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Supplemental Data

Exome Sequencing and Functional Validation in Zebrafish Identify *GTDC2* Mutations as a Cause of Walker-Warburg Syndrome

M. Chiara Manzini, Dimira E. Tambunan, R. Sean Hill, Tim W. Yu, Thomas M. Maynard, Erin L. Heinzen, Kevin V. Shianna, Christine R. Stevens, Jennifer N. Partlow, Brenda J. Barry, Jacqueline Rodriguez, Vandana A. Gupta, Abdel-Karim Al-Qudah, Wafaa M. Eyaid, Jan M. Friedman, Mustafa A. Salih, Robin Clark, Isabella Moroni, Marina Mora, Alan H. Beggs, Stacey B. Gabriel, and Christopher A. Walsh

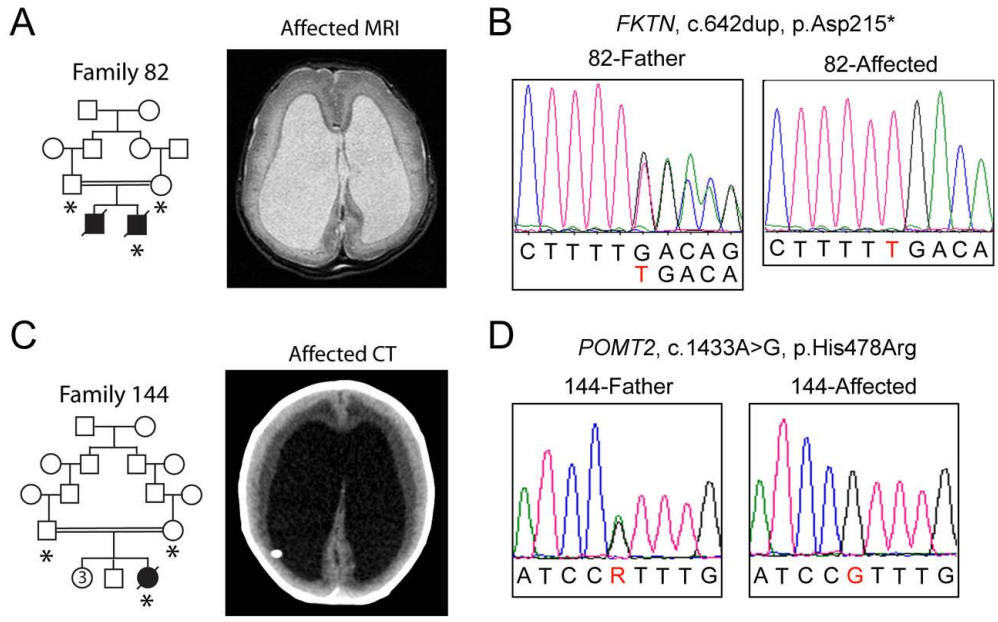

Figure S1. Alleles in *FKTN* and *POMT2* Identified by WES in Individuals Affected by WWS

(A) Pedigree structure of family 82 (DNA was available for individuals with asterisks) and axial MRI image of the propositus showing hydrocephalus and cortical dysplasia.

(B) Sanger sequencing confirmation of the frameshift *FKTN* mutation in the father and the affected individual.

(C) Pedigree structure of family 144 and an axial CT image of the propositus also displaying severe hydrocephalus and thin cortical mantle. The shunt inserted for draining excess cerebrospinal fluid appears as a bright spot on the lower left.

(D) Sanger sequencing confirmation of the novel *POMT2* mutations identified in this family.

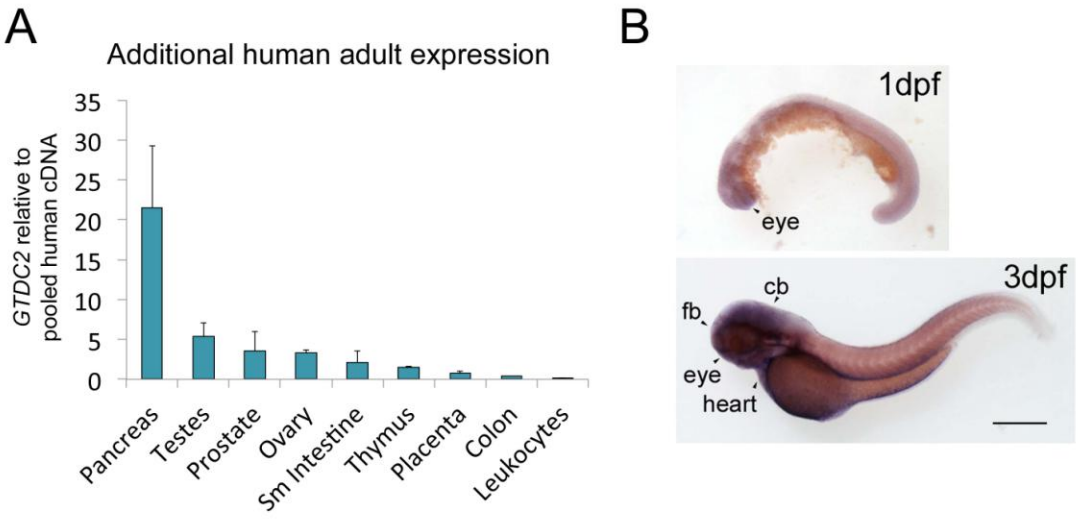

Figure S2. Additional *GTDC2* Expression in Human and Zebrafish

(A) Additional qPCR analysis of *GTDC2* expression in adult human tissues. Highest relative expression was identified in the pancreas and expression levels comparable to those observed in the brain and muscle were also present in the reproductive organs.

(B) Zebrafish *gtdc2* expression is observed throughout the body at 16 hr post fertilization and strongly in the brain (fb = forebrain, cb = cerebellum), eye and heart at 3 dpf. Scale bar: 500 µm.

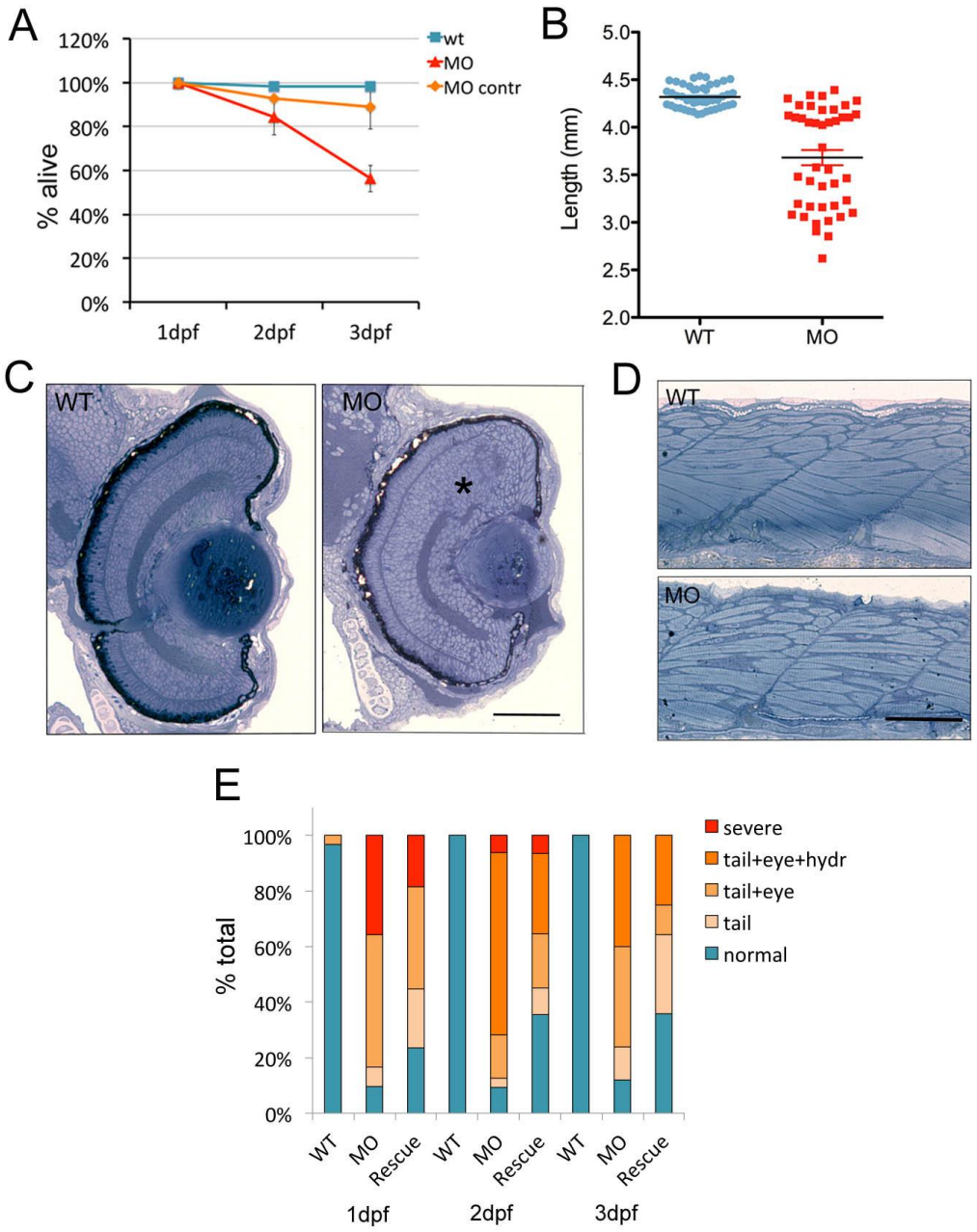

Figure S3. Analysis of the Zebrafish *gtdc2* Morphant Embryos and Larvae

(A) Reduced survival is observed during the first three days post injection in the MO injected embryos when compared to wild-type (wt) and embryos injected with a control MO.

(B) Body length is variable and significantly reduced in 3 dpf *gtdc2* morphants compared to wild-type.

(C–D) Toluidine blue staining shows severe disruptions in organization of the retinal epithelium (asterisk in C; Scale bar: 100 µm) and disorganization of muscle fibers in the *gtdc2* morphants (scale bar in D represents 50 µm).

(E) Developmental analysis of different phenotypes observed in *gtdc2* morphants during the first 3 dpf. Embryos were assigned to different groups depending on which areas were affected: tail = slow moving tail with U-shaped somite and disorganized looking muscle fibers, normal eye and head; tail + eye = affected tail, plus small eye and/or failure of ventral retinal to fuse; tail + eye + hydr = affected tail and eye, plus evident hydrocephalus; severe= stunted tail, no discernible eye and head structures. Rescue with the human *GTDC2* mRNA showed an improvement in the more severe phenotypes. The disappearance of the severe category is due to the death of these embryos between 2 and 3 dpf. Four independent rescue experiments were performed with similar results.

Table S1. Summary of Consanguineous WWS Cohort

Family	Relationship	Affected[a]	Homozygosity	WES	Gene	Reference
3	1st cousin	1	5.9%	Y		
5	2nd cousin	1 (2)	12.8%			
12	1st cousin	1	7.6%		*POMT2*	(6) Fam 7
21	3rd cousin	1	16.4%	Y		
66	unknown	1	7.8%		*FKTN*	(6) Fam 13
70	1st cousin	1 (2)	12.5%			
82	1st cousin	1 (1)	7.4%	Y	*FKTN*	This study
90	1st cousin	1	11.2%	Y	*GTDC2*	This study
94	3rd cousin	1	3.7%			
96	1st cousin	1 (1)	11.2%		*POMT1*	(6) Fam 4
100	1st cousin	1	10.6%	Y	*GTDC2*	This study
120	1st cousin	1 (1)	n/a		*POMT1*	(6) Fam 5
132	1st cousin	2	3.2%		*POMT1*	(6) Fam 6
139	1st cousin	1	9.7%		*POMT1*	(6) Fam 1
140	1st cousin	1	9.9%		*POMT2*	(6) Fam 9
141	1st cousin	1	12.6%		*FKRP*	(6) Fam 11
143	1st cousin	2	n/a		*POMT1*	(6) Fam 2
144	2nd cousin	1	5.3%	Y	*POMT2*	This study
145	1st cousin	2	7.4%	Y		

WES = whole-exome sequencing

[a] in parentheses: additional affected individuals for whom DNA is not available

Table S2. Whole-Exome-Sequencing Statistics

Family	Total Variants	In ROH	Pathogenic/Rare	Correct Inheritance	Missense	Truncating (stop,spl,fs)	Gene
3	56,041	5,040	10	5	5	0	
21	64,860	2,669	2	0	0	0	
82	36,618	1,622	11	9	8	1 fs	*FKTN*
90	34,322	1,866	6	6	4	1 stop, 1 fs	*GTDC2*
100	55,951	8,603	17	5	5	0	*GTDC2*
144	63,080	4,656	16	9	7	1 stop, 1 fs	*POMT2*
145	36,679	602	4	2	2	0	

spl = splicing, fs = frameshift