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Supplemental Data

Mutations in *MED12* Cause X-Linked Ohdo Syndrome

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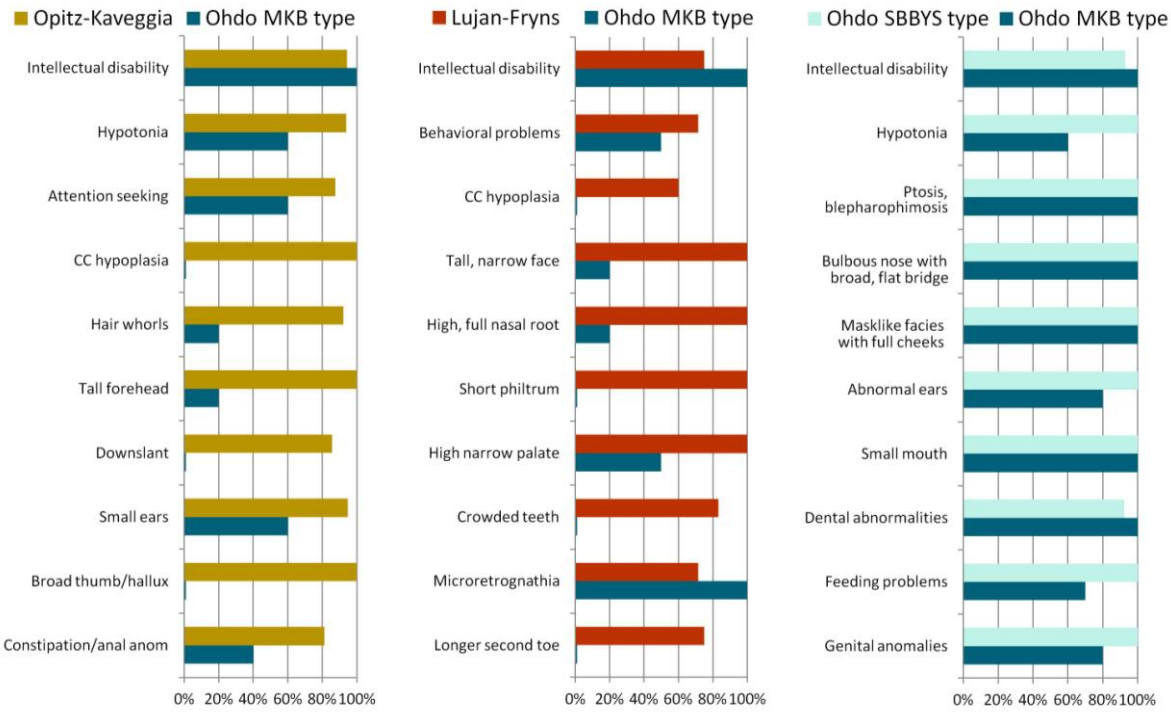

Figure S1. Phenotype Prevalence Plots

Phenotype prevalence plots of persons with Ohdo syndrome MKB type (green) for the ten most prevalent features of the other *MED12*-related disorders Opitz-Kaveggia syndrome[1] (yellow) and Lujan-Fryns syndrome[2] (red) and of Ohdo syndrome SBBYS type[3; 4] (blue). Persons with Ohdo syndrome MKB type show largest overlap to Ohdo syndrome SBBYS type.

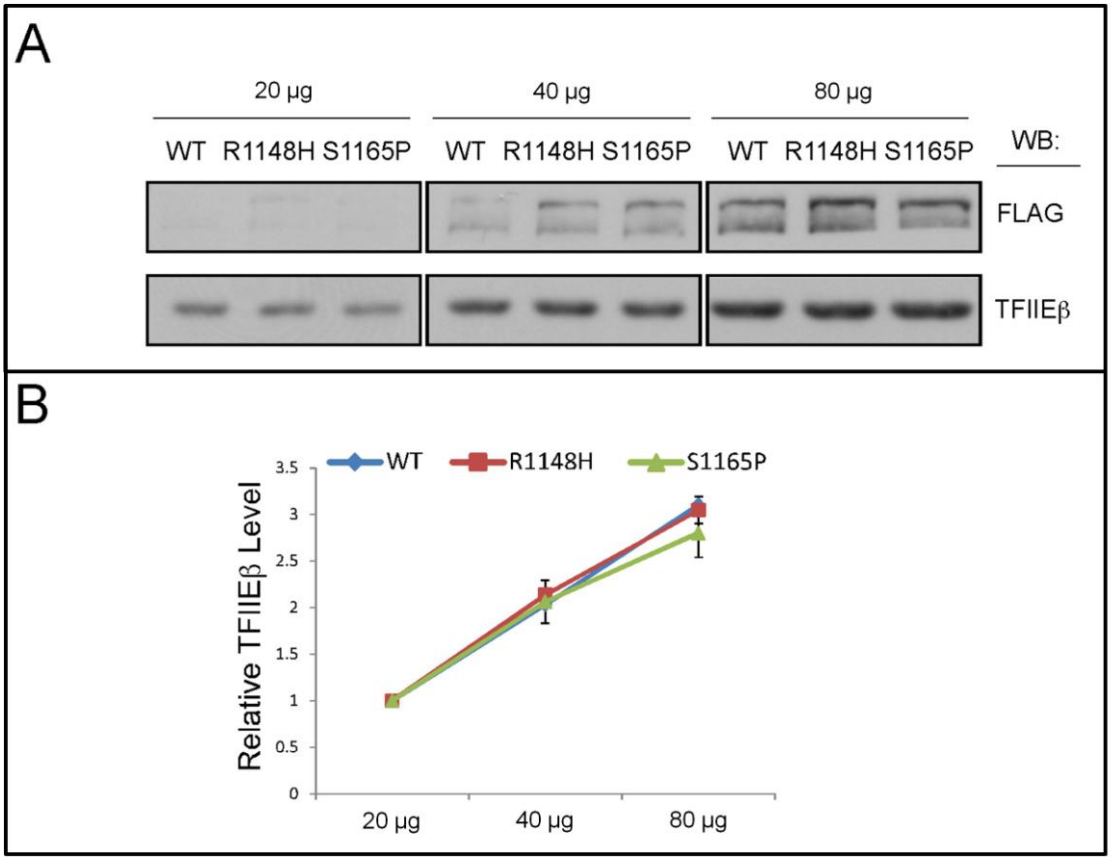

Figure S2. Quantification of Relative TFIIEβ Protein Expression Levels in Serially Diluted Nuclear Extracts from Four Independent Transient Expression Experiments

(A) Representative western blot (WB) of nuclear extracts from HEK293 cells transiently expressing FLAG-MED12 WT or its Ohdo syndrome mutant derivatives p.(Arg1148His) (R1148H) and p.(Ser1165Pro) (S1165P). MED12 was detected using antibodies specific for the FLAG epitope on FLAG-MED12 derivatives and TFIIEβ by antibodies directly directed against this protein.

(B) TFIIEβ levels were quantified using ImageQuant TL software and expressed relative to the level of TFIIEβ in 20 µg nuclear extract. Values represent the average +/- SEM of the four independent transient expression experiments. The linear range of TFIIEβ immunoblot signals occurs between 20 and 80 µg of nuclear extracts. To derive relative FLAG-MED12 WT and mutant protein levels shown in Figure 2, WB signals for FLAG-MED12 derivatives were quantified in 40 µg of nuclear extracts.

Table S1. Detailed Phenotype Description of Persons with Ohdo Syndrome MKB Type and Mutations in *MED12*

	Family	1		2		3
	Individual	III:1	II:3	II:1	II:2	II:1
	Mutation (NM_005120.2)	c.3443G>A		c.3493T>C		c.5185C>A
	Protein change (NP_005111.2)	p.(Arg1148His)		p.(Ser1165Pro)		p.(His1729Asn)
	Inheritance pattern	maternal		maternal		de novo
	Age	4 years	18 years	15 years	13 years	15 years
Growth	Birth weight	-1 SD	-1.3 SD	0 SD	ND	-3 SD
	Height	not short	0 SD	-2 SD	-2.5 SD	-4.5 SD
	Weight	-1.9 SD	-4.5 SD	0 SD	0/+1 SD	0 SD
	Head circumference	-0.3 SD	+0.7 SD	-0.5 SD	-1/-2 SD	-3 SD
Craniofacial	High, prominent forehead	-	high hairline	-	-	+
	Hair whorl/frontal hair upsweep	-	-	-	+	-
	Eyebrows	sparse	sparse	sparse	sparse	sparse
	Blepharophimosis	+	+	+	+	+
	Ptosis	+	+	+	+	+
	Epicanthus	+	+	+	+	+
	Downslanting palpebral fissures	-	-	-	-	-
	Hypertelorism/telecanthus	+	+	-	-	+
	Ocular abnormalities	N	N	S, Mi, H	Mi, H, S	Mi, S
	Broad and flat nasal bridge	+	+	+	+	+
	Bulbous nose	+	+	+	+	+
	Maxillary hypoplasia	+	+	+	+	+
	Small mouth	+	+	+	+	+
	Abnormal palate	ND	ND	-	ND	high, narrow
	Dental anomalies	ND	irregular, wide-spaced	small teeth, supernum tooth	small	+
	Micrognathia	+	+	+	+	+
	Abnormal external ears	low-set, protruding, rounded helices	-	small, posteriorly rotated	small	low-set, posteriorly rotated, small
	Narrow auditory canals	+	-	+	+	+
	Hearing problems	+	+	-	-	+
	Triangular shaped face	+	+	+	+	+
Neurological	Intellectual disability	+	mild	moderate	severe	severe
	Speech abnormalities	no speech	no speech	little speech	little speech	no speech
	Hypotonia	+	-	+	+	-
	Behavioural problems	sensitive, hand flapping	friendly, hyperactive	friendly, difficulty with unexpected situations	autism, occasional aggressive	friendly, rage outbursts
	Seizures	-	+	-	-	-
	Corpus callosum agenesis	-	-	ND	ND	-
Extremities	Thumb/great toe abnormalities	-	-	long	-	short thumbs
	Syndactyly	-	-	-	-	-
	Abnormal patellae	ND	ND	-	-	ND
	Joint laxity	+	+	+/-	+/-	+
	Camptodactyly or contractures	-	+	-	-	ulnar deviated hands, overriding toes
	Short hands/fingers	-	short distal phalanges	long, thin fingers	-	-
	Horizontal palmar creases	-	+	-	-	+
	Congenital heart defects	-	-	-	-	-
GI	Feeding problems	+	+	-	+/-	+
	Anal anomalies	-	-	-	-	-
	Constipation	+	-	-	-	+
GU	Genital anomalies	-	C	C, SS	C, SP	SP
	Renal anomalies	ND	ND	ND	ND	ND
Other		hip dysplasia, scoliosis, died 4yo of septic shock after appendicitis	narrow thorax, severe clinodactyly, mild metaphyseal dysplasia, multiple fractures, died 25 yo of prostate cancer	inguinal hernia, clinodactyly toes III-V	asthma, long face	oligohydramnion, hiatus hernia, thin face

C: cryptorchidism, H: hypermetropia, Mi: microcornea/microphthalmus, N: nystagmus, ND: not determined, S: strabism, SP: small penis SS: shawl scrotum, yo: years old

Table S2. Exome Sequencing Results and Prioritization of Variants of Families 1 and 2

	Family 1	Family 2	Overlapping Genes
Total number of variants	39175	40105	9846
Exonic/canonical splice sites	16820	16655	6108
Non synonymous changes	8197	8118	3411
Not present in dbSNP132	719	744	174
Not present in in-house database	420	438	54
X chromosomal variants	15	15	4
Hemizygous (>70% variation)	4	5	1

Table S3. Variants Remaining after Prioritization in Families 1 and 2

Family	Chr	Position	Gene Name	cDNA Change	Protein Change
1	chrX	44703940	*DUSP21*	c.562A>G	p.(Ile188Val)
	chrX	53573686	*HUWE1*	c.10737C>G	p.(Asn3579Lys)
	chrX	**70348536**	***MED12***	**c.3443G>A**	**p.(Arg1148His)**
	chrX	153609403	*EMD*	c.611G>A	p.(Arg204His)
2	**chrX**	**70348981**	***MED12***	**c.3493T>C**	**p.(Ser1165Pro)**
	chrX	76891467	*ATRX*	c.4638A>C	p.(Lys1546Asn)
	chrX	118250601	*KIAA1210*	c.508C>A	p.(Leu170Ile)
	chrX	125685583	*DCAF12L1*	c.1009G>A	p.(Asp337Asn)
	chrX	134156452	*FAM127C*	c.38C>T	p.(Ala13Val)

MED12 mutations are depicted in bold.

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