OKRAY et al.

SUPPLEMENTAL DATA

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G-insertion identified among previously reported variations in FMR1

(A) Summary of previously unreported intragenic variations in *FMR1* in 7 males. The subject of the study is indicated in bold.

(B) Guanine insertion in exon 15 [1457insG] of FMR1 identified in the subject of the study.

Supplemental Figure 2

Patient C-terminus encodes a functional nuclear localization signal (NLS).

(A) HEK293 cells transfected with vectors encoding mCherry-tagged wild type profilin (*mCherry-Profilin*) or mCherry-tagged profilin fused with the patient NLS sequence (*mCherry-Profilin^{+NLS}*). Wild type profilin is cytoplasmic, however, when fused to the NLS peptide, aggregates in nuclear inclusions. Western blot analysis depicting expression levels of the different FMRP variants studied in the cell culture. A GFP-antibody was used on whole cell lysates of HEK293 cells transfected with the different GFP-FMR1 fusion constructs. Anti-actin was used for validation of protein load. Expression levels do not correlate with localization phenotypes. Scale bar represents 10μm.

Supplemental Figure 3

Sequence alignment of FMR/FXR protein family

(A) Amino acid alignment of human FMRP, FXR1P, FXR2P, mouse FMRP and fly FMRP. N-terminus is highly conserved while the C-terminus is more variable. The protein sequences (accession numbers # AAB28395.2, AAC50155.1, NP004851.2, NP032057.2, AAF54493.2) were aligned with the multiple sequence alignment (MUSCLE) function of MEGA6 (Tamura et al. 2013) using the default parameters and the alignment was corrected manually.

The black asterisk indicates the corresponding location of the patient mutation. The red asterisk shows the location of the C-terminus truncation engineered in the *dfmr1* variants (i.e. *dfmr1*^{ΔCt} and *dfmr1*^{ΔCt} +*NLS*).

Protein levels of *dfmr1* variants tested

(A) A pan-neuronal driver (Elav-Gal4) was used to express the different *dfmr1* variants and protein expression levels were determined via western blot analysis of adult fly brain protein extracts. Anti-Gapdh was used for validation of protein load. The graph shows changes in expression level of the different Dfmrp isoforms relative to wild type. Expression levels do not correlate with axonal phenotypes. For example, the relative expression levels of Dfmrp^{wt+NLS} and Dfmrp $\Delta Ct+NLS$ are comparable, yet unlike Dfmrp^{wt+NLS}, Dfmrp $\Delta Ct+NLS$ fails to cause axonal collapse. Similarly, novel axonal misguidance phenotypes are only observed with Dfmrp $\Delta Ct+NLS$ expression.

Supplemental Figure 5

Increasing Dfmrp expression in LNv neurons does not cause changes in axonal and subcellular localization phenotypes.

- (A) Further increasing expression of wild type Dfmrp in LNv neurons does not alter axonal collapse phenotypes. As observed for one copy of UAS-dfmr1, two copies of UAS-dfmr1 cause strong axonal collapse. However, no misguidance phenotypes are observed (n=20). Scale bar represents 10µm.
- (B) Independent of expression levels, Dfmrp is predominantly cytoplasmic in LNv neurons. Scale bar represents 5μm.

Α

Genomic Position	Туре	Annotation
9896	A>G	intronic
10056	T>C	intronic
16812	G>A	Coding, silent
24421	C>T	intronic
32758	G>T	intronic
32825	delC	intronic
33020	insG	coding, frameshift

В









....RKRTRRKRTWRRLQRKRRSLPNR



В









....RKRTRRKRTWRRLQRKRRSLPNR



В



Α

Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	1 MGGLASGGDV	MEELVVEVRG MADVTVEVRG EPGLPVEVRG MEELVVEVRG MEDLLVEVRL	SNGAFYKAFV SNGAFYKGFI SNGAFYKGFV SNGAFYKAFV DNGAYY <mark>KGQ</mark> V	KDVHEDSITV KDVHEDSLTV KDVHEDSVTI KDVHEDSITV TAVADDGIFV	AFENNWQPDR VFENNWQPER FFENNWQSER AFENNWQPER DVDG-VPE <mark>S</mark> M	QIPFHDVRFP QVPFNEVRLP QIPFGDVRLP QIPFHDVRFP KYPFVNVRLP	PPVGYNKD PPPDIKKE PPADYNKE PPVGYNKD PEETVEVAAP
Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	71 INES-DEVEV ISEG-DEVEV ITEG-DEVEV INES-DEVEV IFEEGMEVEV	YSRANEKEPC YSRANDQEPC YSRANEQEPC YSRANEKEPC FTRTNDRETC	CWWLAKVRMI GWWLAKVRMM GWWLARVRMM CWWLAKVRMI GWWVGIIKMR	KGEFYVIEYA KGEFYVIEYA KGDFYVIEYA KGEFYVIEYA KAEIYAVAYI	ACDATYNEIV ACDATYNEIV ACDATYNEIV ACDATYNEIV GFETSYTEIC	TIERLRSVNP TFERLRPVNQ TLERLRPVNP TIERLRSVNP ELGRLRAKNS	NKPATKDTFH NKTVKKNTFF NPLATKGSFF NKPATKDTFH NPPITAKTFY
1 Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	41 KIKLDVPEDL KCTVDVPEDL KVTMAVPEDL KIKLEVPEDL QFTLPVPEEL	ROMCAKEAAH REACANENAH REACSNENVH ROMCAKESAH REEAOKDGIH	KDFK KAVGAF KDFK KAVGAC KEFK KALGAN KDFK KAVGAF KEF <mark>QRTI</mark> DAG	SVTYDPENYQ RIFYHPETTQ CIFLNITNSE SVTYDPENYQ VCNYS <mark>R</mark> DLDA	LVILSINEVT LMILSASEAT LFILSTTEAP LVILSINEVT LIVI <mark>SE</mark> FEHT	SKRAHMLIDM VKRVNILSDM VKRASILGDM SKRAHMLIDM Q <mark>KR</mark> ASML <mark>K</mark> DM	HFRSLRTKLS HLRSIRTKLM HFRSLRTKLL HFRSLRTKLS HF <mark>RNLSQK</mark> VM
Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	LIMRNEEASK LMSRNEEATK LMSRNEEATK LILRNEEASK LLLRREEAAR	QLESSRQLAS HLECTKQLAA HLETSKQLAA QLESSRQLAS QLETTKLMS <mark>R</mark>	R-FHEQFIVR A-FHEEFVVR A-FQEEFTVR R-FHEQFIVR G <mark>NYVEEFR</mark> VR	EDLMGLAIGT EDLMGLAIGT EDLMGLAIGT EDLMGLAIGT DDLMGLAIGS	HGANIQQARK HGSNIQQARK HGANIQQARK HGANIQQARK H <mark>GSNIQAAR</mark> T	VPGVTAIDLD VPGVTAIELD VPGVTAIELG VPGVTAIDLD VDGVTNIELE	EDTCTFHIYG EDTGTFRIYG EETCTFRIYG EDTCTFHIYG E <mark>K</mark> SCTF <mark>KIS</mark> G
Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	81 EDQDAVKKAR ESADAVKKAR ETPEACROAR EDQDAVKKAR ETEESVORAR	SFLEFAEDVI GFLEFVEDFI SYLEFSEDSV SFLEFAEDVI AML <mark>EYAEE</mark> FF	QVPRNLVGKV QVPRNLVGKV QVPRNLVGKV QVPRNLVGKV QVPRELVGKV	IGKNGKLIQE IGKNGKVIQE IGKNGKVIQE IGKNGKLIQE IGKNGRIIQE	IVDKSGVVRV IVDKSGVVRV IVDKSGVVRV IVDKSGVVRV IVDKSGVVRI IVDKSGVFRI	RIEAE RIEGD RVEGD RIEAE K <mark>VSAIAG</mark> DDE	NEKNYPQEEE NENKLPRED - NDKKNPREE - NEKSYPQEEE QD <mark>QNIPREL</mark> -
3 Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	IMPPNSLPSN IMPPSSLPSN	NS <mark>R</mark> VGPNAPE NS <mark>R</mark> VGPNSSE	E <mark>KKHL</mark> D <mark>IK</mark> EN	STHFSQPNST - <mark>THFSQP</mark> NST	KVQRVLVASS KVQRVLVVSS	VVA <mark>GESQK</mark> PE IVA <mark>GGPQK</mark> PE	L <mark>KAWQGMVPF</mark> GMVPF GMVPF P <mark>K</mark> AWQGMVPF AHV <mark>P</mark> F
4 Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	21 VFVGTKDSIA VFVGTKESIG IFVGTRENIS VFVGTKDSIA VFIGTVESIA	NATVLLDYHL NVQVLLEYHI NAQALLEYHL NATVLLDYHL NA <mark>K</mark> VLLE <mark>YHL</mark>	NYLKEVDQLR AYLKEVEQLR SYLQEVEQLR NYLKEVDQLR SHL <mark>K</mark> EVEQLR	LERIQIDEQL MERIQIDEQL LERIQIDEQL LERIQIDEQL QE <mark>K</mark> MEIDQQL	R <mark>QIGASSR</mark> PP RQIG <mark>S</mark> RQIGLGFNPP RQIGASSRPP R <mark>AIQESSMGS</mark>	PN <mark>RTDN</mark> GSG <mark>R</mark> GSGGSD PN <mark>R</mark> TD <mark>N</mark> TQSFPVTRRS	E <mark>KSYVT</mark> DDGQ KAGYSTDE <mark>SS</mark> EKGYVTDDGQ E <mark>RGYSSDIES</mark>
4 Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	GMGRGS SSSLHATRTY GMGRGS VRSMRGG	- RPYRNRGHG - RSYSGRGRG GGSYGGRGRG - RPYRNRGHG GGG <mark>ORGRVR</mark> G *	RRGPGYTS RRGPNYTSGY RRTGGPAY RRGPGYTS RGGGGPGGGN FMR/FXR R	GTNSEASNAS GTNSELSNPS GPSSDVSTAS GTNSEASNAS GLNQ <mark>R</mark> YHNNR 2GG BOX	ETESDH ETESER ETESEK ETESDH RDEDDYNSRG	RDELSDW KDELSDW REEP RDELSDW DHQRDQQRGY	SLAPTEEE <mark>R</mark> E SLAGEDN-RD N <mark>R</mark> AGPGD-RD SLAPTEEE <mark>R</mark> E ND
Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	ST SFLEREGDG SFLEREGDG SFLEREGDG 	- RRRGGGGRG - RRRP-GGRG - RRRPIGGRG - RRRGGGGRG GSYRGGGGGA *	QG GRGRG R <mark>SVS</mark> GGRGRG RGPPPAP - RP QG GRGRG GGPGNNR - RG	GG GPRGG <mark>KSSIS</mark> TSRYNSSSIS GG GIN RR PPRND	FKGNDD SVLKDPDSNP SVLKDPDSNP FKGNDD OONGRD	HSETDNEP YSLLDNTEED YSLLDTSEPE HSETDNEP -YQHHNHTTE	QTADTDASES PPVDSEPGEP EV <mark>R</mark> ET <mark>R</mark> EMSS
Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	HHSTN PPASA VERADSNSSY	RNPREA RRRRSR RRRRSR RNPREA EG <mark>SS</mark> RRRRRQ	KGRTTDGSLQ RRRTDEDAVL RRRTDED <mark>RTV</mark> KG <mark>RTADG</mark> KNNN <mark>GPSNTN</mark>	I EVDCNNER MDGMTESDTA MDGGLESDGP GAVANNNNKP	SVHTKTLONT SVNENGLVTV NMTENGLEDE SLOSA QSAQQPQQQQ	SEGS SEQYIS SEPQR SEGS PPAPGNKAAL	RLETGKDEN – RAESQSEQE – RNESERERE RLETGKDEN – NAGDASKQNS
/ Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	-NLPRETLAK GNRTDGSISG GNANAAGGAS	N <mark>KK</mark> D <mark>RQPVTVADY</mark> KP <mark>K</mark> DAS <mark>R</mark> NGD	- EMAKDVI EE ISKAESQSKQ KQQAGTQQQQ	<mark>QKKEK</mark> HGPSEKAING RPPLERTKPS QKKEK PSQVQQQQAA	PD <mark>SVDG</mark> P TSASG D EDSLSGQKGD PDSVDG QQQQPKPR-R	DISKLORTPG SVSKLPKGPS N <mark>K</mark> NRSNNHTD	EE <mark>MINTLNEE</mark> ENGELSA QPS <mark>GQQQLA</mark> E
Hs-FMR1 Hs-FXR1 Hs-FXR2 Mm-FMR1 Dm-FMR1	771 QQPLVN NTQEAAVLN PLELGSMVN LQPLVN NVKKEGLVN	g VP g VS g VS g VP g TS					

Α





Pdf-Gal4, UAS-CD8-GFP x ...

В

Α

Pdf-Gal4, UAS-CD8-GFP x ...

