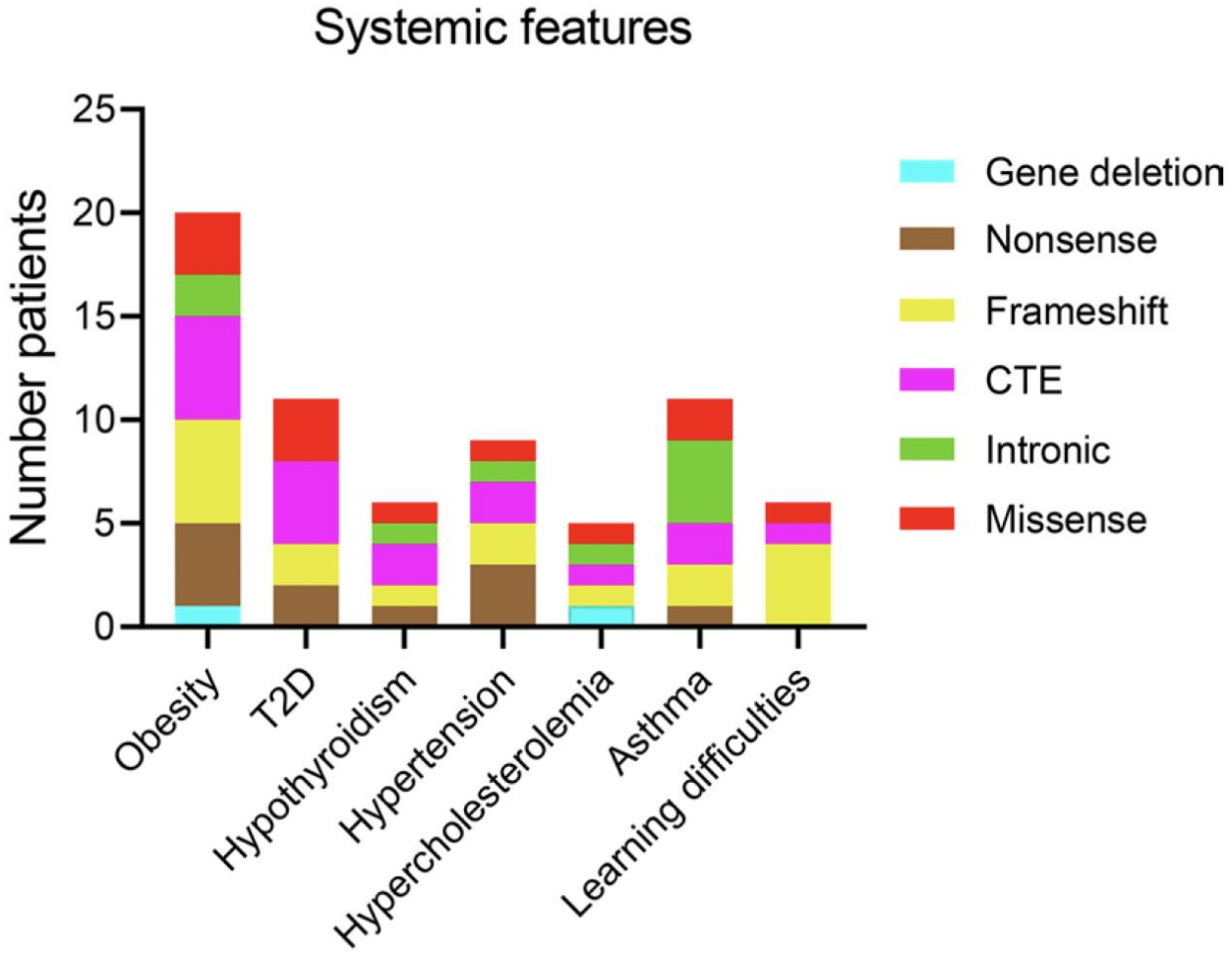


- Midline structures affected: hypoplasia of the olfactory bulb, pineal gland, anterior and posterior commissures; and optic chiasm
  - Abnormal central auditory processing (difficulty localizing sounds and understanding speech in noise)
  - Olfactory dysfunction and anosmia
  - Sleep disturbance

JCI insight
RESEARCH ARTICLE

Longitudinal genotype-phenotype analysis in 86 patients with *PAX6*-related aniridia

- Systemic evaluation identified:
  - Type 2 diabetes in 12.8% (n=11)- twice the UK prevalence
  - Obesity in 23.3% (n=20)
  - Learning difficulties in 7% (n=6)
  - Autism in 2.3% (n=2)

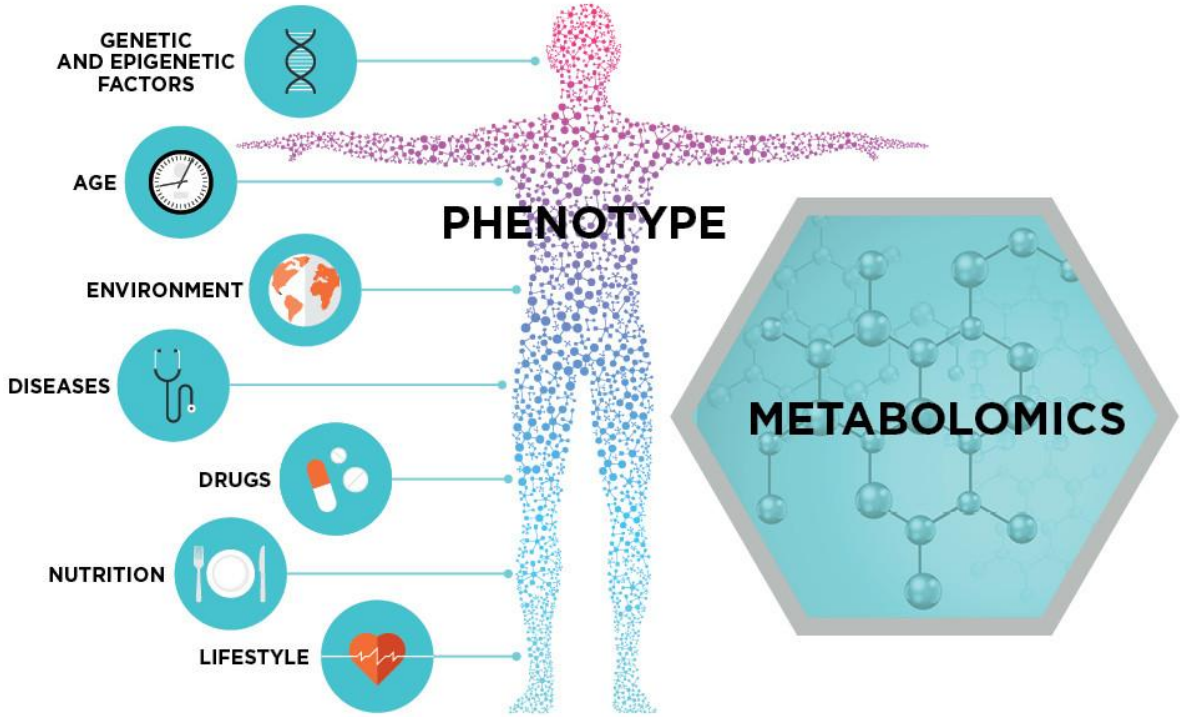


Moorfields Eye Hospital NHS Foundation Trust

THE FRANCIS CRICK INSTITUTE

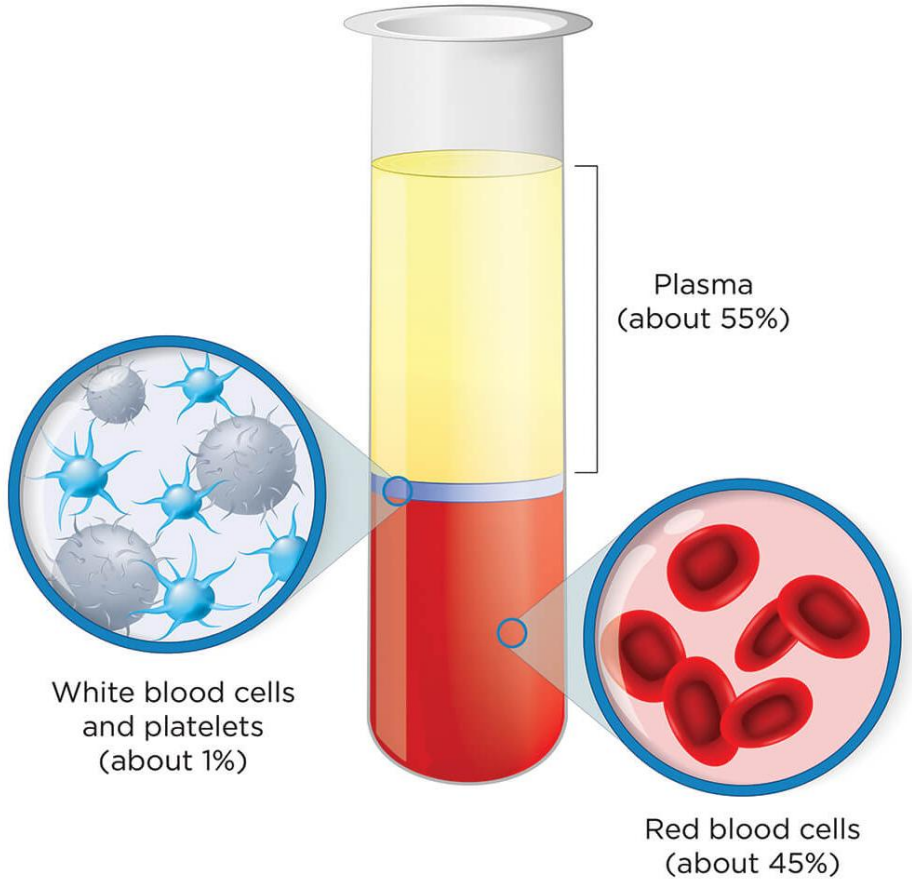
UCL

Investigate the systemic effects of *PAX6* haploinsufficiency through whole metabolomic profiling of aniridia patients



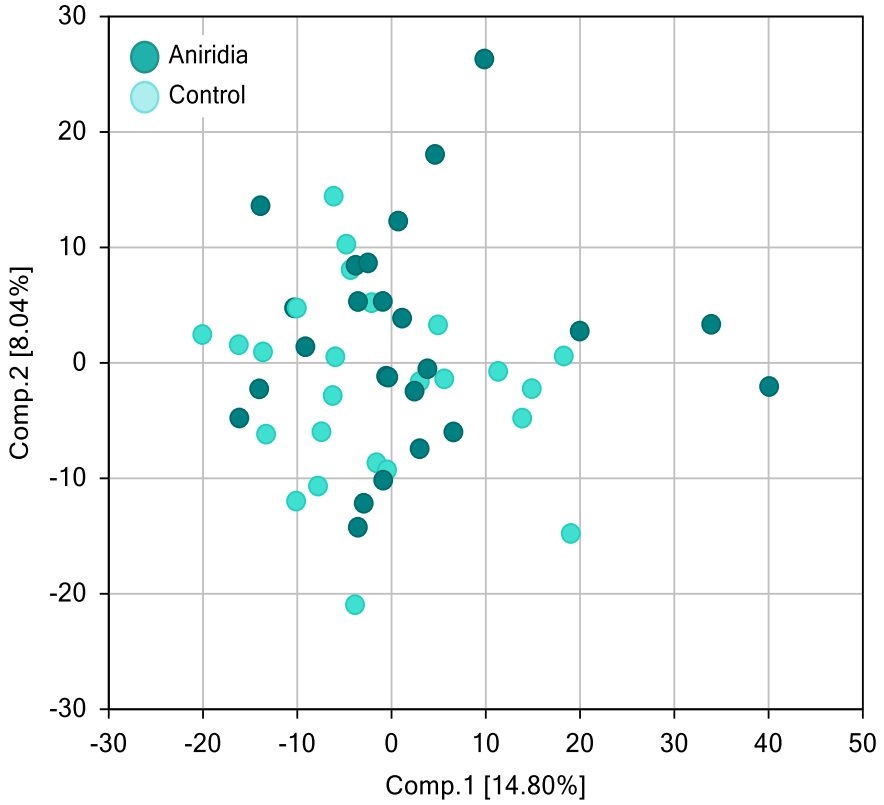
Methods

- 25 aniridia patients versus 25 age and gender matched healthy controls
- Looks at >1000 metabolites in the blood plasma including proteins, carbohydrates, lipids, vitamins, caffeine etc (Metabolon Inc)
- Asked each patient to fill out a food frequency questionnaire to ensure the results were not skewed by the diet
- Patients with diabetes or those on statins were not included



Cohort description

- 7 children/18 adults
- Mean age 31 years
- 48% male
- BMI (kg/m^2)
  - Aniridia group 27.7 ±6.1*
  - Control group 23.99 ±5.75



PCA plot showed overlap between aniridia and controls, this improved when age was factored in

Reported clinical features in aniridia cohort

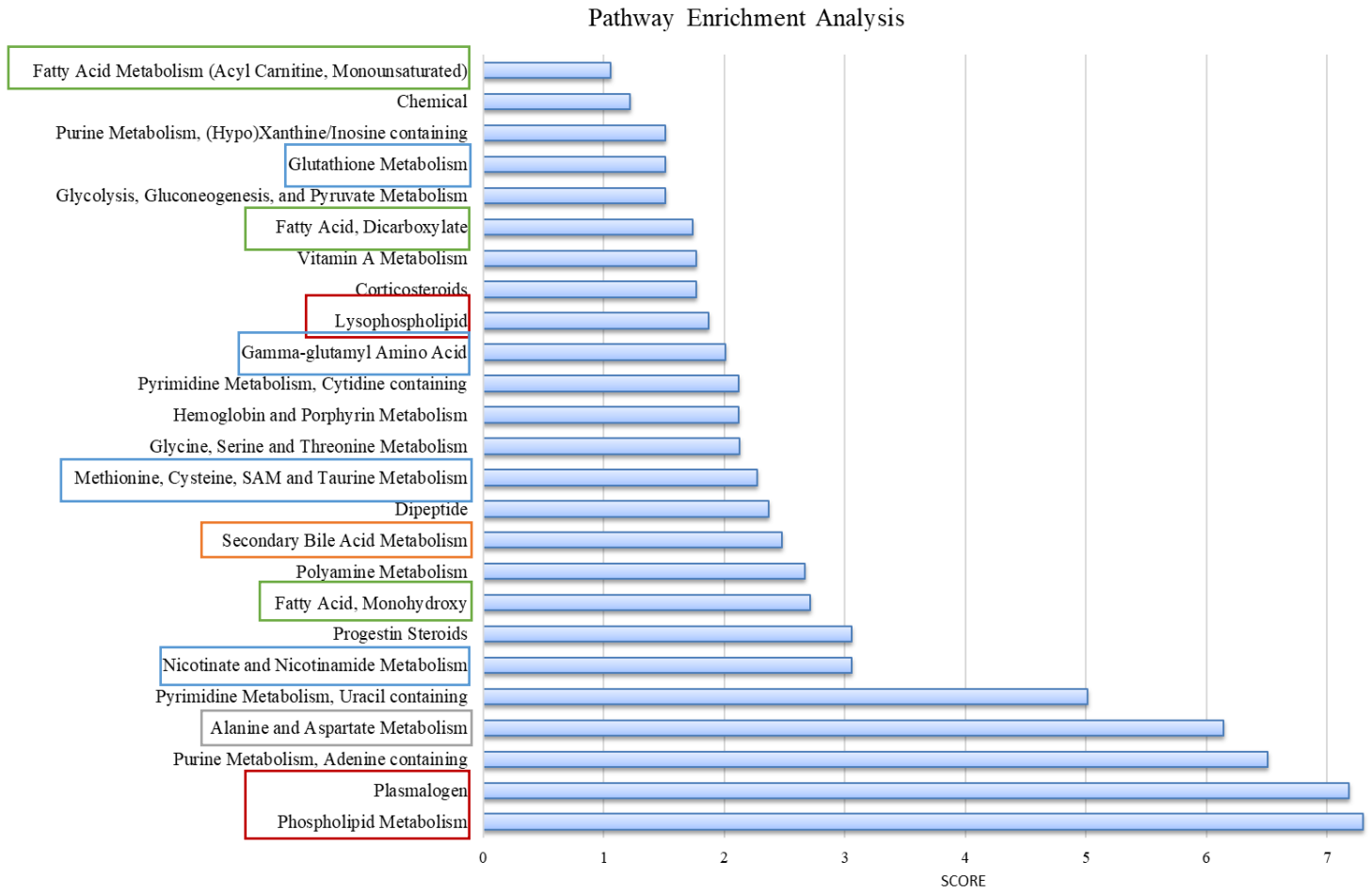
- 44% of aniridia patients had obesity vs 16% in controls

Extra-ocular features	Patients	Controls
Obesity	11/25	4/25
Gastric reflux	2/25	
Hypertension	1/25	2/25
Central auditory processing disorder	2/25	-
Depression	2/25	
Anxiety	3/25	
Learning difficulties	2/25	
ADHD, ASD	2/25, 1/25	1/25
Pineal gland hypoplasia	1/25	-
Sleep disturbances (melatonin supplement)	3/25	1/25

Affected pathways

There were over 94 metabolites that were different, main disrupted groups:

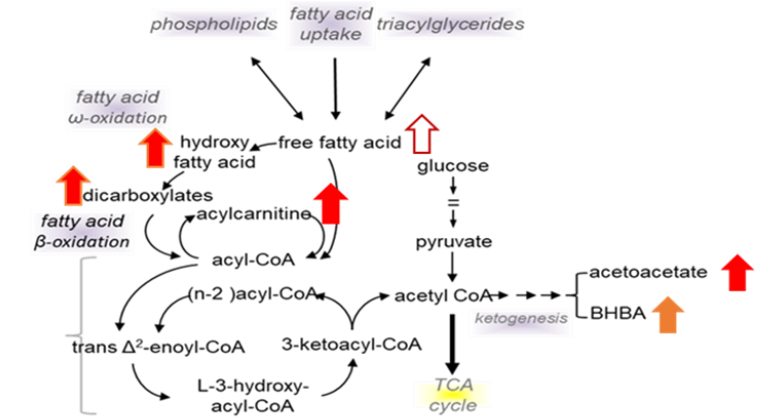
1. Lipid oxidation
2. Oxidative
3. Complex lipids
4. Neuroactive metabolites
5. Microbiome-related metabolites



Lipid metabolism disruption

Dicarboxylate FAs, monohydroxy FAs and carnitines are increased in aniridia

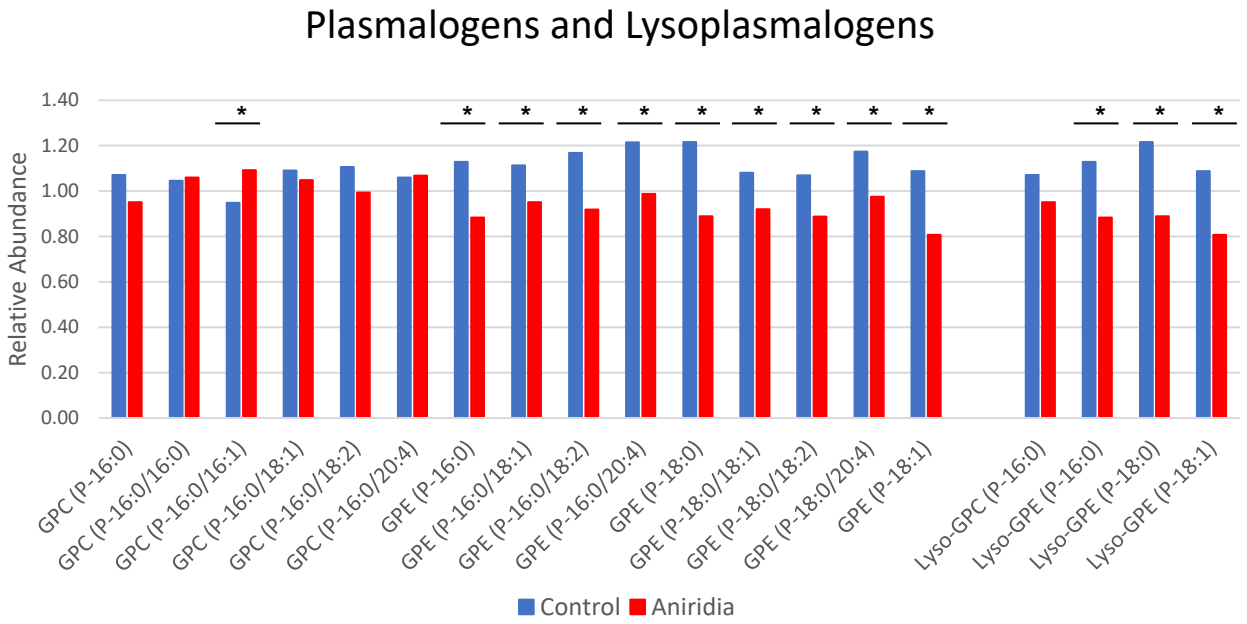
- Markers of fatty acid metabolism were disrupted in aniridia patients
- Increased carnitines have been linked to diabetes and heart failure



Red = increased levels in aniridia

Plasmalogens

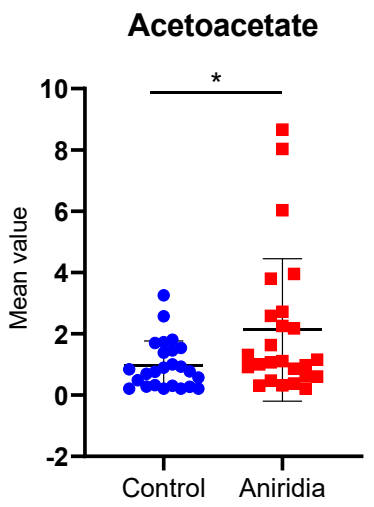
- Decreased plasmalogens levels
- Low levels are a biomarker for Zellweger syndrome and rhizomelic chondrodysplasia punctata (cataracts)
- Reduced levels have been detected in patients with metabolic syndrome, T1D and T2D, and hyperlipidemia

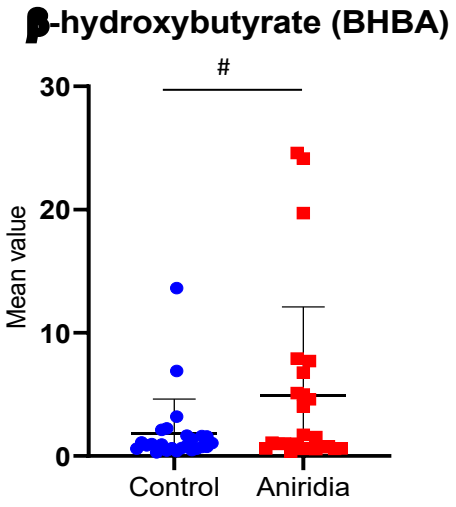


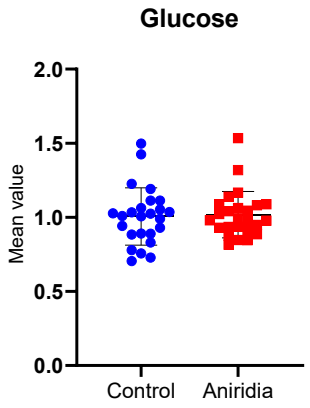
Significantly reduced in aniridia patients

Ketone bodies

- We found an accumulation of ketone bodies despite no changes in glucose levels
- Other raised markers linked to ketoacidosis
- Taken together suggest aniridia patients could be in an insulin resistant/prediabetic stage

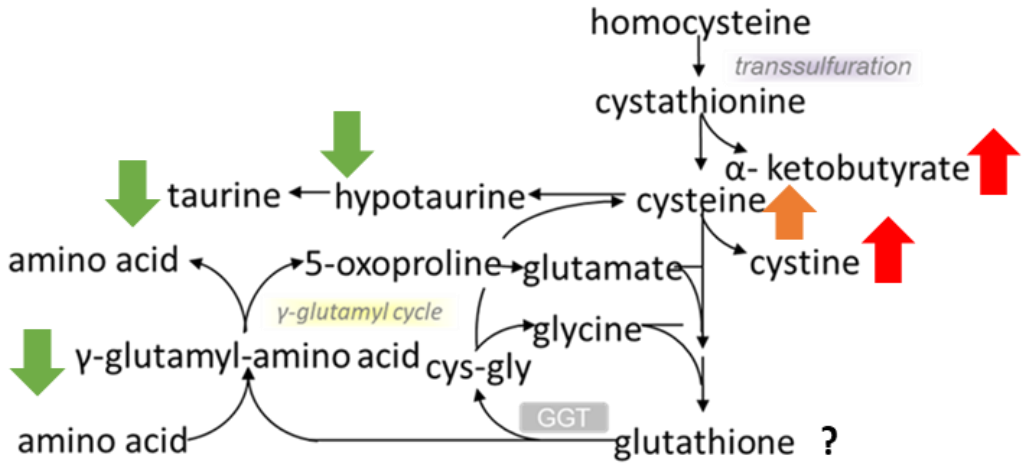






Oxidative stress

- We detect high levels of oxidative stress in the blood plasma
  - Antioxidant taurine and its precursor hypotaurine are significantly reduced

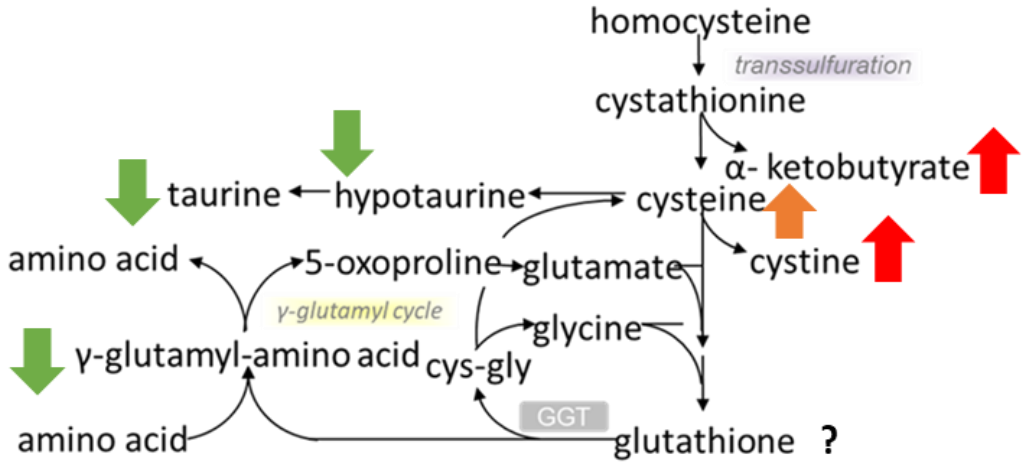


Green = decreased levels in aniridia

Red = increased levels in aniridia

Taurine

- Important in bile acid synthesis and stimulates insulin release
- Altered plasma taurine levels have been associated with neurological disorders like depression, schizophrenia or autism spectrum disorder, but also with metabolic diseases like hypertension, obesity
- Taurine supplementation showed promising benefits against several of these diseases, so there may be therapeutic potential for aniridia

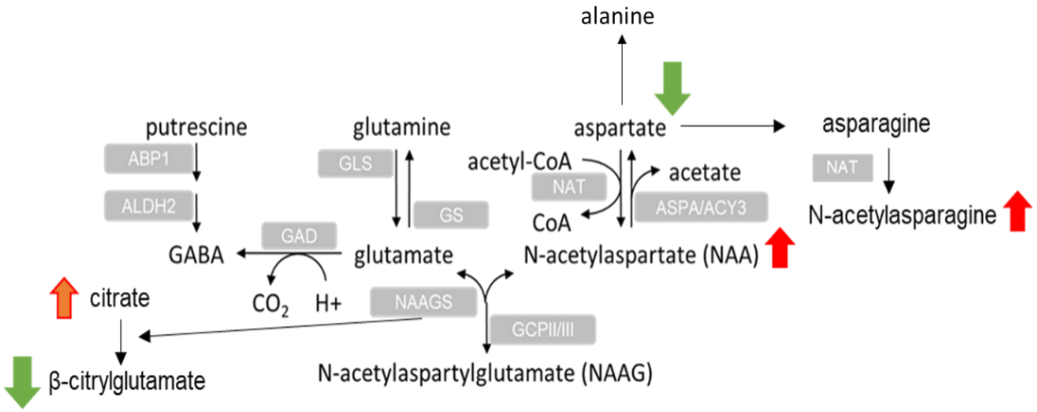


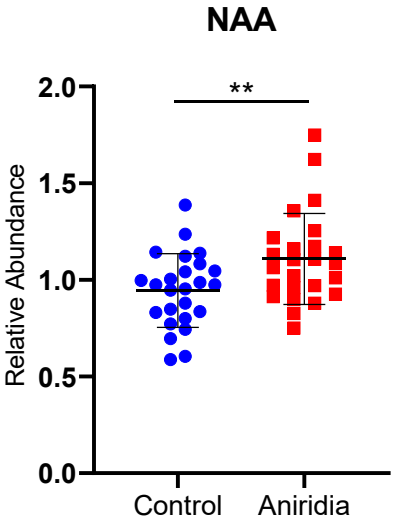
Green = decreased levels in aniridia
Red = increased levels in aniridia

Neuroactive metabolites

Green = decreased levels in aniridia
Red = increased levels in aniridia

- Aspartate levels were reduced but with increased N-acetylaspartate (NAA)
- NAA is one of the most prevalent neurotransmitters in humans
- Elevated NAA levels are markers for Canavan disease, an infantile lethal neurodegenerative disease
- Increased levels of NAA have also been detected in the brain of female children diagnosed with ADHD



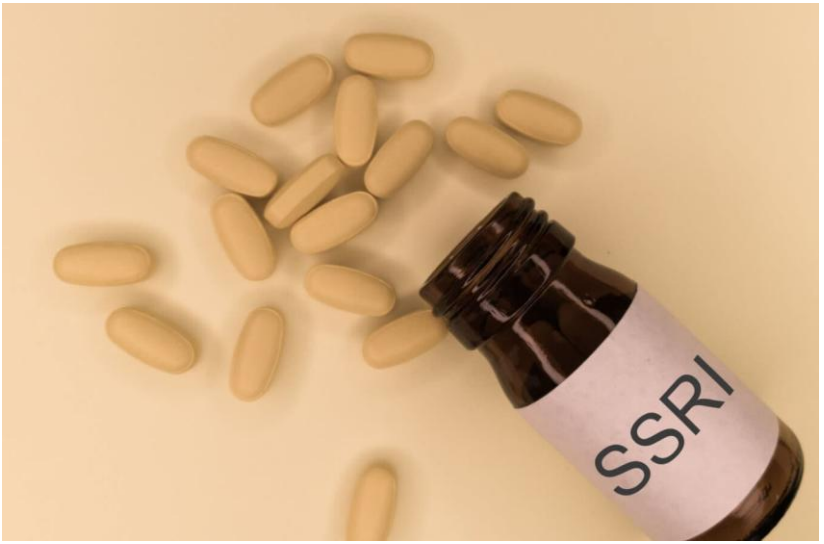


Aniridia, autism and ADHD

Dr Ngozi Oluonye
Paediatric Neurodisability
Consultant, MEH & GOSH

Therapies under investigation…

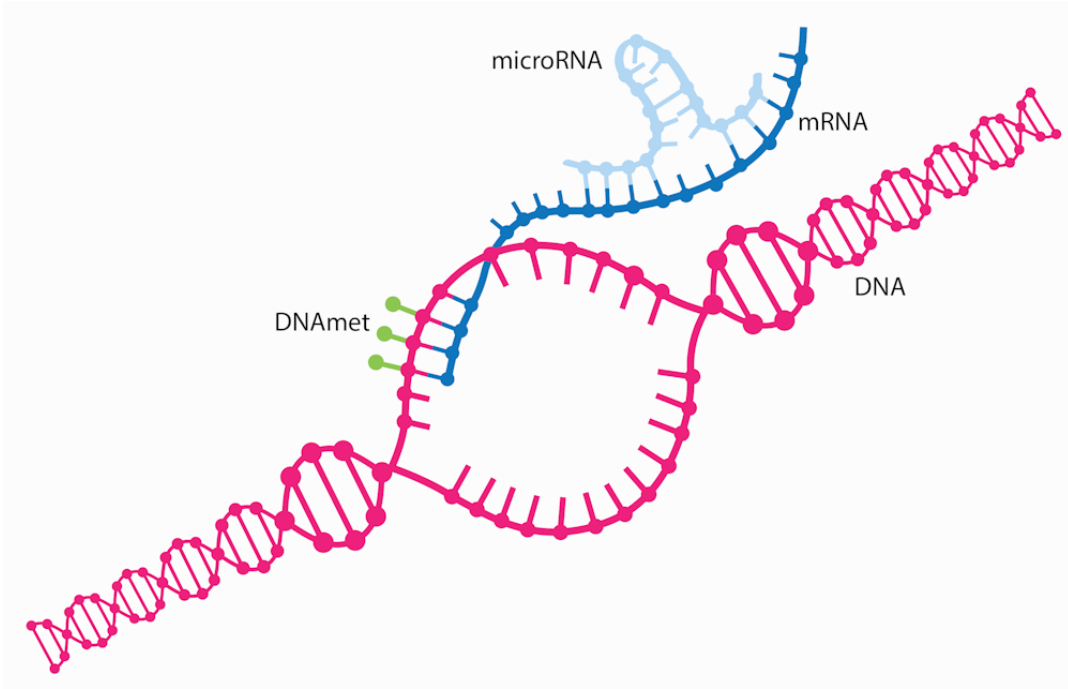
Anti-serotonin drugs, like duloxetine and ritanserin, have been found to increase production of PAX6 in corneal limbal epithelial stem cells



Therapies under investigation…

A regulatory microRNA, miR-204-5p, has been found to increase PAX6 in corneal limbal stem cells and mature differentiated corneal epithelial cells

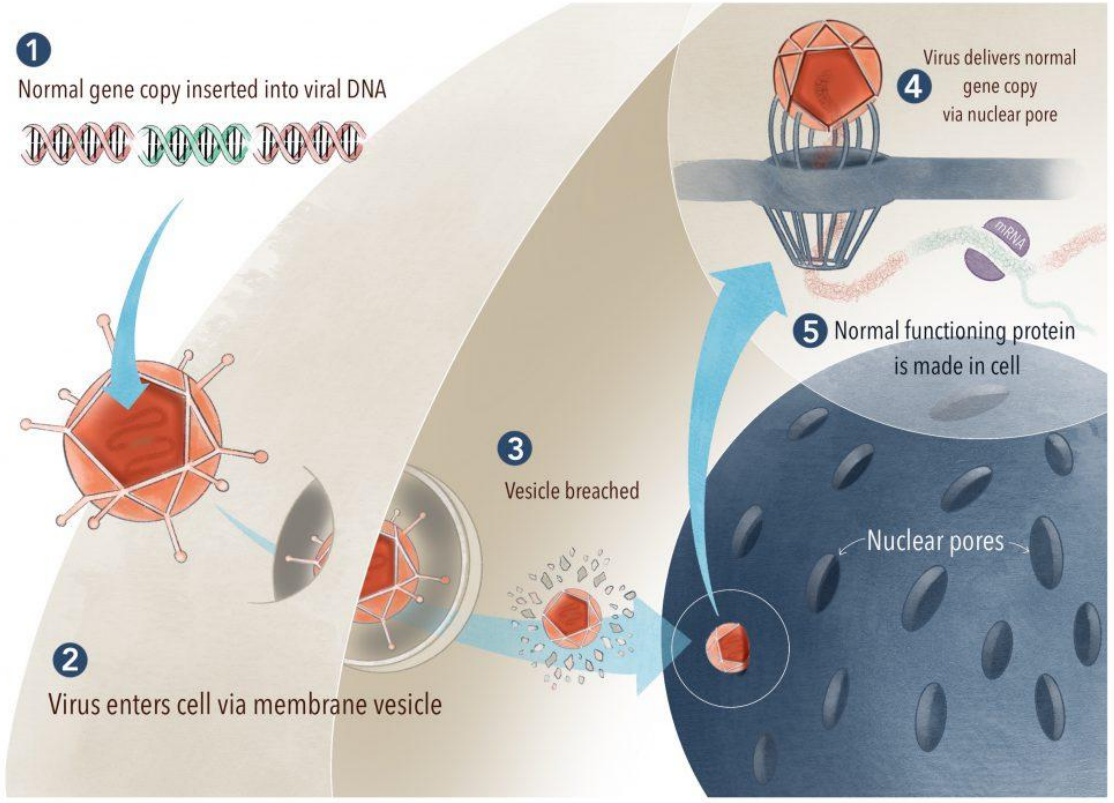
BUT there was no significant change in human limbal epithelial cells, human limbal stem cells or in the corneas of Pax6 mice



Therapies under investigation…

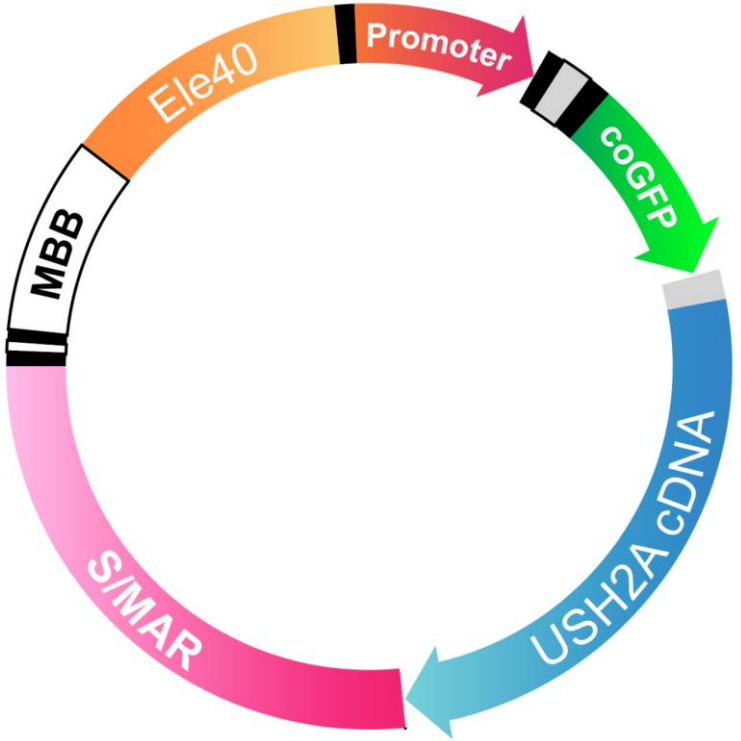
- A gene therapy would be the obvious strategy but viral vectors can provoke immune responses and inflammation, but researchers are looking at this approach (retired Dr Elizabeth Simpson showed *PAX6* expression in mouse models for 5 months)

- Alternative strategies…



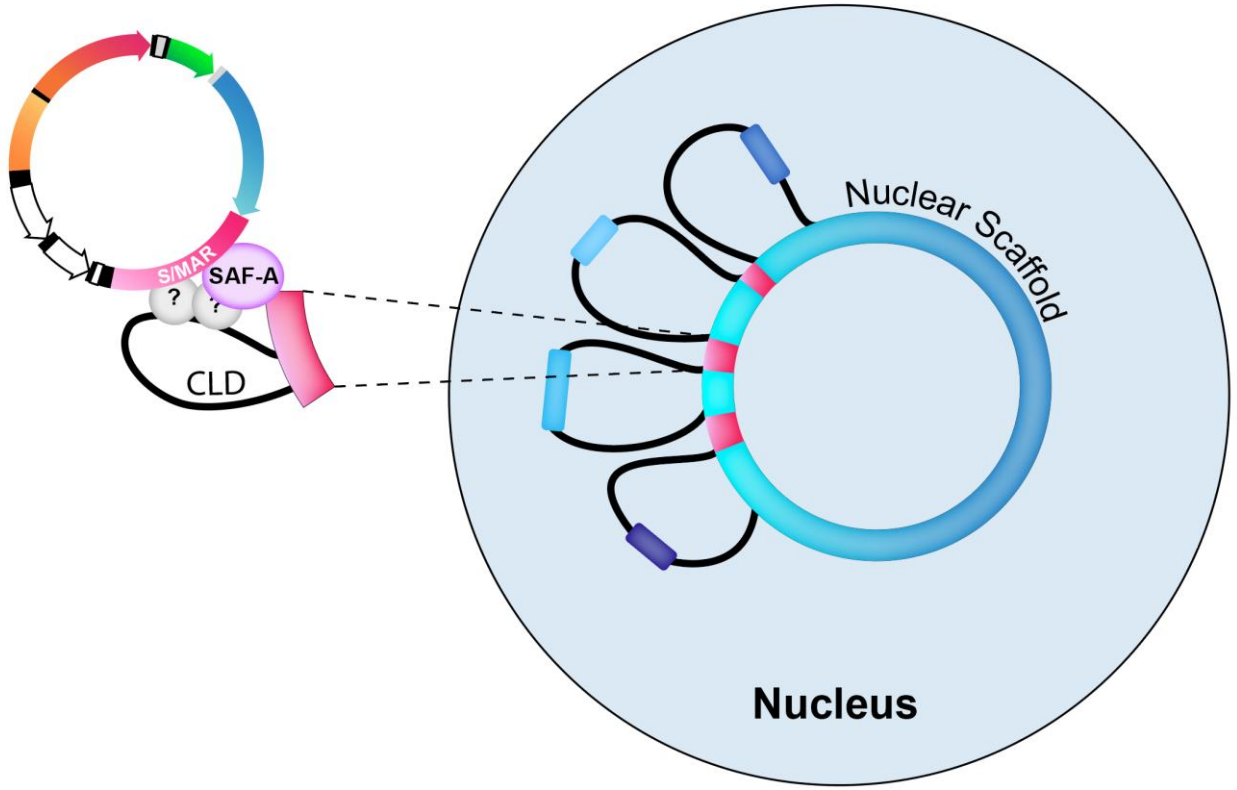
My Lab: Non viral gene therapy

- Circular piece of human DNA- plasmid
- Composed of only human elements- reduced immune reaction
- Does not integrate into genome
- Can hold unlimited size genes *(USH2A* is 16,000 letters) even pieces of chromosome!



Scaffold matrix attachment region

- Special ingredient…Scaffold matrix attachment region (S/MAR)
- Found in our cells – helps to fold DNA
- Protects the plasmid from degradation

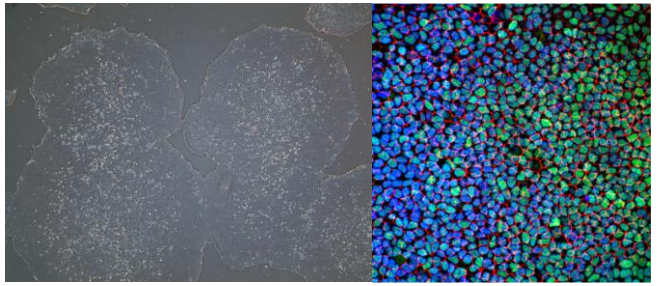


Moorfields Eye Hospital NHS Foundation Trust

THE FRANCIS CRICK INSTITUTE

UCL

Disease models- zebrafish, mouse, patient cells and rabbit



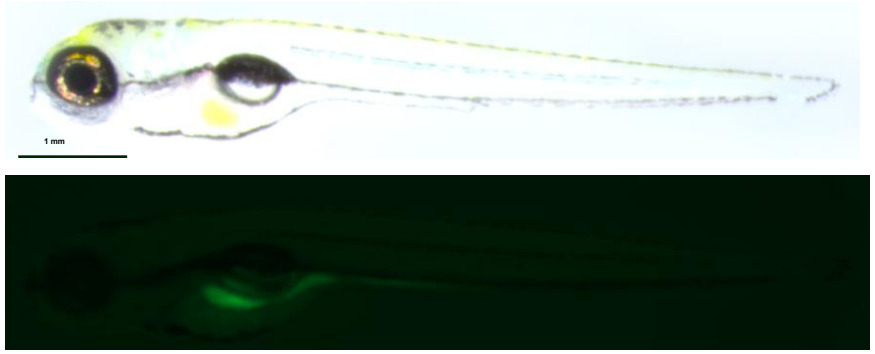
Moorfields Eye Hospital NHS Foundation Trust

THE FRANCIS CRICK INSTITUTE

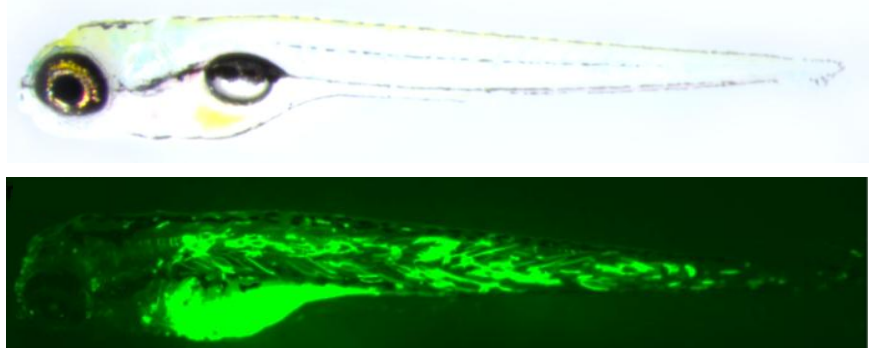
UCL

S/MAR vector delivery to zebrafish

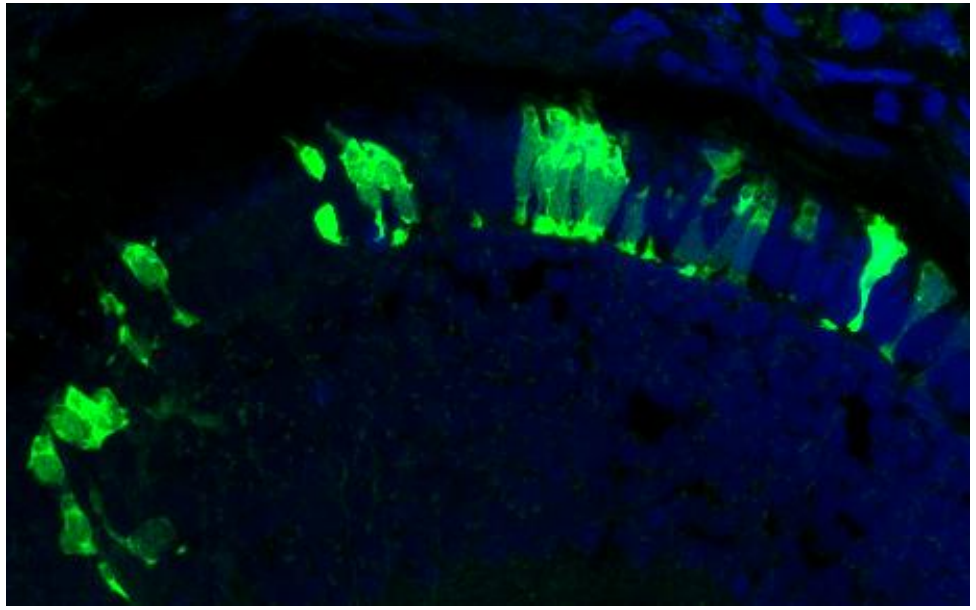
Wild-type



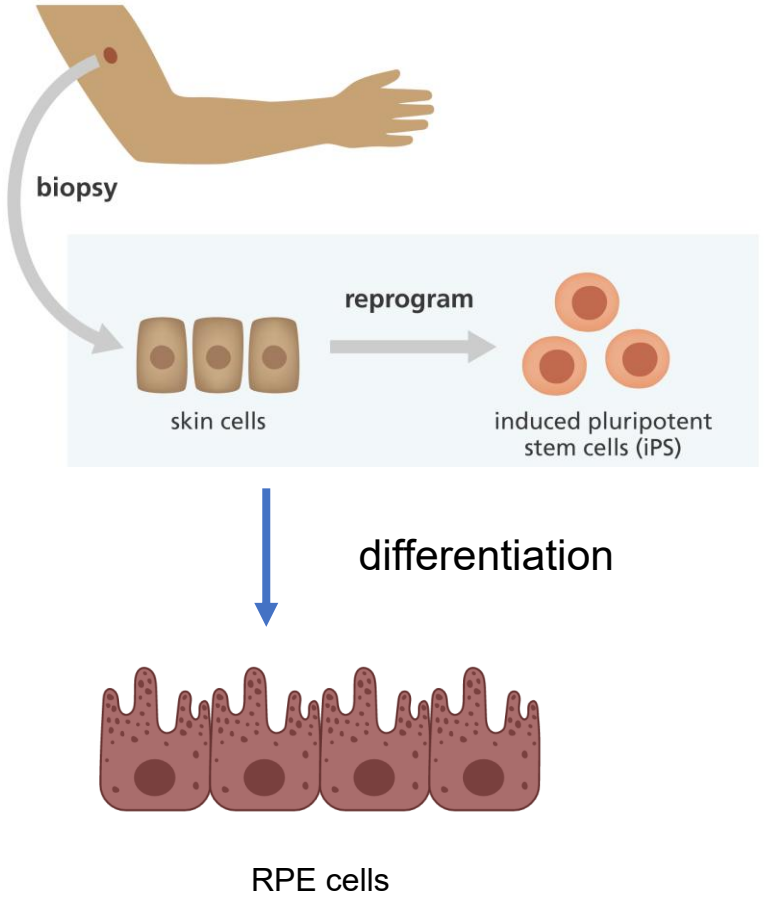
Injected wild-type



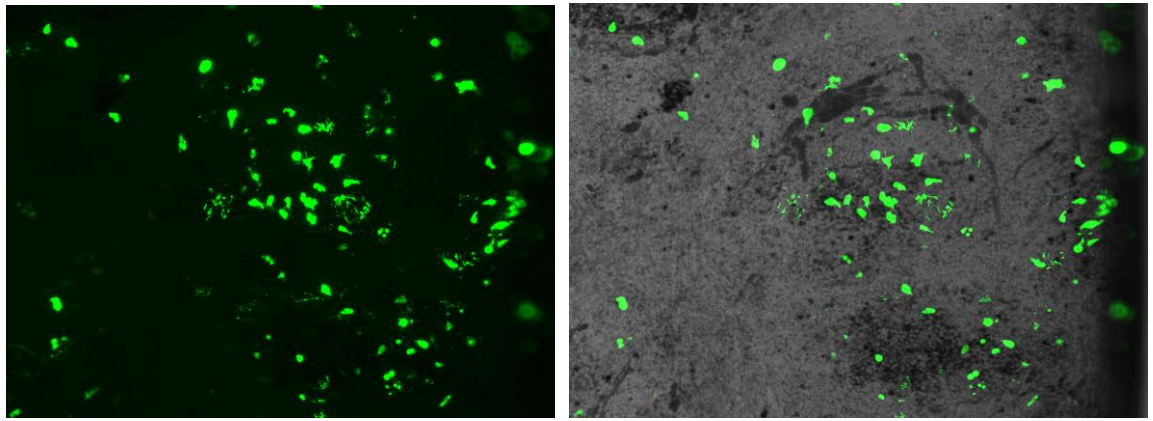
Photoreceptors in the retina (up to 1 year)



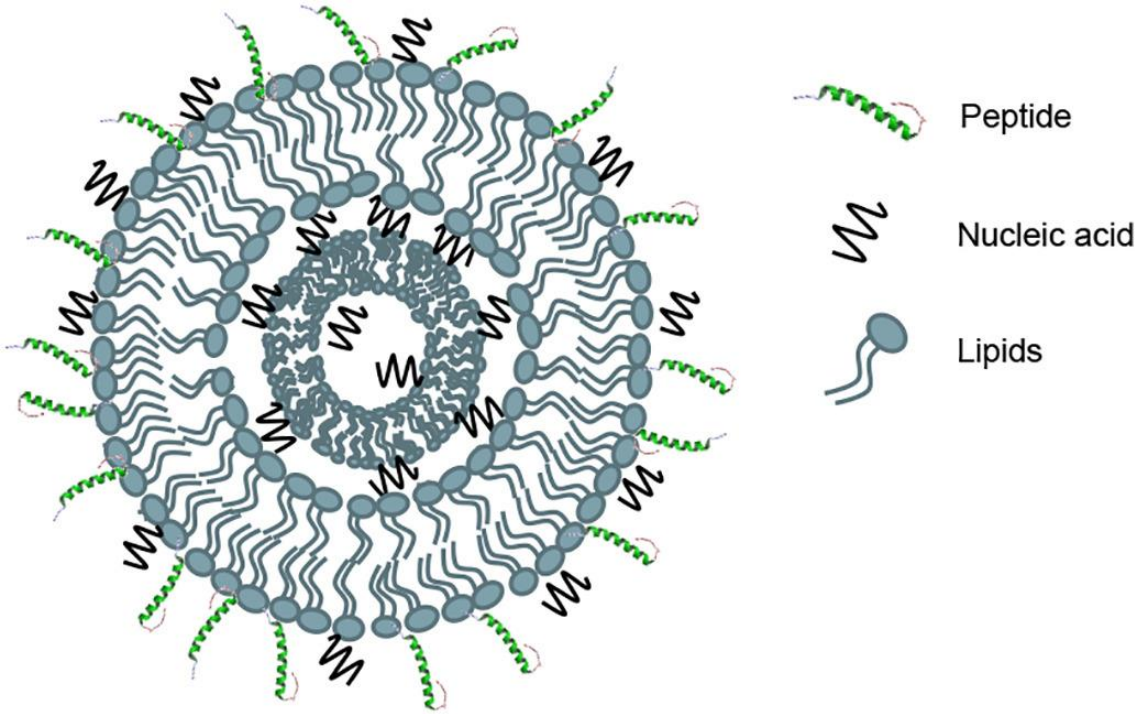
S/MAR vector delivery to patient cells

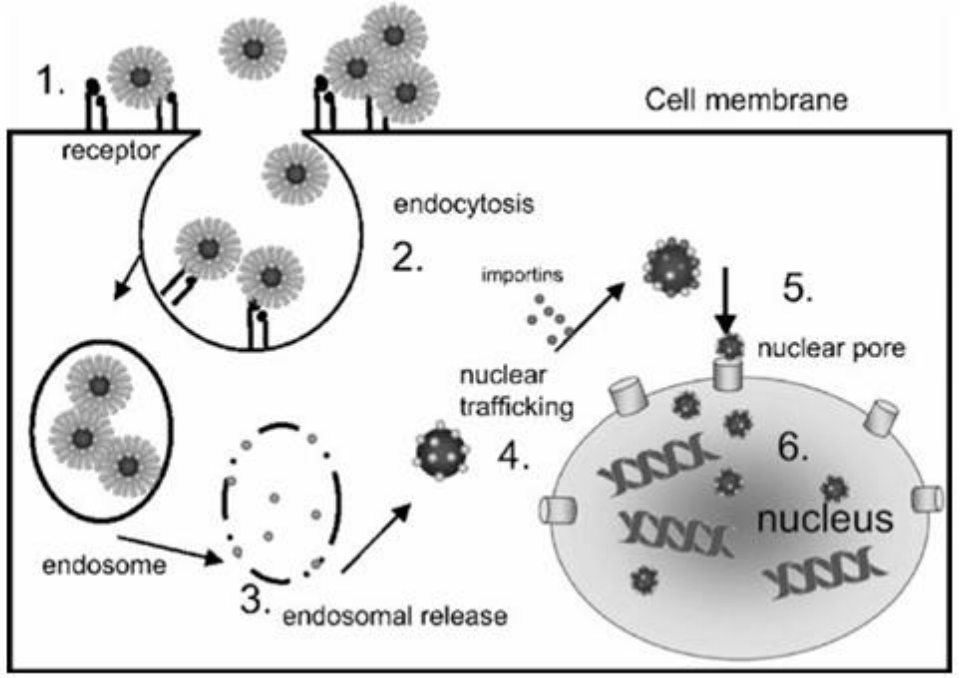


48 hours post-transfection



How do we get the DNA into cells?





Grant-Serroukh et al. *J Control Release*. 2022

Hart S. *Cell Biol Toxicol*. 2010

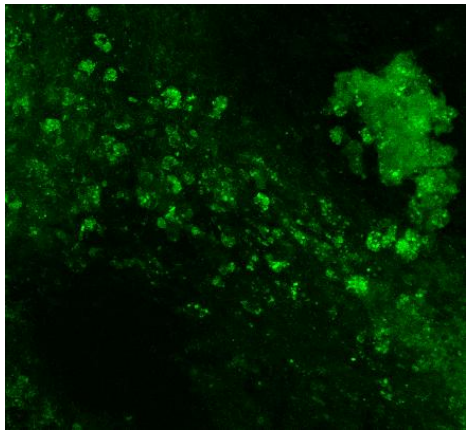
Moorfields Eye Hospital NHS Foundation Trust

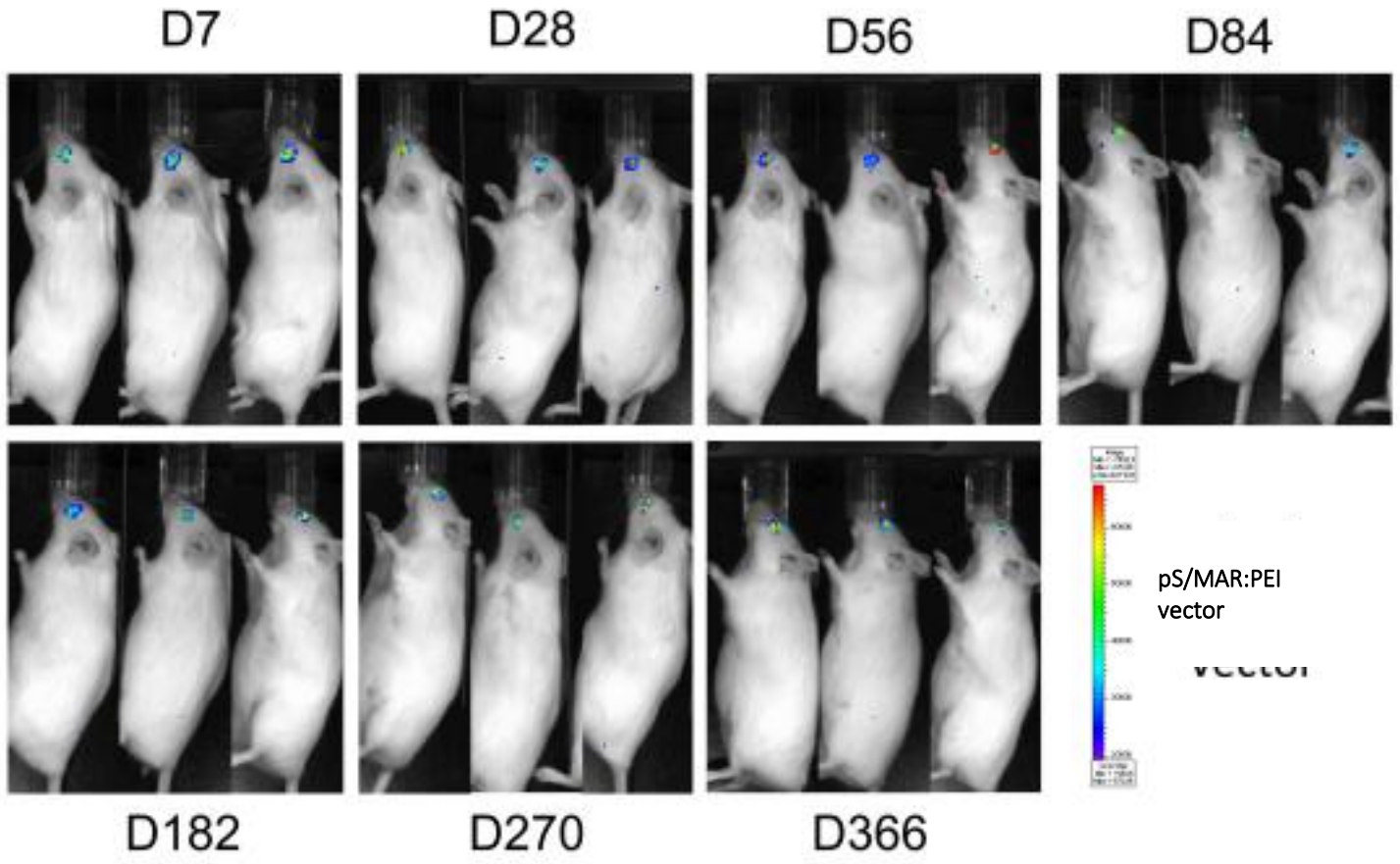
THE FRANCIS CRICK INSTITUTE

UCL

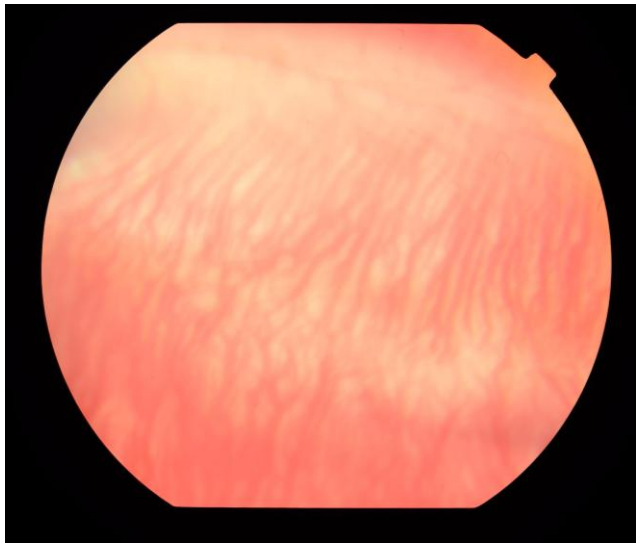
Long-term gene delivery to the mice

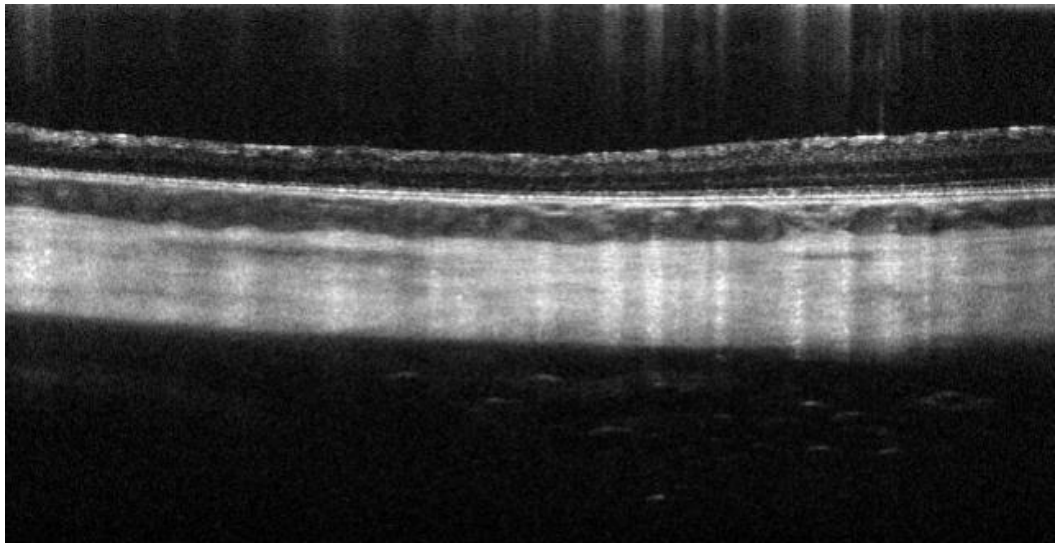
Retina flatmount

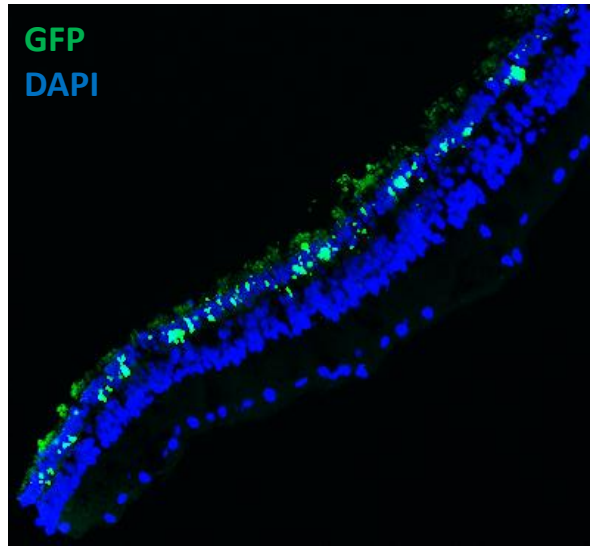




Gene delivery to the rabbit



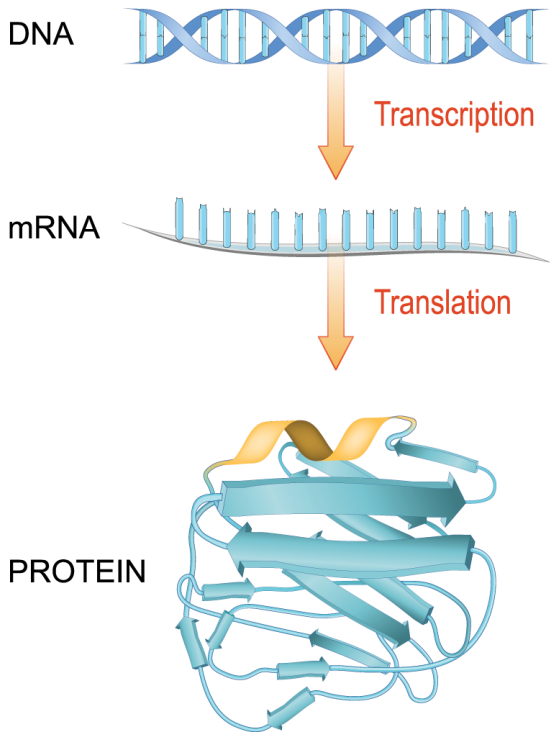


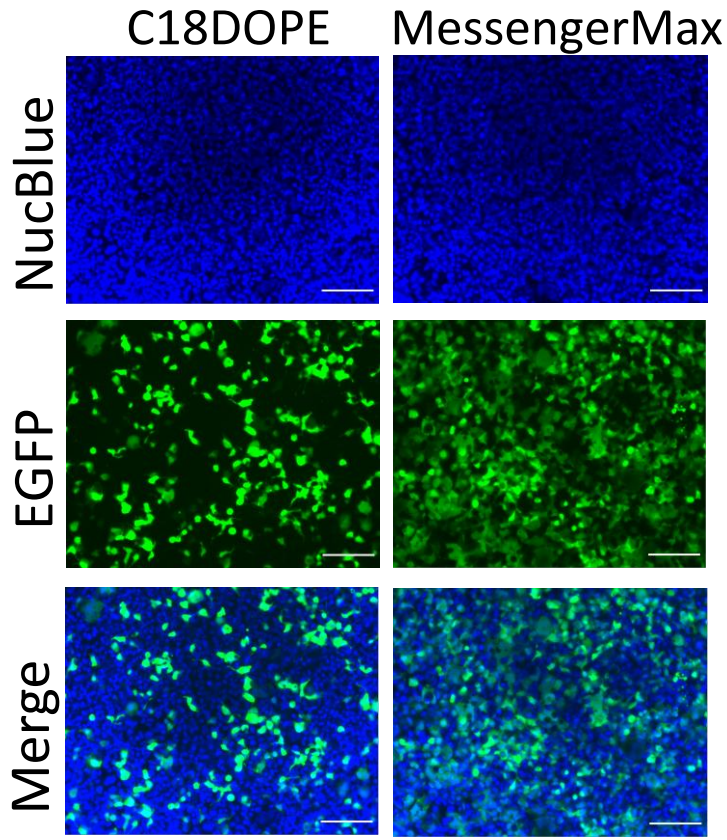


- Showing promise, may be applicable to WAGR
- Responsibility to prove safety and efficacy before moving to clinical trials

Other approaches we could look at for *PAX6* therapies

- mRNA therapy





How can individuals with WAGR syndrome participate in research?

- Contact me- m.moosajee@nhs.net
- Register for Research Opportunities at Moorfields- ROAM
- Stay in touch with IWSA and Aniridia Network
- Help fundraise!

- We will be sending research studies to IWSA to distribute

www.gene.vision

A resource on rare genetic eye disorders for everyone

- Reliable and up-to-date
  - Written and edited by specialists
  - For patients and health care professionals

- Relevant information
  - Condition, latest research
  - Practical aspects: Education, employment, family support etc
  - Links to relevant organisations and charities

- Optimum accessibility for the visually impaired
  - Accessibility consultants
  - Tested with real-world patients

NHS National Institute for Health Research

RETINA UK
WORKING FOR PEOPLE WITH INHERITED SIGHT LOSS

Wilms tumor, aniridia, genitourinary anomalies and range of developmental delay (WAGR) syndrome: for patients

Quick links

Conclusions

- Genetics team see the patient and the family as a whole, detail their features through careful phenotyping, make molecular diagnoses, provide psychosocial support
- It allows us to assemble the correct MDT for optimising patient care
- Getting a genetic diagnosis can help patients access treatments, clinical trials and the latest research
- We can learn a lot from advances in aniridia but we need more focus on WAGR

Acknowledgements

- Keep in touch: m.moosajee@nhs.net
- Follow on Bluesky @ProfMariya.bsky.social

Clinical Team:

Dr Ngozi Oluonye
Dr Elly Schiff
Julie Broadbent
Sakina Rajabali
Meei Wong
Mrs Jean Cavanagh

Lab Team:

Post-docs

Dr Maria Toms
Dr Cecile Mejecase
Dr Sara Vasquez
Dr Jose-Martin
Dr Vitalii Kalynovskyi
Dr Mubeen Khan

Clinical research fellows

Dr Lee Jones
Dr Randa Li

PhD students

Mutian Lian
Dr Daniel Jackson
Katy Linkens
Omayma Al-Saei
Kishan Devraj
Dr Ana Rodriguez-Martinez
Dr Nicola Cronbach
Dr Paulina Ocieczek

Research Assistants

Elham Shadman
Jieren Bornia