Supplementary Table 5. All published noncoding small variants with details of information used to apply pathogenicity criteria.

Ref.	Location		# affected with variant, sex	CGG repeats	Other testing done, variants	Inheritance in proband	Patient and/or functional data	ACMG criteria	Conclusion
	DNA	r./p.							
[1]	g.147909332G>A	N/A (2.5 kb upstream of r.1, loss of EcoRI site)	1F	23, 32		Paternal (unaffected)	(1 het in gnomAD)	BS2	VUS
[2] #2	g.147911760_2dup(GGC) (promoter ~-154)		1M	31	FMR1 only		Absent in unaffected sister Normal FMRP (lymphocytes), normal gel shift and reporter assays *in vitro*	BS1 BS2 BS3	BEN
[3]	g.147911760_2dup(GGC) (promoter ~-154)		8 samples (male DD cohort)	nl	FMR1 only		(gnomAD AF 2.5% with many hemizygotes, rs200904100)		
[4]	g.147911767C>G (promoter ~-149)		1M	31	FMR1 only		Present in unaffected MGF (and mother, mat aunt, and sister)	BS2 BS3	BEN
[2] #1	g.147911767C>G (promoter ~-149)		1M	29	AFF2 repeats and FMR1		Normal gel shift and gene reporter assays		
[3]	"c.-332G>C" (promoter)		1M (male DD cohort)	nl	FMR1 only		Reduced reporter expression to 5.9% of WT (10 hemizygotes in gnomAD, rs922007219)	BS1 BS2 PS3	VUS
[3]	"c.-293T>C" (promoter)		1M (male DD cohort)	nl	FMR1 only		Reduced reporter expression to 29.2% of WT (7 hemizygotes in gnomAD, rs1222840333)	BS1 BS2 PS3	VUS
[3]	c.-254A>G		1M (male DD cohort)	nl	FMR1 only		Reduced reporter expression to 36.2% of WT (8 hemizygotes in gnomAD, rs1217601043)	BS1 BS2 PS3	VUS
[5]	CGG repeat 8 CGG>CCG (c.-107G>C)	N/A (new EagI site)	N/A (healthy female, FHx ID)	nl	FMR1 repeat region only		Present in 2 unaffected males, opposite side of family from members with ID	BS2 BS4	BEN

[6]	CGG repeat 26 of 31 CGG>CCG	N/A (new EagI site)	1M	31	FMR1 partial sequencing only	Maternal (unaffected)	Normal % of FMRP+ lymphocytes, but statistically significant decrease to 76% normal FMRP level in lymphoblastoid cell line	None	VUS
[7]	c.-4_+1delGAAGA	p.(M1=), no start loss because c.-9_-5 is also GAAGA (amplification failure in PCR assay)	1M	nl	FMR1 only	Maternal (98% FMRP+ lymphocytes)	Normal FMRP level in lymphoblastoid cell line; 80% lymphocytes FMRP+	BS3	VUS
[8]	c.18G>T	p.(V6=)	2M (unrelated)	nl	FMR1 only	Maternal (unaffected) in 1	Normal intron 1 splicing in lymphoblastoid cell line (1 patient), normal FMRP Western blot in lymphoblastoid cell line (1 patient)		
[9]	c.18G>T	p.(V6=)	4/508 male ID/DD cohort					BS1 BS2 BS3	BEN
[3]	c.18G>T	p.(V6=)	13 samples, 19 controls				Observed in multiple unaffected controls		
[8]	c.51+88_89del	(intron 1)	1M (clinical FXS testing)	nl	FMR1 only		Absent in affected brother	BS4	VUS
[10]	c.51+423G>C	(New BssHI site on Southern)	1M	42	FMR1 partial sequencing				VUS
[11]	c.51+730A>G	(New EcoRI site on Southern)	1M (clinical lab testing)	nl	FMR1 only	Maternal (unaffected)	Present in 1 brother, ? phenotype		VUS
[12]	c.52-47A>G		1M (ID, 2 FXS features)		FMR1 sequencing-by-hybridization		(221 hemizygotes in gnomAD, rs80358323)	BS1 BS2	BEN
[13]	c.52-1_52delinsTA	2 abnormal transcripts	1M + mother	nl	FMR1 partial sequencing	Maternal (affected)	No normal transcripts; 2 abnormal transcripts with skipped exon 2 and skipped exons 2-3; FMRP absent in lymphoblastoid cell line	PVS1 PS3 PP1	PATH

[12]	c.105-179G>T		1M (ID, 2 FXS features)		FMR1 sequencing-by-hybridization		(305 hemizygotes in gnomAD, rs80358324)	BS1 BS2	BEN
[3]	c.105-8A>C		6/963 samples (male DD cohort)	nl	FMR1 only		(103 hemizygotes in gnomAD, rs183745963)	BS1 BS2	BEN
[14] #3	c.420-8A>G	r.419_420ins420-7_420-1 (p.(M140Ifs*3))	1M (ID cohort)	nl	451-gene ID exon panel	Maternal (unaffected, no XCI skewing)	Cryptic splice acceptor leading to retention of 7 nt from intron 5 (blood)	PS3 PM2	LPATH
[15]	c.513+27T>C	No change found (intron 6)	1 sample (ID, 1 FXS feature cohort)	nl	FMR1 only		No change in exon 6-7 junction on RT-PCR product sequencing	BS3	VUS
[3]	*c.630+438A>C*		1M (DD cohort)	nl	FMR1 only				VUS
[3]	*c.631-840G>A*		1M (DD cohort)	nl	FMR1 only				VUS
[16,17]	c.801G>A (IVS8-1)	r.631_801del (p.(S211_G267del)); exon 8 skipping	1M (ID cohort)		56-gene ID panel	De novo with maternity/paternity confirmed	No FHx DD (parental first cousin consanguinity) Exon 8 skipping with no normal RT-PCR product; rat model with deletion of exon 8 is affected	PS2 PS3 PM2	PATH
[18]	c.879A>C (IVS9-2)	p.(V293=); reported abnormal splicing intron 9	1F (autism/ ID cohort)	nl	FMR1 partial (SSCP with subclone sequencing)		Inclusion of intron 9 sequence in 23/36 subclones from peripheral blood cDNA	BS2	VUS
[15]	c.879A>C (IVS9-2)	p.(V293=); no splicing abnormality found	1M (ID, 1 FXS feature)	nl	FMR1 only	Maternal (unaffected)	No abnormal splice amplicons found; normal exon 9-10 junction in RT-PCR product sequence in blood; FMRP present in blood homogenate (1 hemizygote in gnomAD, rs782013865)		
[3]	c.880+885A>G		1M (DD cohort)	nl	FMR1 only		(24 hemizygotes in gnomAD, rs781933453)	BS1 BS2	BEN

[19]	c.881-1G>T	? (exon 10 splice acceptor)	1M (clinical suspicion)		Genome sequencing	Maternal (1:99 skewed XCI, unaffected)		PVS1 PM2	LPATH
[14]	c.990+1G>A	r.881_990del (p.(K295Nfs*11)) (exon 10 skipping)	1M (ID cohort)	nl	451-gene ID exon panel	De novo	Exon 10 skipping in blood	PVS1 PS3 PM2 PM6	PATH
[3]	*c.990+4T>C*	? (intron 10)	1M (DD cohort)	nl	FMR1 only			PM2	VUS
[20]	c.990+14C>T	r.881_990del (p.(K295Nfs*11)) (exon 10 skipping)	3 unrelated males (ID, FXS feature cohort)	nl	SSCP exons 1-10 and 15 with sequencing; c.1637G>A in one patient		Exon 10 skipping on peripheral blood RT-PCR product sequencing in 2 probands (TN-183, TN-351)	BA1 BS2	BEN
[21]	c.990+14C>T		81 control individuals				Observed in many controls from general population		
[9]	c.990+14C>T		45/508 in ID/DD cohort						
[22]	c.990+14C>T		7M/4F among 88 patients with ASD				Statistically significant (p=0.0123) higher frequency in ASD patients vs controls Stably inherited in unaffected family members		
[23]	c.990+14C>T						No significant transmission disequilibrium (p=0.26) Allele frequency 65% (22/34) in East Asian controls; concluded that previously observed association with ASD was false positive due to population stratification (gnomAD AF >10%, rs25714)		
[9]	c.1189-39A>G		1M (ID/DD cohort)		FMR1 melting and		(2 hemizygotes in gnomAD, rs781962133)	BS2	VUS

				sequencing only			
[3]	c.1472-521C>G	1M (DD cohort)		FMR1 only	(1 hemizygote in gnomAD, rs1557181482)	BS2	VUS
[3]	c.*23T>C	1M (DD cohort)		FMR1 only			VUS
[9]	c.*60G>C	1M (ID/DD cohort)		FMR1 melting and sequencing only	(46 hemizygotes in gnomAD, rs782402226)	BS1 BS2	BEN
[9]	c.*68T>C	1M (ID/DD cohort)		FMR1 melting and sequencing only	(2 hemizygotes in gnomAD, rs781983693)	BS2	VUS
[15]	c.*312_313dupT	1 (ID, 1 FXS feature cohort)	nl	FMR1 only		PM2	VUS
[24], [3]	c.*746T>C	2M (proband and half-brother from DD cohort)	nl	FMR1 only	Faster mRNA decay with FMRP level 80% of normal in lymphoblastoid cell line; variant sufficient and necessary to decrease reporter gene expression *in vitro;* loss of metabotropic glutamate receptor-stimulated upregulation of reporter expression in transfected mouse neurons (72 hemizygotes in gnomAD, rs183130936)	PS3 PP1 BS1 BS2	VUS
[15]	c.*760C>A	1 (ID, 1 FXS feature cohort)	nl	FMR1 only	(1 hemizygote in gnomAD, rs184987604)	BS2	VUS
[3]	c.*1867G>A	12/963 samples (DD cohort)	nl	FMR1 only	(466 hemizygotes in gnomAD, rs148216485)	BS1 BS2	BEN
[3]	c.*2035C>T	3/963 samples (DD cohort)	nl	FMR1 only	(39 hemizygotes in gnomAD, rs140123351)	BS1 BS2	BEN

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