Supplementary Table S3. All published coding region small variants with details of information used to apply pathogenicity criteria.

Ref.	Location		# affected with variant, sex	CGG repeats	Other variants in pt.	Inheritance in proband	Segregation	Patient and/or functional data	ACMG criteria [1]	Conclusion
	c.	p.								
[2]	c.80C>A	p.S27*	1M, 1F	29		Mat (affected)		FMRP absent	PVS1 PS3 PM2 PP1	PATH
[3]	c.188_193del	p.(D63_E64del)	1M	nl		De novo confirmed			PS2 PM2 PM4 PP3	LPATH
[4]	c.229delT	p.(C77Afs*5)	1M	nl	Maternal PATH 308 kb del involving *PTCHD1-AS* (autism)	De novo confirmed			PVS1 PS2 PM2 BP5	PATH
[5] #1	c.373delA[a]	p.(T125Lfs*35)	1M	nl		De novo		mRNA + (RT-PCR), FMRP absent (lymphoblastoid cell line)	PVS1 PS3 PM6	PATH
[6], [7][b]	c.377T>C	p.(F126S)	1M		*KDM5C* I1349M (maternal), *ALG13* H771R (maternal)	De novo			PS2 PM6 PP3	LPATH
[8], [9]	c.413G>A	p.R138Q	1M, 1F	nl		Maternal (affected, learning disability/anxiety)	MGF and father (not tested) also had learning problems	AMPA signaling and RNA binding unchanged (transduced mouse neurons); deficient presynaptic function (Drosophila)	PS3 PP1 PP4; 7 hemizygotes in gnomAD v3 exomes (rs200163413)	LPATH
[10]	c.413G>A	p.R138Q	1M	24	(1st cousin consanguinity)	Maternal	Present in sister (no details)	Classic FXS presentation		
[11]	c.413G>A	p.R138Q	N/A					ID/ASD-like features (knock-in mouse model)		
[12]	c.413G>A	p.R138Q	1F	29, 37		Absent in mother; healthy father not tested				
[13,14]	c.677G>A	p.(R226K)	1M		7 variants, no diagnosis				PM2 PP3	VUS
[13]	c.767A>G	p.(D256G)	1M		5 variants, no diagnosis				PM2 PP3	VUS

[15]	c.797G>A	p.(G266E)	1M	23		Maternal (unaffected)	Absent in 3 unaffected brothers	Deficient RNA binding, AMPA trafficking (transduced mouse neurons)	PS3 PM2 PP3	LPATH
[16], [17]	c.911T>A	p.(I304N)	1M	nl	Known PHKA2 deficiency (glycogen storage disease IXa1)	De novo confirmed		mRNA present (RT-PCR), deficient association with polyribosomes (lymphoblastoid cell line)	PS2 PS3 PM2 PP3	PATH
[18] #1	c.1021-1028delinsTATTGG	p.N341Yfs*7	2M	nl		Mother not tested; had epilepsy	Present in unaffected sister, absent in 2 unaffected brothers	mRNA +, FMRP absent (lymphoblastoid cell line)	PVS1 PS3 PP1	PATH
[19]	c.1325G>A	p.(R442Q)	2M + mother, grandmother		11 kb paternal deletion of part of *MYH7*	Maternal (affected)	Present in 2 affected brothers and mildly affected mother	FMRP nl in hair roots (4 family members); abnormal protein localization (transfected HEK cells)	PS3 PM2 PP1 PP3	LPATH
[20]	c.1444G>A	p.(G482S)	1M						PM2 BP4	VUS
[21]	c.1550C>T[c]	p.(P517L)	1M	36		?			PM2	VUS
[20]	c.1601G>A	p.(R534H)	2M (unrelated), 1F			Maternal (unaffected) in both families			PM2	VUS
[22]	c.1610dup	p.(G538Rfs*24)	1M	41		?		mRNA 60% decreased and FMRP >90% decreased (lymphocyte cell line) Truncated protein abnormal localization and function (transfected HEK cells, Drosophila)	PVS1 PS3 PM2	PATH
[23]	c.1618G>A	p.(G540E)	1M	nl					PM2 PP3	VUS
[24] TN351	c.1637G>A	p.(R546H)	1M	nl	*FMR1* c.990+14C>T (see Table 4)	?			PM2	VUS

[a] New BglII restriction site.

[b] All references in literature are to a variant identified in the DDD study that was not listed in that publication but corresponds to DECIPHER patient #259197.

[c] Reported as NM_001185081.1:c.1216C>T (p.(Q406*)) on an alternate reading frame from the reference transcript.

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