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Supplemental Data

Monoallelic and Biallelic Mutations in *MAB21L2* Cause a Spectrum of Major Eye Malformations

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Supplemental Data

Figure S1 Alignment of MAB21L2 orthologs

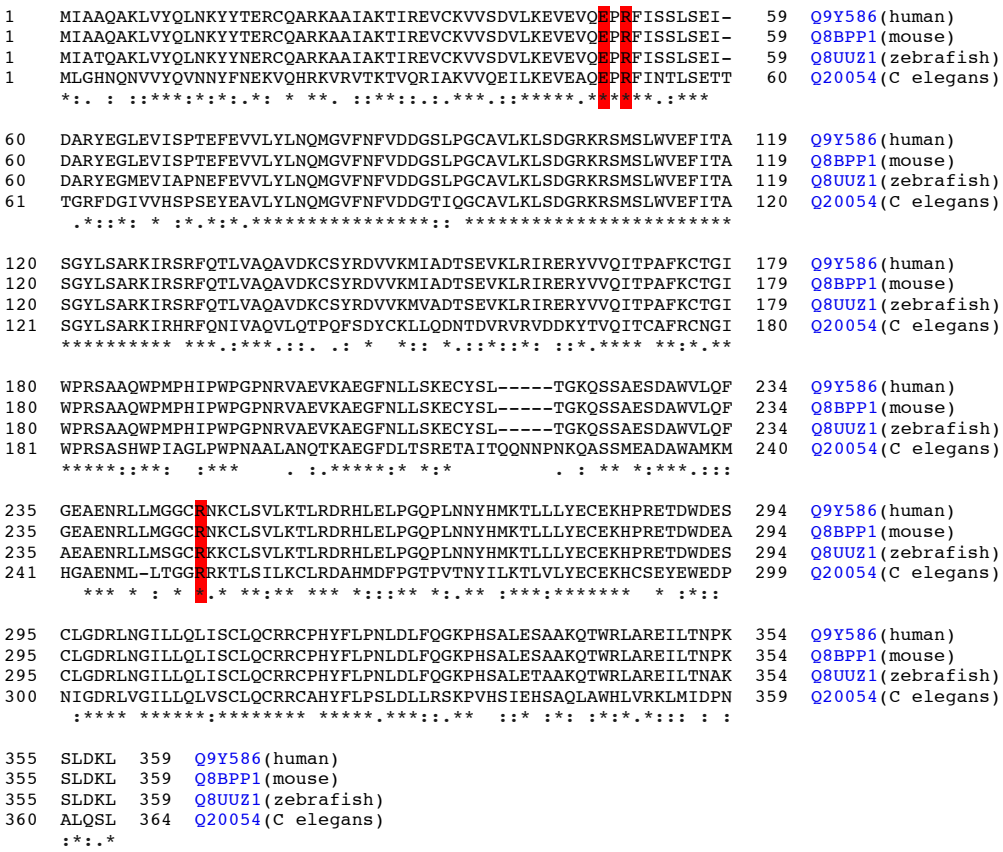

Figure S1 legend: Alignment of orthologous MAB21L2 proteins from human, mouse, zebrafish and C elegans showing cross species conservation of primary amino acid sequence. The residues in the sequences that are equivalent to those mutated in the human protein are indicated by the red highlight. The UniProt accession codes for each of the proteins is given to the right of the alignment. The alignment was performed on the UniProt web site using the default parameters.

Figure S2 MAB21L2 lacks Nucleotidyl Transferase activity

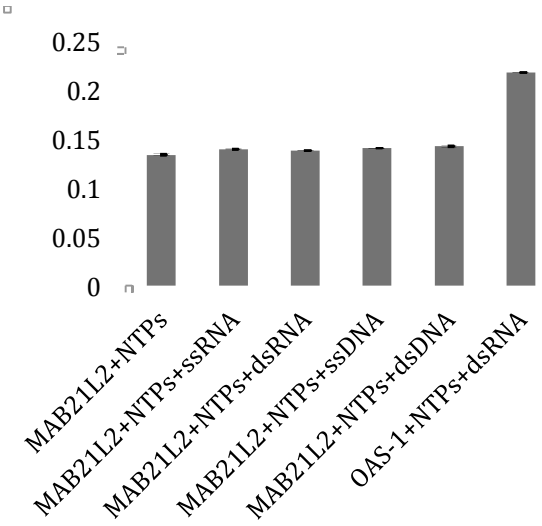

Figure S2 legend: A graph showing the absence of nuceotidyl transferase activity in MAB21L2 purified protein. OAS protein purified in the same way as MAB21L2 is a positive control and when incubated with an equal mixture of NTP (ATP, CTP, GTP, UTP) and double-stranded RNA (dsRNA) significant pyrophosphate release is detected indicating nucleotidyl transferase activity. MAB21L2 showed no activity above background with NTPs using dsRNA, dsDNA, single stranded RNA (ssRNA) or ssDNA as an activator. The error bars represent standard errors.

Figure S3

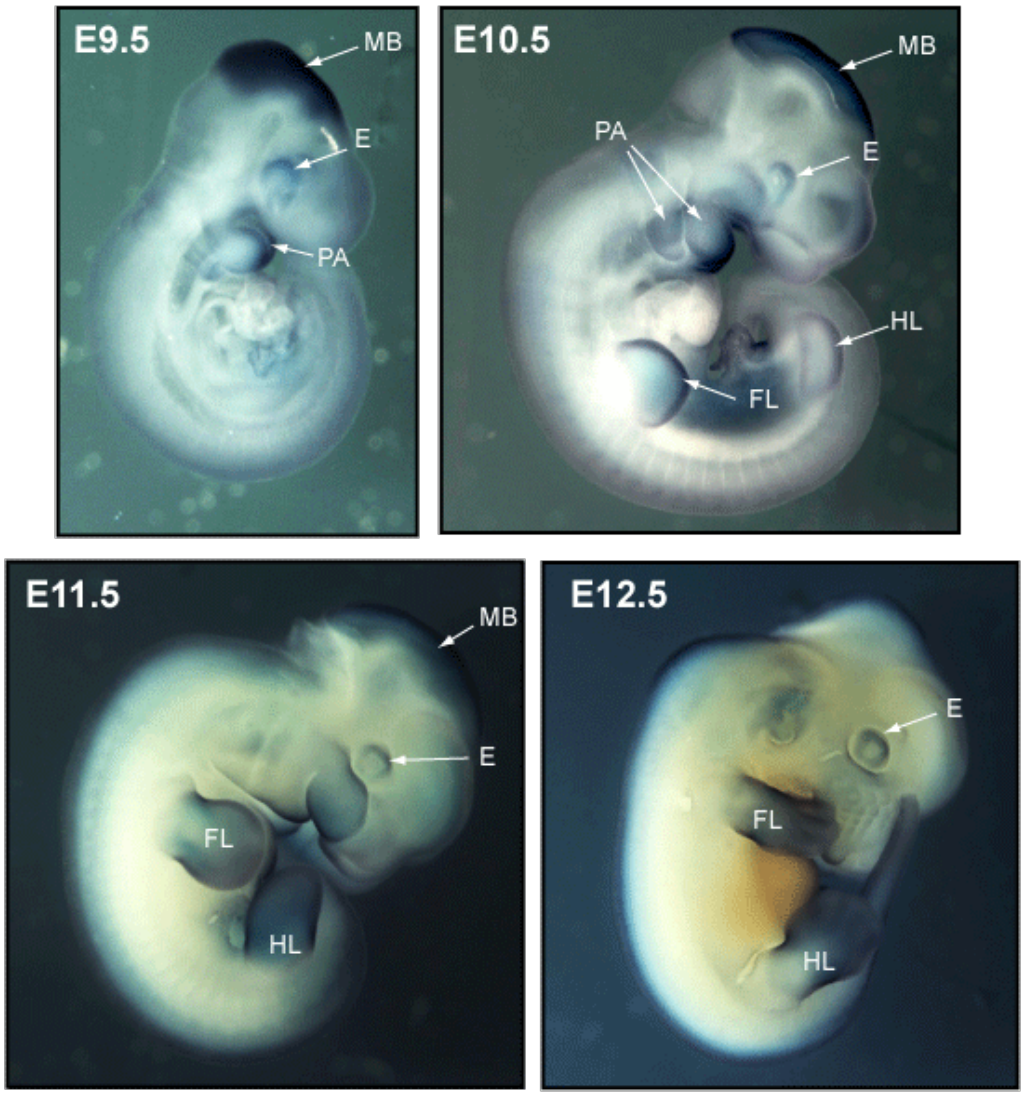

Figure S3. Developmental expression of *Mab21L2* in early mouse embryos at embryonic stage (E)9.5, E10.5, E11.5 and E12.5 by whole mount *in situ* hybridization showed specific expression domains at all stages. In particular, transcripts were identified in the developing eyes (E), forelimbs (FL), hindlimbs (HL), pharyngeal arches (PA) and midbrain (MB) regions. Midbrain expression was particularly strong at E9.5-E10.5.

Figure S4

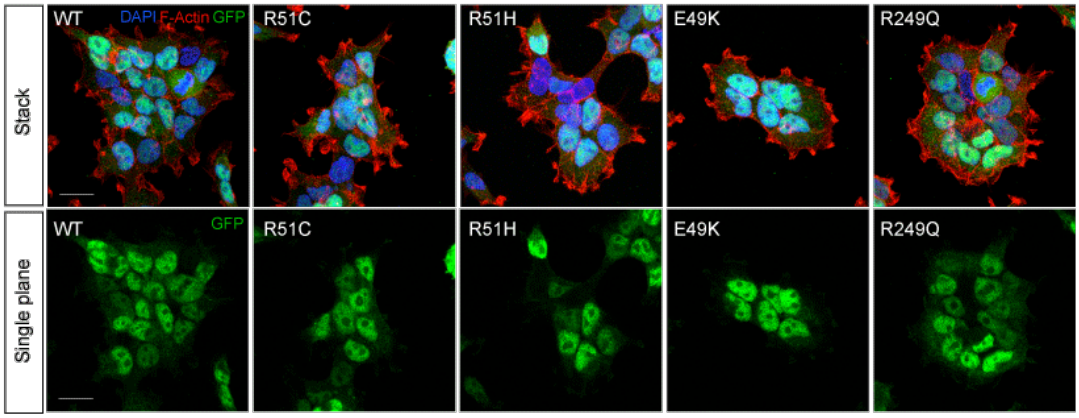

Figure S4: Immunofluorescence analyses with stacked (top) and single-plane (bottom) confocal microscopy images of MAB21L2-GFP stable cell lines indicated predominantly nuclear localization of WT and mutant MAB21L2-GFP (green) alleles with anti-GFP antibody (Clonetech Living Colours JL-8). Filamentous actin was stained red (Alexa Fluor 555-Phalloidin, Life Technologies) and DAPI (blue) was used to counterstained nuclei. Scale bar = 20 µm.

Table S1 Shared Heterozygous Variants in Family 1463 and Homozygous Variants in Family 4468

ID	Gene	genomic	mut	Genotype	Consequence	SIFT	PolyPhen
1463	ABCA6	chr17 g.67084341C>A	1222G>V	0/1	Missense	deleterious(0.01)	probably_damaging(0.998)
1463	ANKRD50	chr4 g.125592016C>A	806A>S	0/1	Missense	tolerated(0.89)	possibly_damaging(0.888)
1463	AURKB	chr17 g.8109861C>G	212G>R	0/1	Missense	deleterious(0.01)	probably_damaging(1)
1463	CCDC87	chr11 g.66359810T>C	226N>S	0/1	Missense	tolerated(0.25)	benign(0.002)
1463	CDH13	chr16 g.82892049A>C	90E>A	0/1	Missense	tolerated(0.19)	possibly_damaging(0.913)
1463	CHRNE	chr17 g.4805555G>C	101P>A	0/1	Missense	deleterious(0.01)	probably_damaging(0.998)
1463	CTU2	chr16 g.88781064C>T	424T>I	0/1	Missense	tolerated(0.19)	benign(0)
1463	FAM196B	chr5 g.169310185G>A	240R>C	0/1	Missense	tolerated(0.06)	benign(0)
1463	FRMPD1	chr9 g.37740753C>T	743T>I	0/1	Missense	tolerated(0.05)	benign(0.059)
1463	HMGB2	chr4 g.174253258CTCATCT>C	199-201EDE>E	0/1	In-frame deletion		
1463	IFT122	chr3 g.129207162CTTGG>C	690-691	0/1	Frameshift coding		
1463	IFT122	chr3 g.129207157GCTT>G	688-689AC>G	0/1	In-frame deletion		
1463	IFT122	chr3 g.129207169GTCACAGAC>G	692-694	0/1	Frameshift coding		
1463	IL12RB1	chr19 g.18174698G>T	536R>S	0/1	Missense	deleterious(0.05)	possibly_damaging(0.487)
1463	LIN9	chr1 g.226496911C>T	127R>H	0/1	Missense	deleterious(0.01)	unknown(0)
1463	MAB21L2	chr4 g.151504333G>A	51R>H	0/1	Missense	deleterious(0)	probably_damaging(1)
1463	METTL4	chr18 g.2544214G>A	418S>L	0/1	Missense	deleterious(0)	probably_damaging(1)
1463	MUC4	chr3 g.195512179G>A	2091	0/1	Possible Missense		
1463	MVD	chr16 g.88723943G>C	102L>V	0/1	Missense	tolerated(0.3)	benign(0)
1463	NEB	chr2 g.152522837C>T	1600V>I	0/1	Missense	tolerated(0.25)	probably_damaging(0.971)
1463	NLRC4	chr2 g.32476662G>T	91H>N	0/1	Missense	tolerated(0.35)	benign(0.073)
1463	PSMB5	chr14 g.23503876G>C	72	0/1	Possible Missense		
1463	PTCHD3	chr10 g.27703152G>T	10P>T	0/1	Missense		benign(0)
1463	SLC9A9	chr3 g.143551039C>G	67R>P	0/1	Missense	deleterious(0)	probably_damaging(0.999)
1463	SLCO2B1	chr11 g.74873700G>C	6G>A	0/1	Missense	deleterious(0)	probably_damaging(0.987)
1463	SPON1	chr11 g.14279384G>A	477V>I	0/1	Missense	tolerated(0.38)	possibly_damaging(0.619)
1463	TMEM116	chr12 g.112371748G>C	133H>Q	0/1	Missense	tolerated(0.43)	benign(0.174)
4468	LTB4R2	chr14 g.24780056GCA>G	N149fs	1/1	frameshift_deletion		
4468	MAB21L2	chr4 g.151504921	R247Q	1/1	Missense	deleterious(0.97)**	probably_damaging(1)
4468	NXF3	chrX g.102332646	V494L	1	Missense		neutral (0.24)
4468	TMPRSS9	chr19 g.2424094	G818fs	1/1	frameshift_insertion		

1/1 = homozygous; 1/0 = heterozygous; 1 = hemizygous; ** dbNSFP v1.3 annotates this variant with a SIFT Score (1-SIFT) of 0.97 and a prediction "Deleterious". dbNSFP v2.0 and dbNSFP v2.3 annotate this variant with a SIFT score of 0.14 (1-SIFT score = 0.86) and a "Tolerated" prediction. The current web version of SIFT (http://provean.jcvi.org/genome_submit.php) gives a SIFT score of 0.000 with a "Damaging" prediction and a PROVEAN score of -2.812 with a "Deleterious" prediction. Mutation taster and Polyphen2 predicted "disease causing" and "probably damaging", respectively.

Table S2: Clinical Features and Genotypes of Individuals with *MAB21L2* mutations

Family	131		1463		676		4480		4468			
Proband(s)	II.1		III.1		II.1		II.1		II.1		II.2	
chr4 genomic variant hg19	g.151504326G>A		g,151504333G>A		g,151504332C>T		g,151504332C>T		g.151504921G>A			
Mutation	c.145G>A p.(Glu49Lys)		c.152G>A p.(Arg51His)		c.151C>T p.(Arg51Cys)		c.151C>T p.(Arg51Cys)		c.[740G>A];[740G>A] p.[(Arg247Gln)];[(Arg247Gln)]			
Inheritance	unknown		paternal		de novo		de novo		maternal & paternal – parents have normal eyes			
Sex	Male		Male		Female		Male		Male		Male	
Genotype	heterozygous		heterozygous		heterozygous		heterozygous		homozygous		homozygous	
Birth Weight [z score]	NR		3033g @ 40 [-1.1]		3260g@38		3360 g		NR		3900g @ 40 [+0.88]	
Maternal/Paternal Age at birth	33/37		24/30		NR/NR		30/33		18/NR		20/NR	
Age at last assessment	39 y		13 y		10 y		24 y		5y		3y	
Height[z score]	NR		154.9cm [-0.29]		100cm[-6.7]		121 cm [-8.3]		112cm [-0.07]		97cm [+0.44]	
Weight[z score]	NR		63.5kg [+1.73]		NR		52 kg [-2.9]		17.5kg [-0.92]		13.8kg [-0.55]	
OFC[z score]	60 cm [+1.8]		NR		57 cm [+2.59 SD]		55 cm [-1.1]		51cm [-1.2]		48cm [-2.4]	
Eye	R	L	R	L	R	L	R	L	R	L	R	L
Anophthalmia	-	-	-	-	+	+	+	+	-	-	-	-
Microphthalmia	+	+	+	+	N/A	N/A	N/A	N/A	-	-	-	-
Coloboma	+	+	+	?	N/A	N/A	N/A	N/A	+	+	+	+
Microcornea	+	+	+	?	N/A	N/A	N/A	N/A	-	-	-	-
Sclerocornea	-	-	-	+	N/A	N/A	N/A	N/A	-	-	-	-
Vision	None	None	6/60	None	None	None	None	None	Yes	Yes	Yes	Yes
Skeletal												
Rhizomelia	No		No		Severe Bilateral		Arms & legs		None		None	
Joint contractures	No		Knees & Hips Bilaterally		All large joints		All large joints		None		None	
Hypoplastic femoral condyles	Unknown		Bilateral				Yes		None		None	
Details	Recurrent patella dislocations, 3/4 syndactyly of hands, 2/3 syndactyly of feet		Bowing of both legs noted as infant, calf wasting and pes planus		Short truck, normal sized hands and feet		Short humeri and femora,short tibiae, thin radius		None		Mild shortness of the long bones with decreased tubulation	
Other												
Hypospadias	No		+		N/A		No		No		No	
Undescended testes	Bilateral		No		N/A		No		No		No	
Precocious Puberty	No		No		7 years		No		No		No	
Intellectual disability	None		None		Moderate ID with autistic spectrum disorder		Moderate		None		None	
Other features									Strabismus on left, facial dysmorphism		Strabismus on right, facial dysmorphism	

NR = not recorded; N/A = not applicable; + = feature present; - = feature not present

Table S3: List of candidate genes used in UK10K exome analysis:

Inclusion criteria for this were; site- and stage-specific expression during early eye development in mouse embryos AND/OR the observation of a major eye malformation in any vertebrate animal model AND/OR mutation identification in humans with major eye malformations.

Gene symbol	Gene name	MIM number
ATOH1 (*MATH1*)	atonal homolog 1 (Drosophila)	601461
ATOH7 (*MATH5*)	atonal homolog 7 (Drosophila)	609875
BMP4	bone morphogenetic protein 4	112262
BMP7	bone morphogenetic protein 7	112267
FGF10	fibroblast growth factor 10	602115
FGF19 (*Fgf15*)	fibroblast growth factor 19	603891
HES1	hes family bHLH transcription factor 1	139605
HESX1	HESX homeobox 1	601802
LHX1	LIM homeobox 1	601999
MAB3: 21L1	mab-21-like 1 (C. elegans)	601280
MAB21L2	mab-21-like 2 (C. elegans)	604357
MAF1	MAF1 homolog (S. cerevisiae)	610210
mbx1	MADS-box transcription factor Mbx1	N/A (*S. pombe*)
NEUROG2 (*NGN2*)	neurogenin 2	606624
OTX1	orthodenticle homeobox 1	600036
OTX2	orthodenticle homeobox 2	600037
PAX2	paired box 2	167409
PAX6	paired box 6	607108
POU4F1	POU class 4 homeobox 1	601632
POU4F2	POU class 4 homeobox 2	113725
RAX	retina and anterior neural fold homeobox	601881
RXR: RXRA; *RXRB*; *RXRG*	retinoid X receptor, alpha; retinoid X receptor, beta; retinoid X receptor, gamma	180245; 180246; 180247
SOX1	SRY (sex determining region Y)-box 1	602148
SOX2	SRY (sex determining region Y)-box 2	184429
SOX3	SRY (sex determining region Y)-box 3	313430
SOX14	SRY (sex determining region Y)-box 14	604747
SOX21	SRY (sex determining region Y)-box 21	604974
SHH	sonic hedgehog	600725
SIX3	SIX homeobox 3	603714
SIX6	SIX homeobox 6	606326
STRA6	stimulated by retinoic acid 6	610745
TBX2	T-box 2	600747
TBX3	T-box 3	601621
TBX5	T-box 5	601620
VAX1	ventral anterior homeobox 1	604294
VAX2	ventral anterior homeobox 2	604295
VSX1	visual system homeobox 1	605020
VSX2 (*CHX10*)	visual system homeobox 2	142993

Table S4 Coverage and Depth of Exome Sequencing

Family	Median Depth	Coverage at >8X
4468	97-112x	90-93%
676	49-102X	>94%
1463	110-112X	>90%