Exome sequencing and functional validation in zebrafish identify novel genes for dystroglycanopathies

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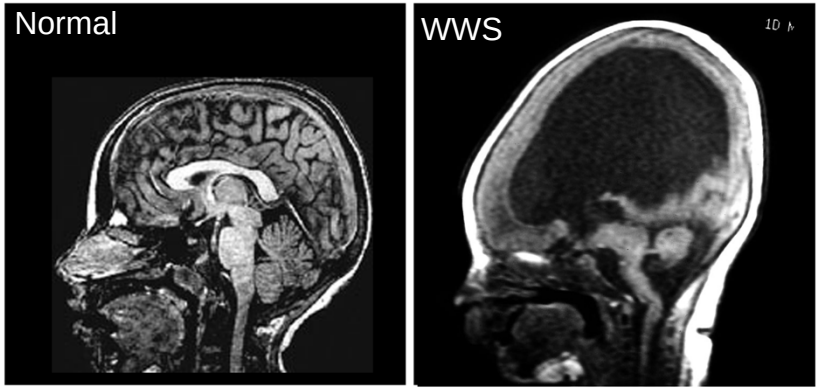
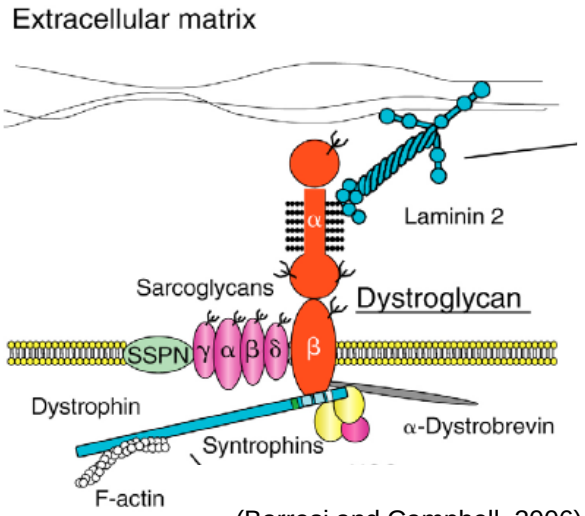
Dystroglycanopathies affect muscle, eye and brain development

- Core features:
  - Congenital muscular dystrophy
  - Brain malformations: hydrocephalus, gyral defects
  - Ocular malformations in the retina, lens and cornea



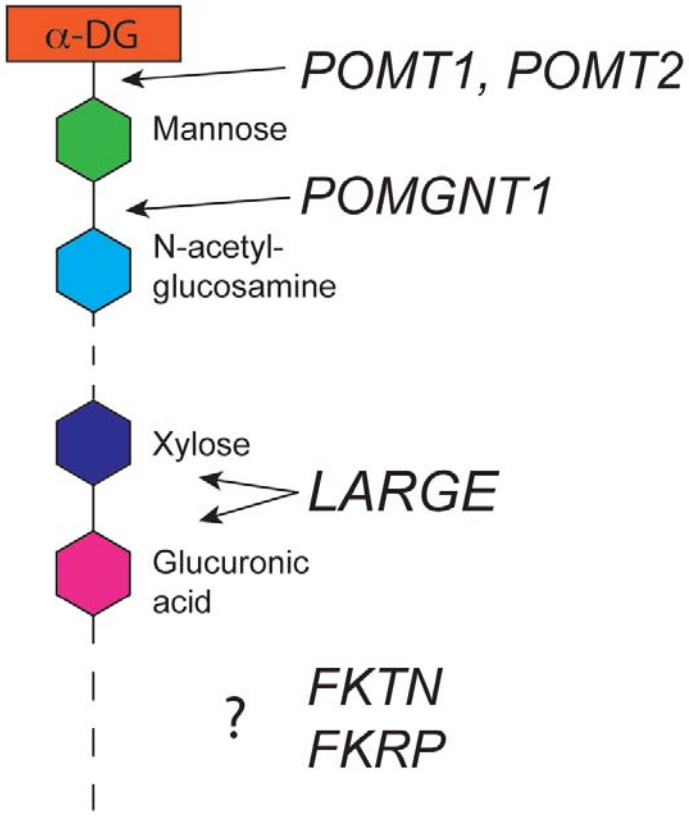
- Dystroglycan is a widely expressed transmembrane glycoprotein
- α-dystroglycan (α-DG) interacts with extracellular proteins like laminin via its glycosylated moieties



(Barresi and Campbell, 2006)

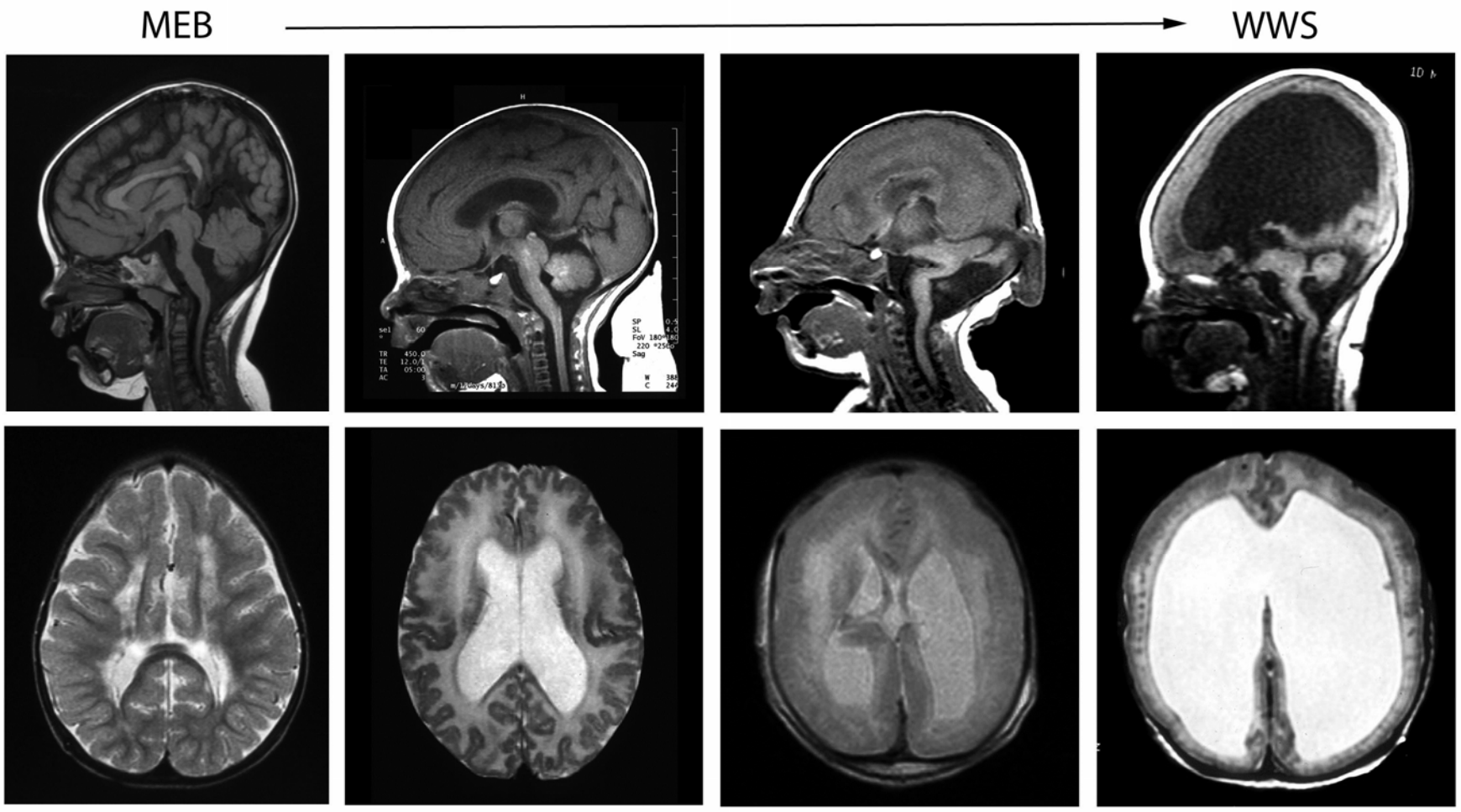
Dystroglycanopathies are extremely genetically and clinically heterogeneous

- Mutations in 10 genes cause dystro-glycanopathies and all affect α-DG glycosylation
- Some genes are glycosyltransferases, others are suspected to have a role in glycosylation
- α-DG glycosylation is essential for it function, e.g. laminin binding
- Mutations in these genes explain only a portion of the cases

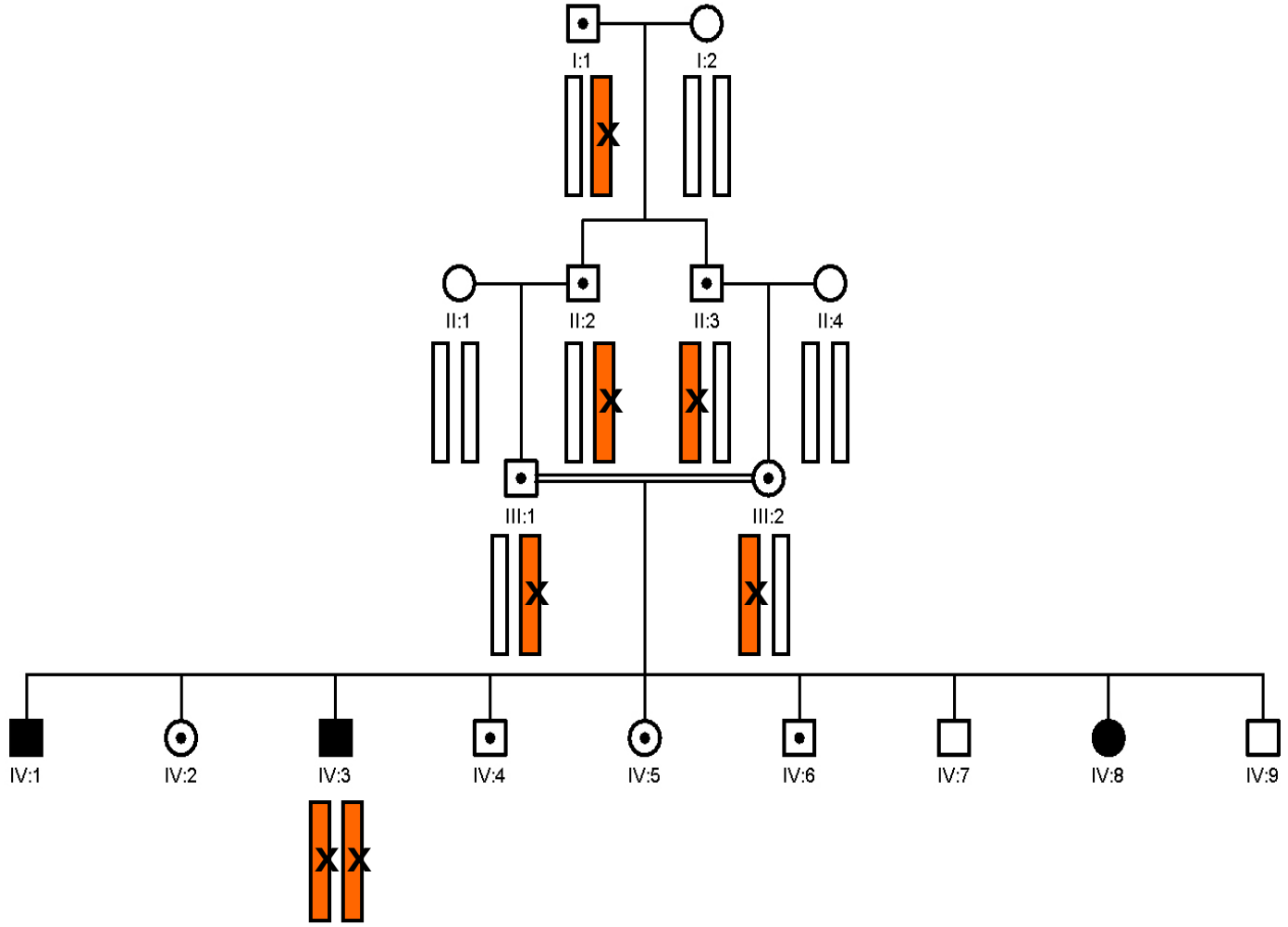


- Mutations in the same gene cause a spectrum of phenotypes affecting the brain-eye-muscle or only the muscle

Brain phenotypes range greatly in severity

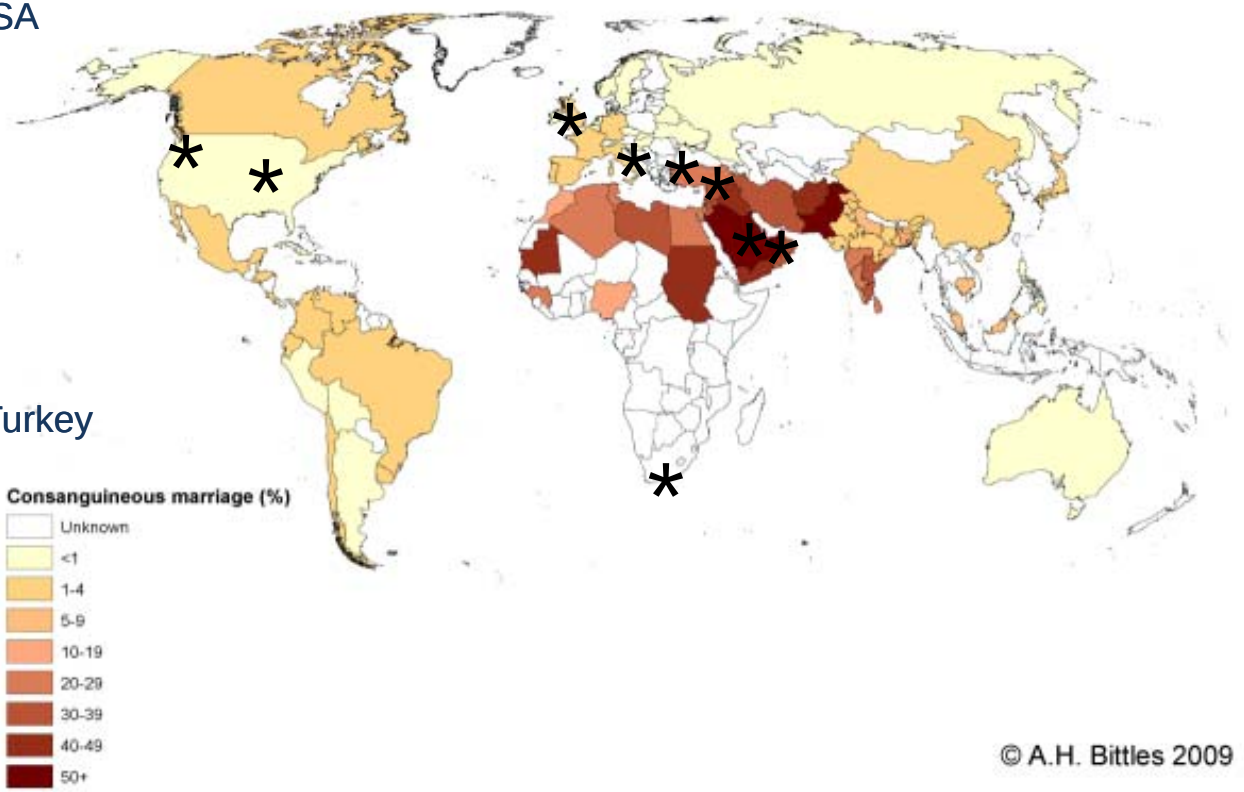


Homozygosity mapping provides linkage information at the single family level



Identifying patients with rare diseases requires a large network of collaborators

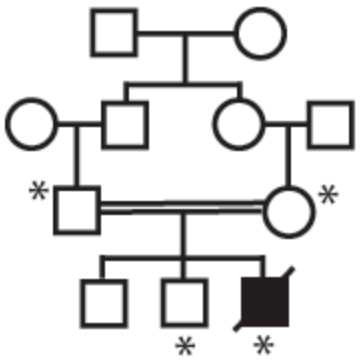
Dr. L. Al Gazali, UAE
Drs. F. Al Kuraya, M. Salih, KSA
Dr. A. Al-Qudah, Jordan
Dr. L. Basel-Vanagaite, Israel
Dr. K. Campbell, USA
Dr. W.B. Dobyns, USA
Dr. A. Krause, South Africa
Dr. M. Mora, Italy
Dr. F. Muntoni, UK
Dr. A. Rajab, Oman
Drs. M. Tekin and M. Topcu, Turkey

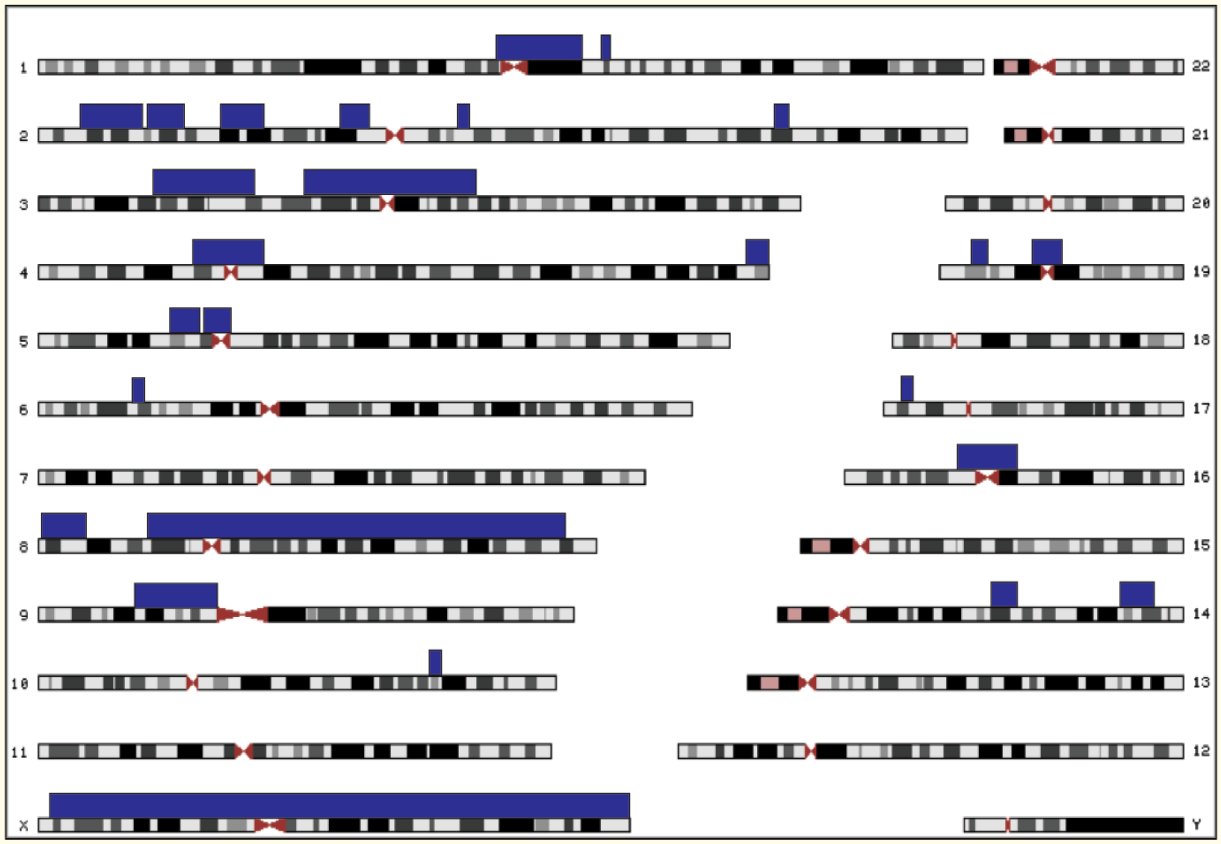


- Many of our collaborators are based in countries with high rate of consanguineous marriages: consanguinity yields more informative families

Homozygosity mapping in a dystroglycanopathy family

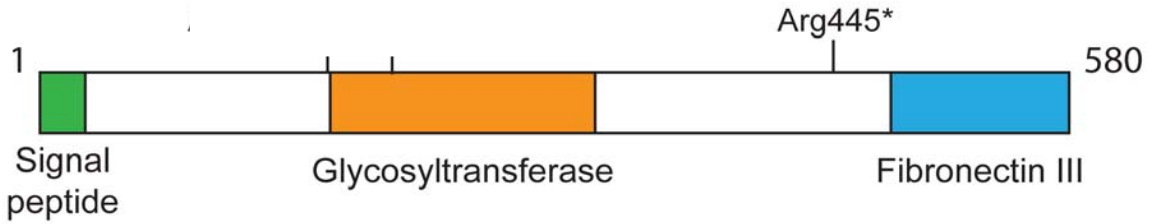
- 1st cousin union affected with Walker Warburg Syndrome
- 9.6% homozygosity - 2480 genes





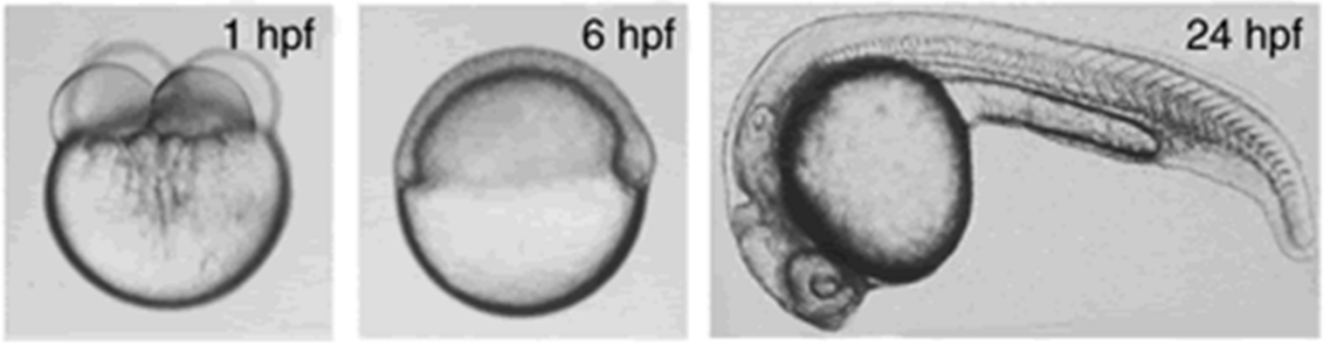
Homozygosity mapping and exome sequencing identify a new disease gene

- Exome sequencing yields 30-50,000 variants per individual
- A need for an appropriate filtering strategy
  - In region of homozygosity: focus on the 2480 genes
  - Rare or novel
  - Pathogenic: missense, stop, splicing, frameshift
- This approach reduced the number of candidate genes from 2480 to 6!
- A stop codon in the glycosyltransferase *GTDC2* appeared to be the best candidate (Manzini et al, 2012)

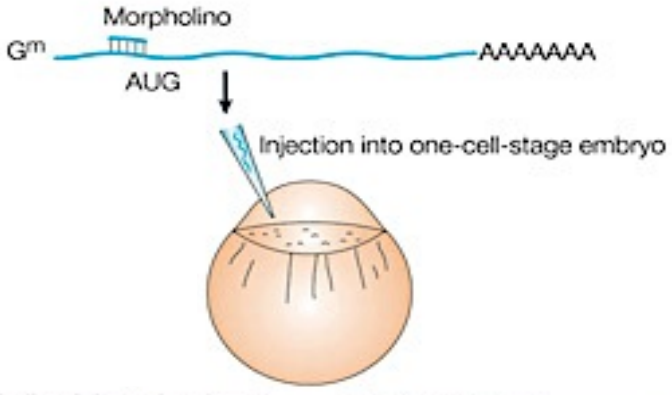


The zebrafish larva as a functional model for variant validation

- The zebrafish larva has prominent eyes and muscle, which develop over a few days



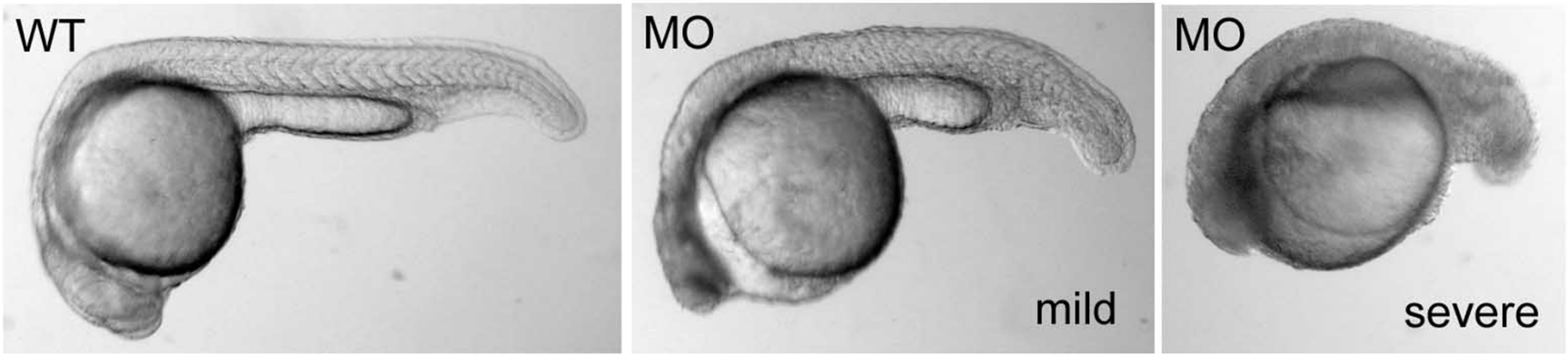
(Zhang et al, 2003)



(Langenau and Zon, 2005)

gtdc2 loss of function severely affects development

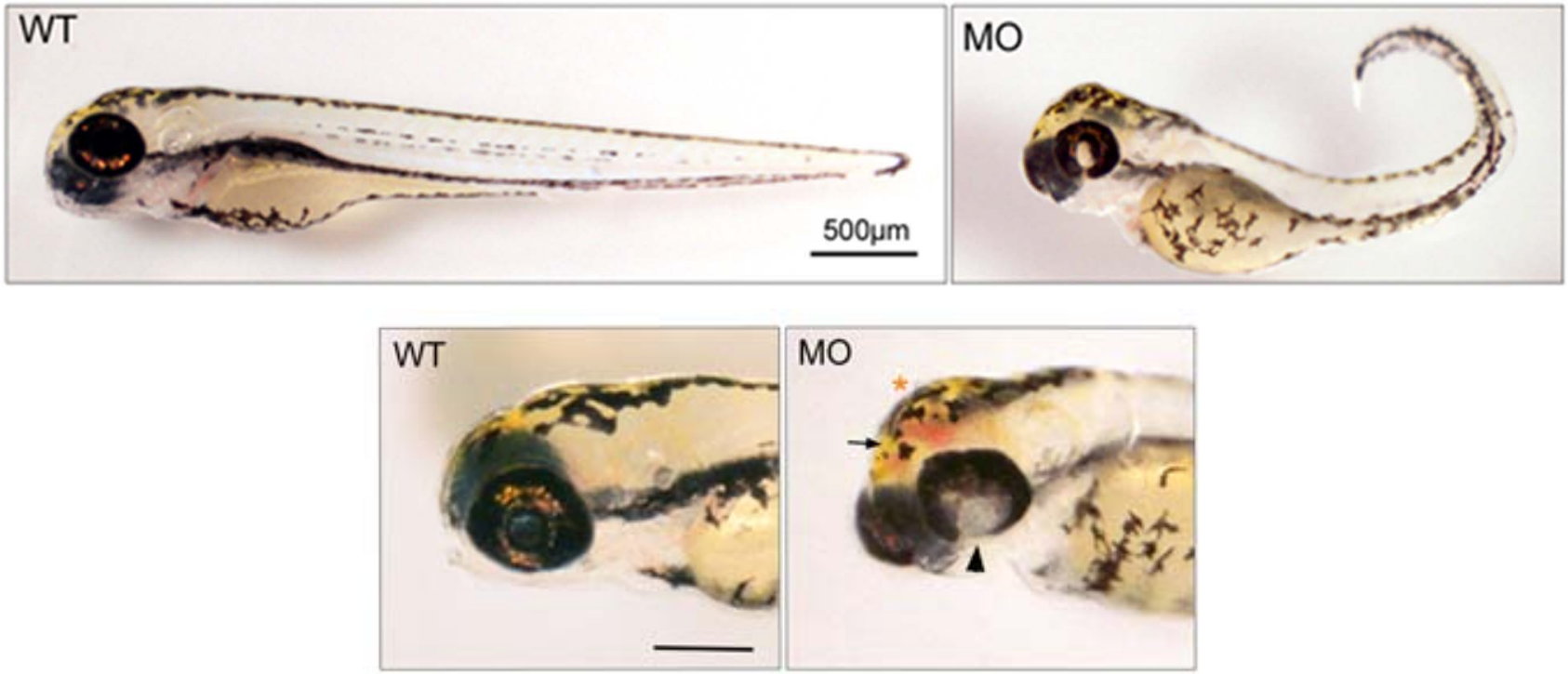
- Morphants for *gtdc2* show severe developmental defects from 1dpf
  - Stunted growth
  - Disorganized muscle fibers
  - Reduced mobility
  - Stunted head and eye development



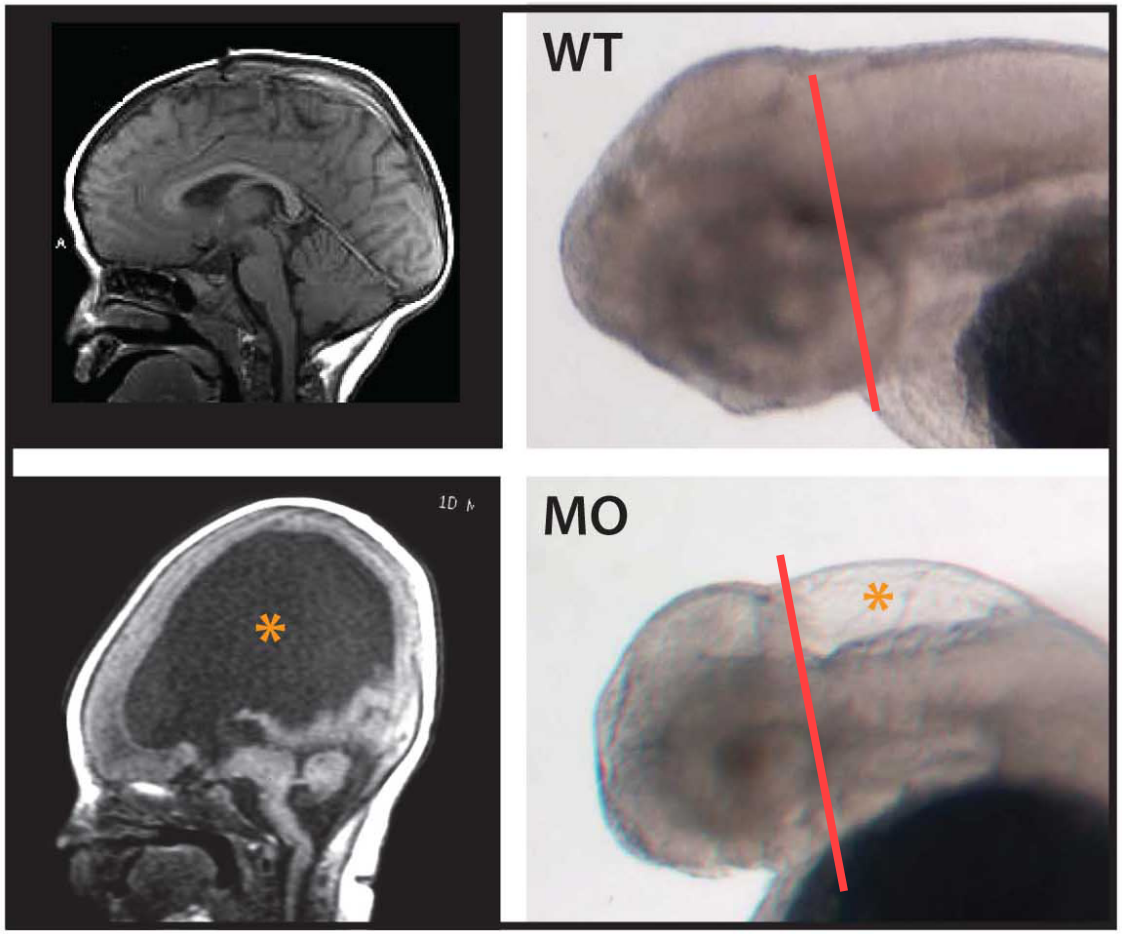
- Survival reduced to 50% by 3 dpf

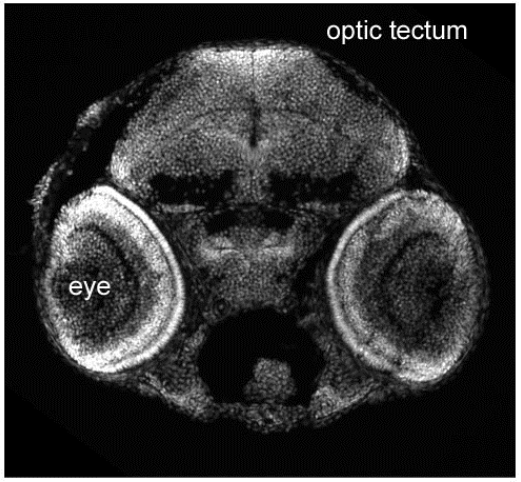
gtdc2 loss of function severely affects development

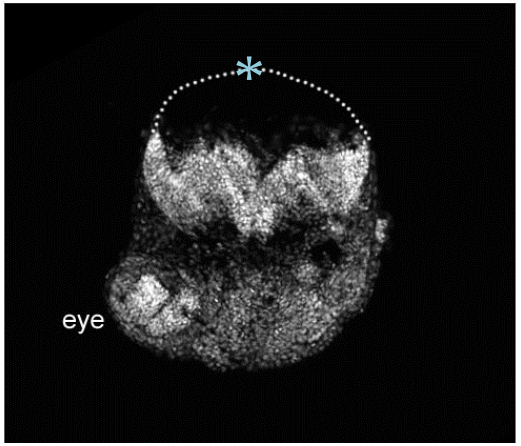
- 3dpf morphant larvae are short, with a bent tail and display impaired mobility
- Normal ventral retinal fusion is prevented



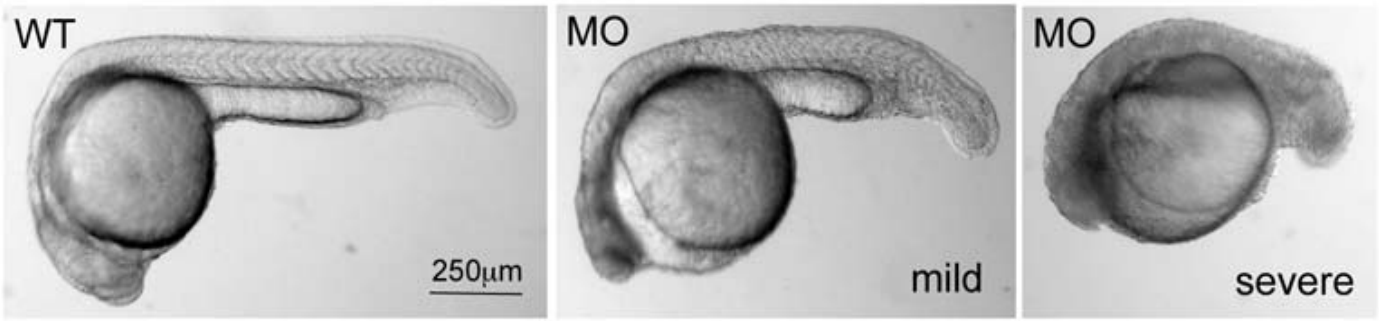
gtdc2 morphants also show severe hydrocephalus

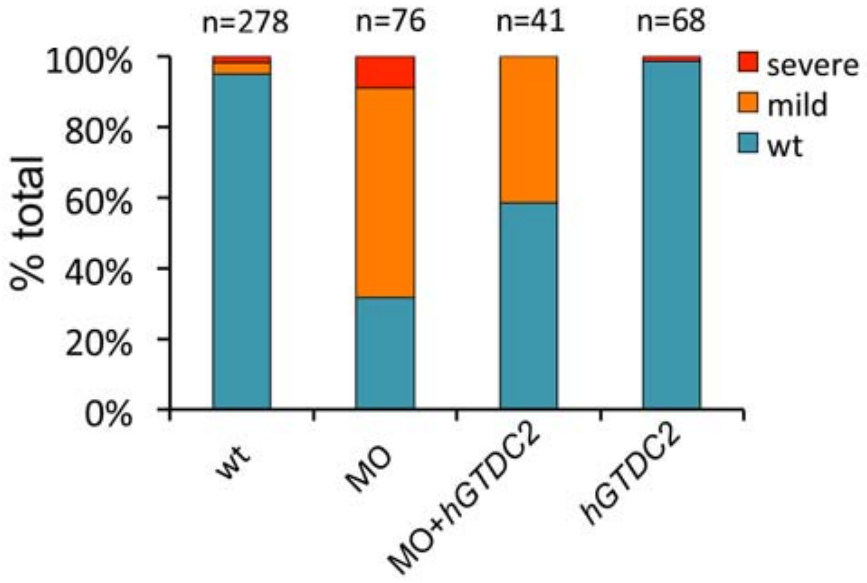






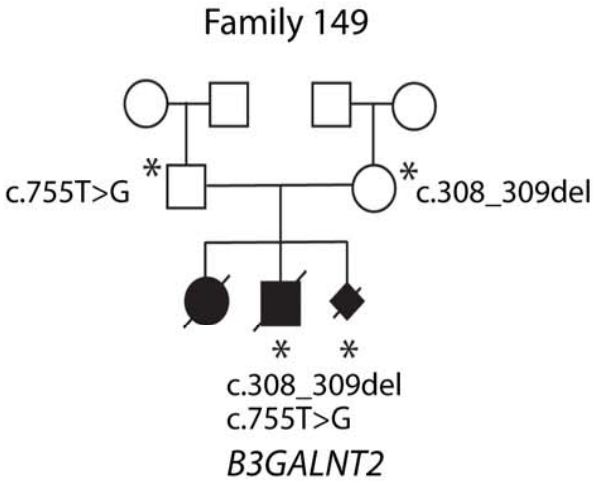
Human *GTDC2* mRNA rescues the morphant phenotype





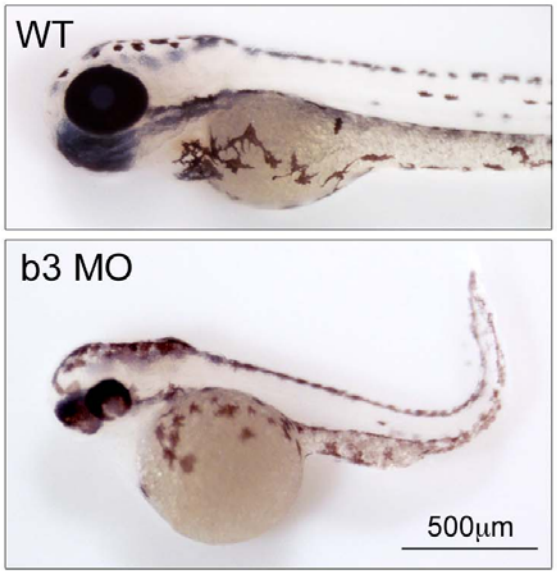
Non-consanguineous families can also be easily studied

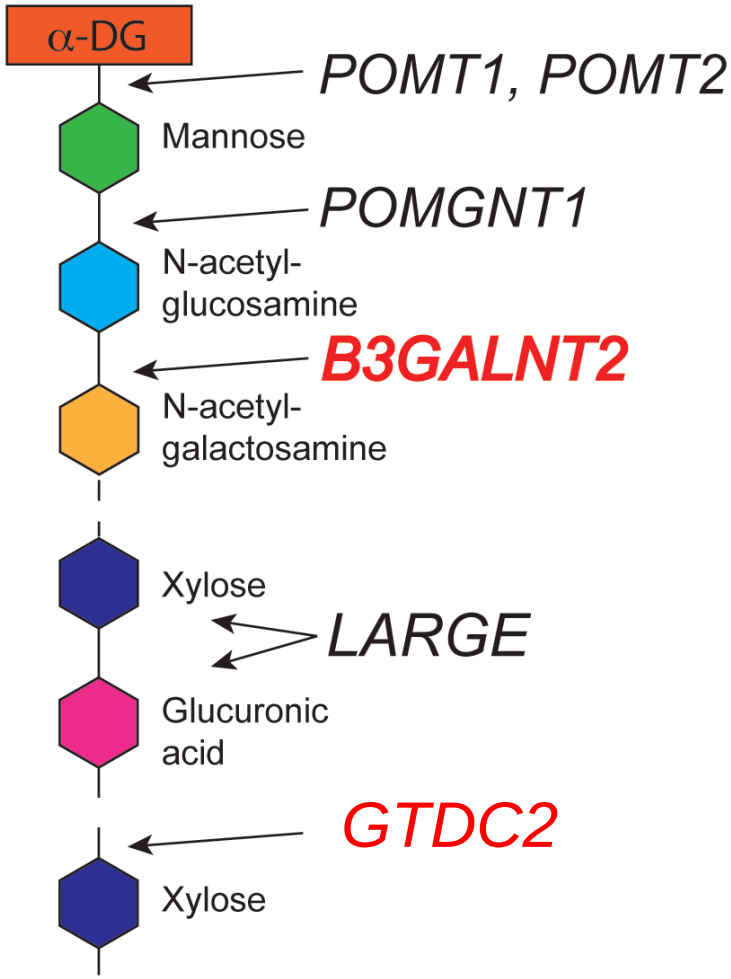
- Unrelated parents: two children and one fetus affected with WWS
- Only one gene with compound heterozygous mutations shared by all affected children: *B3GALNT2*
- Three additional families with alleles in the same gene



B3GALNT2 is another new dystroglycanopathy gene

- *B3GALNT2* is another glycosyl-transferase to add to the puzzle
- Zebrafish MO knockdown analysis is consistent with the findings from other dystroglycanopathies





Conclusions: Combination of human genetics and zebrafish validation to identify novel disease genes

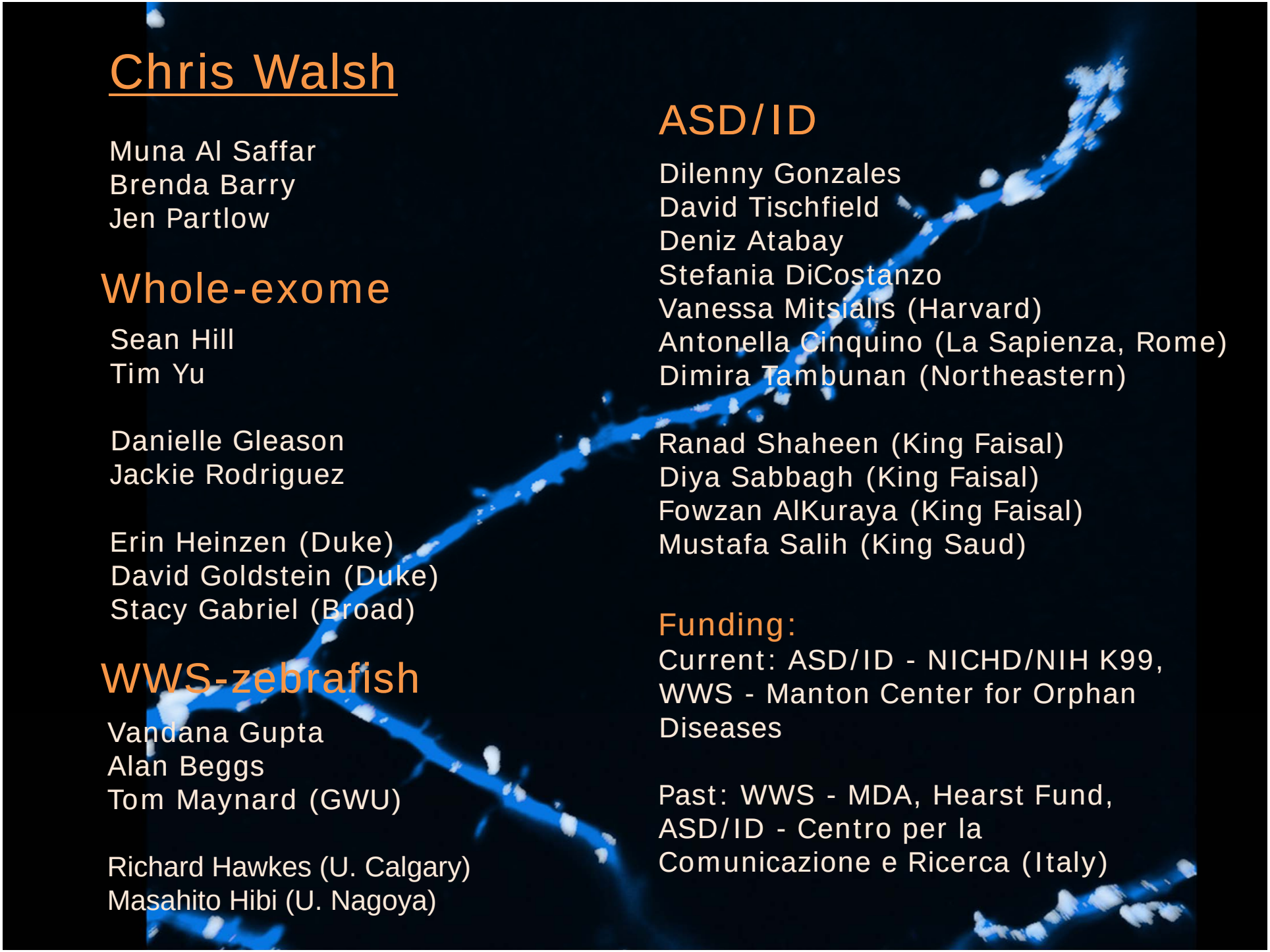
- Homozygosity mapping and WES in consanguineous families can identify disease-causing mutations in rare, severe disorders

- WES is effective even in non-consanguineous unions with multiple affected children

- The zebrafish embryo is a rapid validation model for candidate mutations: muscle, eye and brain phenotypes are comparable to the ones observed in human patients

- *GTDC2* and *B3GALNT2* are novel dystroglycanopathy genes identified using this approach

- Multiple new candidates can be tested in this fashion



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Whole-exome

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WWS-zebrafish

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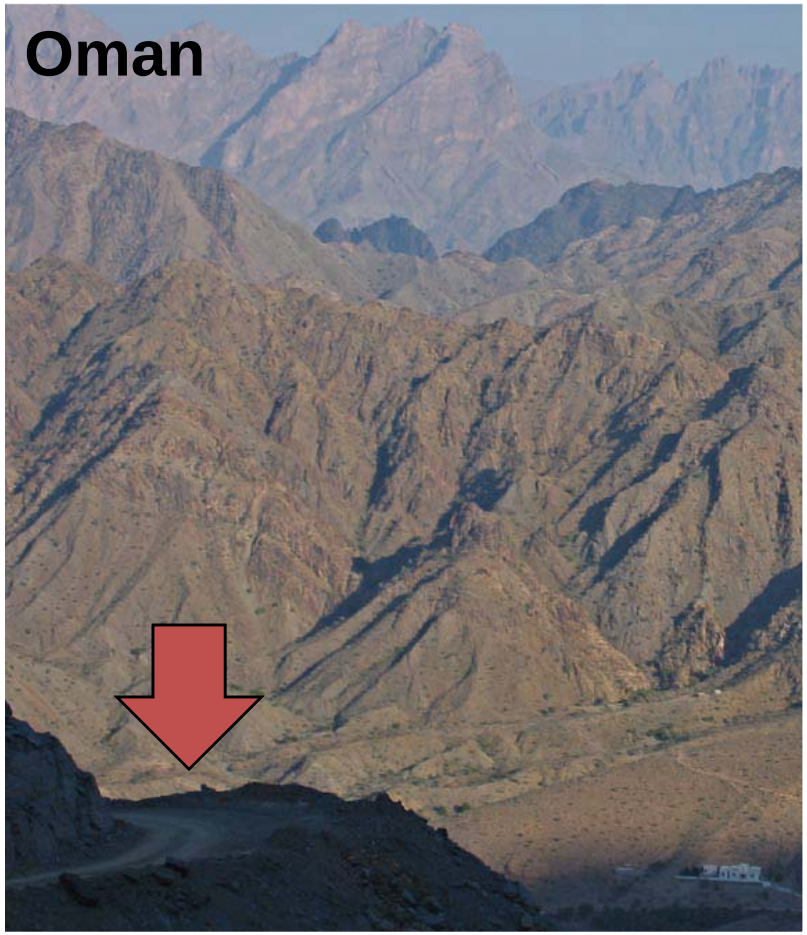
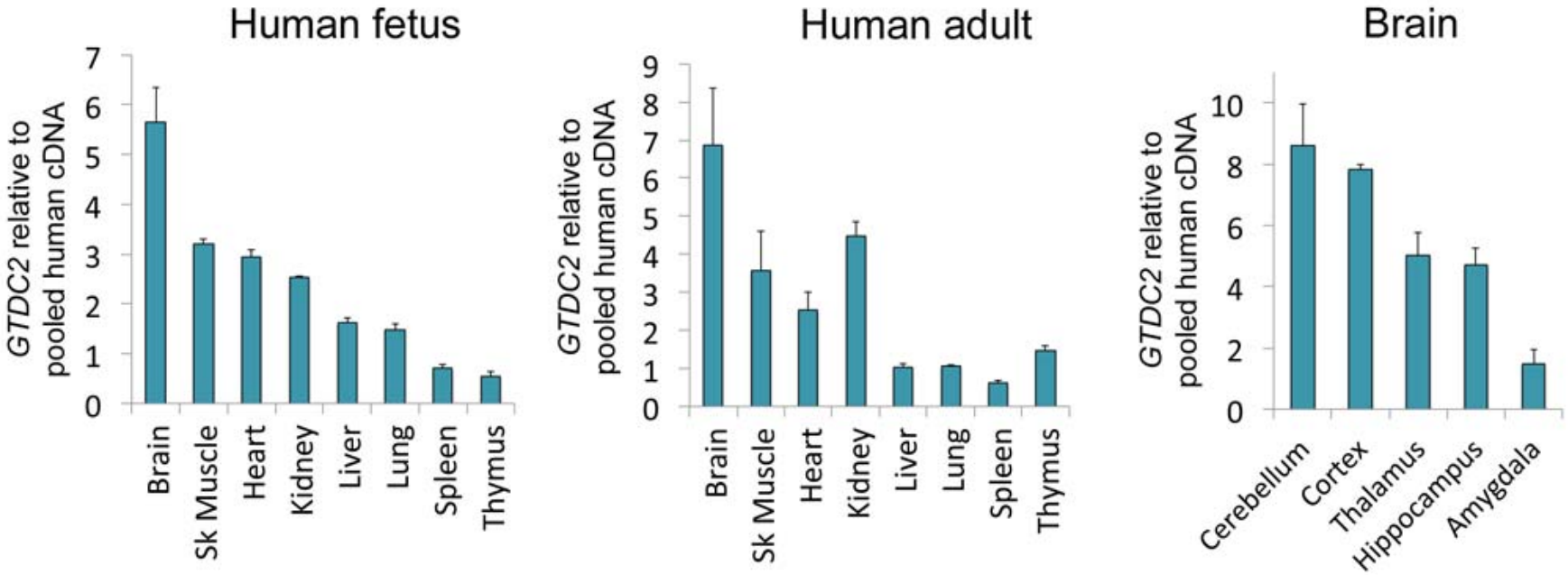
Our clinical team



CHB Team: Ganesh Mochida, Ramzi Nasir, Muna Al Saffar, Jen Partlow, Wen-Hann Tan

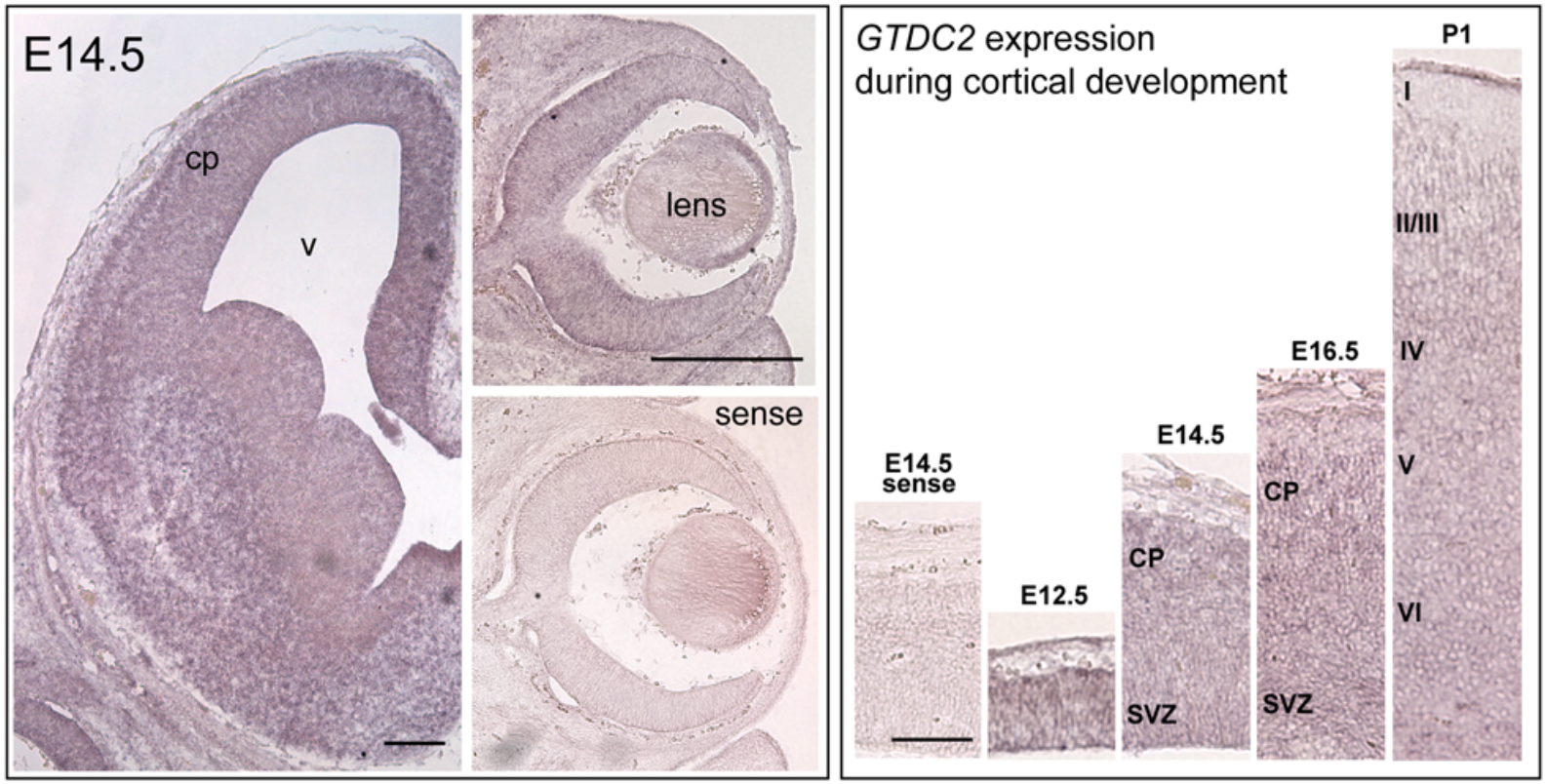
GTDC2 is expressed in the brain and muscle during human development

- high brain and muscle expression in fetal and adult tissues
- highly expressed in the heart and kidneys, which are also affected in the patients



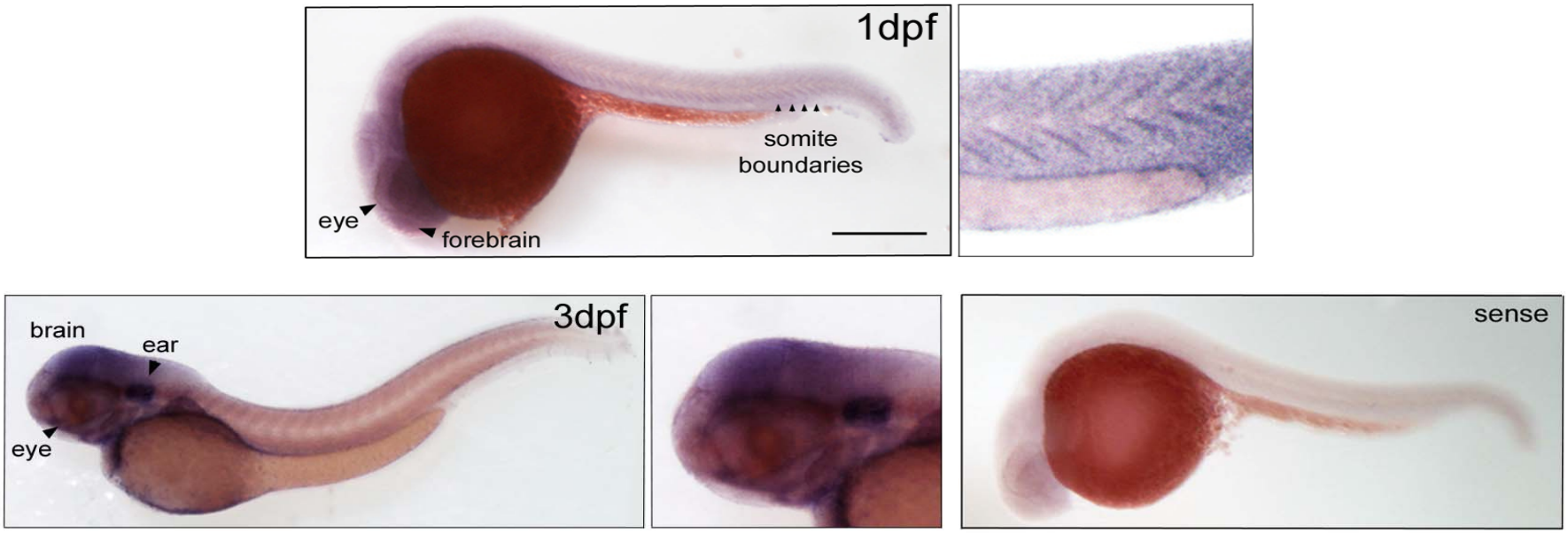
Gtdc2 is expressed in the brain and eye during mouse development

- high brain expression during embryonic development
- expressed in the eye, in the retina, lens and cornea
- cortical expression is highest during proliferation and migration

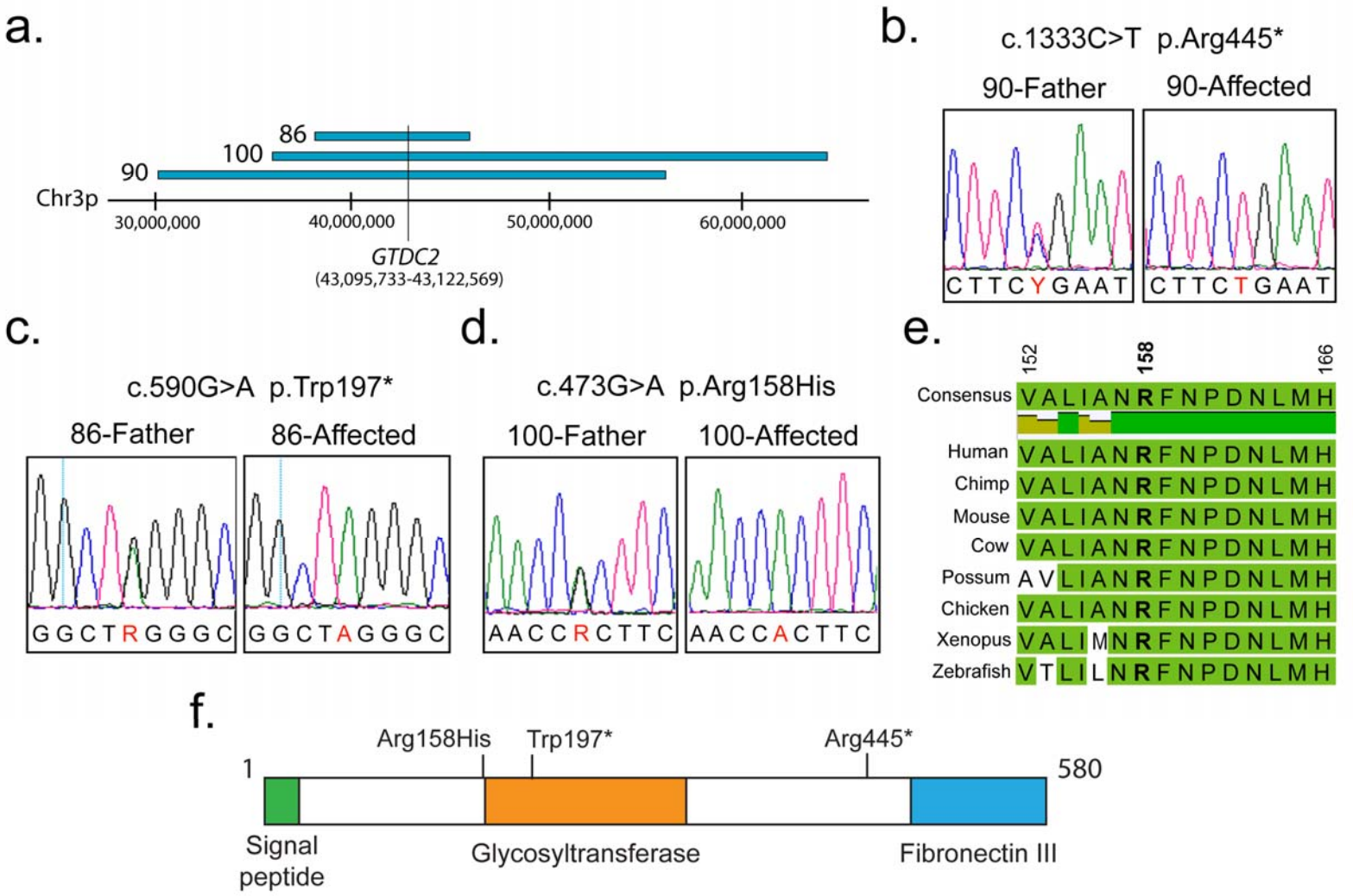


gtdc2 mRNA is highly expressed in the brain, eye and muscle

- *gtdc2* is expressed throughout development and mostly in the brain, eye, and somite boundaries at 1dpf
- at 3dpf highest expression is in the brain, eye and ear

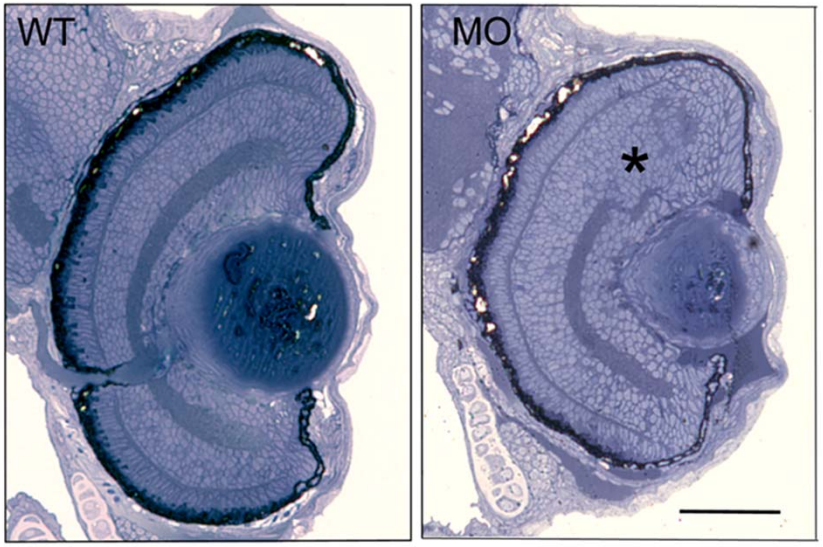


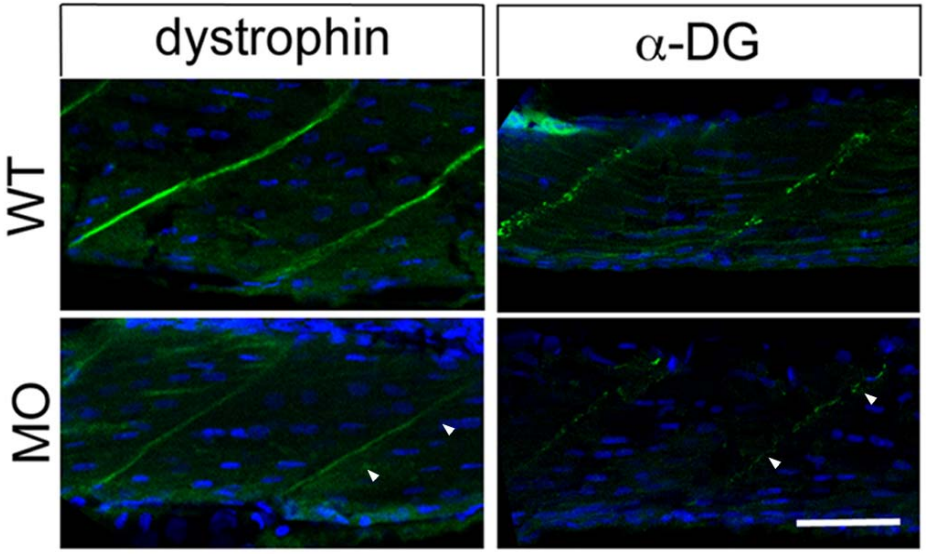
Three independent alleles in *GTDC2* in our patient cohort



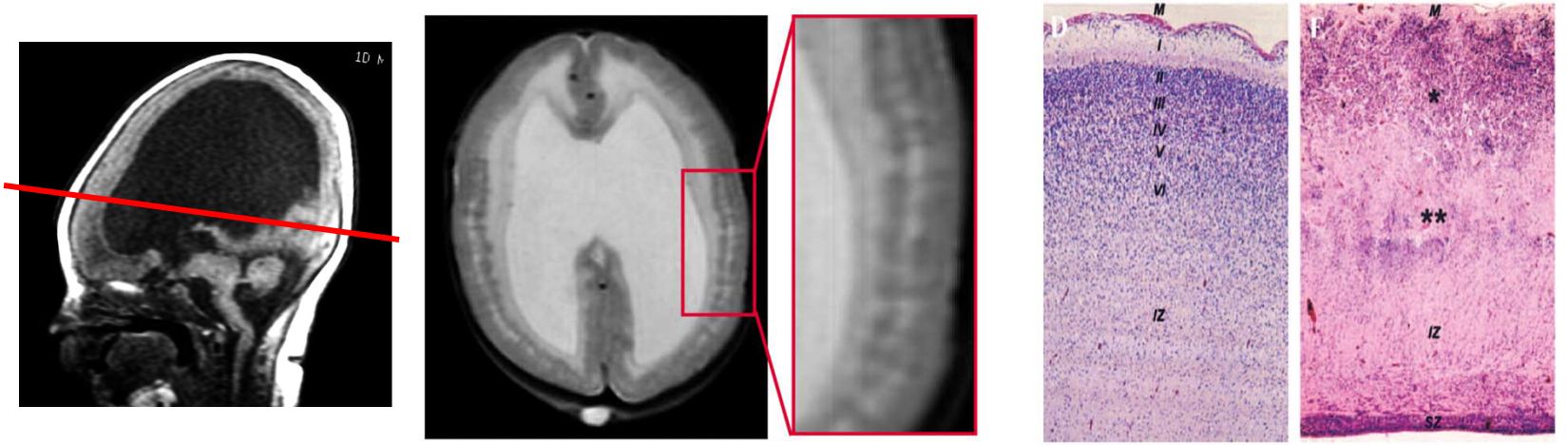
gtdc2 loss of function disrupts organization of the retina and the muscle

- Retinal lamination defects are observed in *gtdc2* morphant eyes
- Both dystrophin and glycosylated dystroglycan are greatly reduced at the myosepta

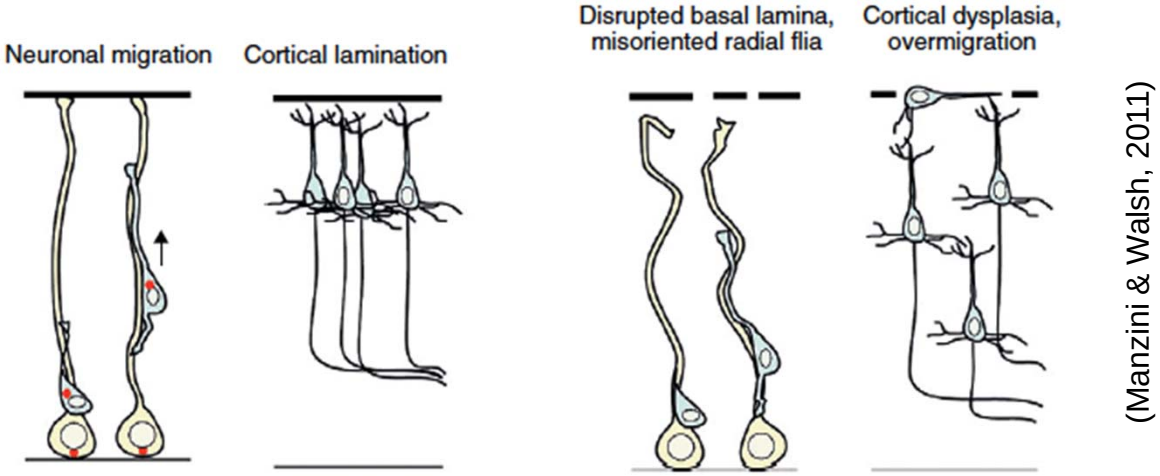




Neuronal migration and differentiation are severely disrupted



(Beltran-Valero et al, 2002)



(Manzini & Walsh, 2011)

Post-doctoral positions available

Manzini lab
The George Washington University Medical Center
Department of Pharmacology and Physiology
Department of Integrative Systems Biology
The Institute of Neuroscience

Position is fully funded for 2 years starting summer of 2013

Topics:

- The ECM in neuromuscular disorders
- Role of endosomal trafficking in cognitive development

Email CV and reference information to:
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How do you approach a heterogeneous disorder?

- Collect a large cohort of patients with accurate clinical description
- Study the frequency and distribution of mutations in known genes to provide genetic testing guidelines
- Study families with no mutations in known genes to identify novel disease causes

Summary: WES identifies *GTDC2* as a new WWS gene

- WES can rapidly provide a molecular diagnosis for genetic disorders
- Several patients in our cohort had mutations in known disease genes changing the diagnosis or expanding the phenotypic spectrum
- We identified *GTDC2* as a novel WWS gene
- In consanguineous families an average of 5 variants per family is identified
- **A validation plan must be in place to analyze the function of these variants**