Supp. Table S1. All Newly- and Previously-Reported Mutations in *EFTUD2*

ID	Exon / Intron	Mutation Class	gDNA [hg19; NC_000017.10]	Predicted cDNA [NM_004247.3]	mRNA Effect	Predicted Protein [NP_004238.3]	Segregation	Remarks	Reference
1	exon 1 - exon 28	whole-gene deletion	ND	ND	r.0?	p.0?	Unknown	arr cgh 17q21.31(CTD-2145G14)x1; 46,XY	this report
2	exon 1 - exon 28	whole-gene deletion	g.42778736_43233331del	c.-256599_*149906del	r.0?	p.0?	Unknown	Coordinates approximate	Gandomi et al 2013
3	exon 1 - exon 28	whole-gene deletion	g.42792021_43147305del	c.-170573_*136621del	r.0?	p.0?	De novo	Coordinates approximate	Lines et al 2012
4	exon 1 - intron 3	multi-exon deletion	g.42963474_42997474del	c.-20742_271+479del	r.0?	p.0?	De novo	Coordinates approximate	Gordon et al 2012
5	exon 1 - intron 4	multi-exon deletion	g.42962474_42985474del	c.-8742_350+150del	r.0?	p.0?	De novo	Coordinates approximate	Gordon et al 2012
6	intron 11-exon 28	multi-exon deletion	g.42010525_42949809del	c.994+5_*918117del	r.0?	p.0?	Unknown	Identified by microarray (Coordinates approximate)	this report
7	intron 19 - exon 28	multi-exon deletion	g.42923422_42935059del	c.1963-534_*5220del	r.0?	p.0?	Unknown	Identified by reduced NGS read depth	Lines et al 2012
8	exon 2	frameshift	g.42971878del	c.12del	r.(?)	p.(Leu5Tyrfs*41)	Unknown		this report
9	exon 2	splice site (exonic)	g.42971785C>T	c.105G>A	r.7_111del	p.Thr3_Asp37del	De novo	Verified by RT-PCR (as per clinical laboratory report)	this report
10a (proband), 10b (sibling), 10c (mother)	intron 2	splice site	g.42964120T>C	c.106-2A>G	r.spl?	p.?	Germline (inherited) (10a, 10b); unknown (10c)		this report
11	exon 3	nonsense	g.42964026G>C	c.198C>G	r.(?)	p.(Tyr66*)	Unknown		Lehalle et al 2014
12	exon 3	nonsense	g.42964016C>A	c.208G>T	r.(?)	p.(Glu70*)	De novo		this report
13	intron 3 - exon 4	splice site	g.42962694_42962713del	c.272-11_280del	r.272_350del	p.Glu91Valfs*6	Unknown	Verified by RT-PCR (Supp. Fig S2)	this report
14	intron 4	splice site	g.42961093C>T	c.351-1G>A	r.spl?	p.?	De novo		Voigt et al 2013
15	intron 4	splice site	g.42961093C>G	c.351-1G>C	r.spl?	p.?	Unknown		Lehalle et al 2014
16	intron 4	splice site	g.42961093C>A	c.351-1G>T	r.spl?	p.?	De novo		Luquetti et al 2013
17a (proband), 17b (mother)	intron 5	splice site	g.[42960528delT; 42960533G>A]	c.[427-2delA;427-7C>T]	r.spl?	p.?	Germline (inherited) (17a); unknown (17b)		this report; Guion-Almeida et al 2009 (clinical description)
18a (proband), 18b (mother)	intron 6	splice site	g.42960460C>T	c.492+1G>A	r.spl?	p.?	Germline (inherited) (18a); de novo (18b)		this report

ID	Exon / Intron	Mutation Class	gDNA [hg19; NC_000017.10]	Predicted cDNA [NM_004247.3]	mRNA Effect	Predicted Protein [NP_004238.3]	Segregation	Remarks	Reference
19	exon 7	nonsense	g.42959085G>T	c.498C>A	r.(?)	p.(Cys166*)	Unknown		Lehalle et al 2014
20	intron 7	splice site	g.42958013C>T	c.529-1G>A	r.spl?	p.?	Unknown		Lehalle et al 2014
21	exon 8	nonsense	g.42957947A>C	c.594T>G	r.(?)	p.(Tyr198*)	De novo		Voigt et al 2013
22	exon 8	frameshift	g.42957939dup	c.602dupA	r.(?)	p.(Asn201Lysfs*13)	De novo		Vincent et al. 2015
23	intron 8	splice site	g.42957921C>T	c.619+1G>A	r.spl?	p.?	De novo		Gordon et al 2012
24	exon 9	missense substitution	g.42957003T>C	c.623A>G	r.(?)	p.(His208Arg)	De novo		Gordon et al 2012
25	exon 9	missense substitution	g.42956956C>T	c.670G>A	r.(?)	p.(Gly224Arg)	Unknown		Gordon et al 2012
26	exon 9	frameshift	g.42956928del	c.698del	r.(?)	p.(Glu233Glyfs*3)	De novo		Need et al 2012
27	intron 9	splice site	g.42956923del	c.702+1del	r.spl?	p.?	Unknown		Lehalle et al 2014
28	intron 9	splice site	g.42956919C>G	c.702+5G>C	r.(spl?)	p.?	De novo		Lehalle et al 2014
29, 30	intron 9	splice site	g.42956919C>T	c.702+5G>A	r.(spl?)	p.?	De novo (30); unknown (29)		this report
31	exon 10	nonsense	g.42953426C>A	c.745G>T	r.(?)	p.(Glu249*)	De novo		Lehalle et al 2014
32, 33, 34	exon 10	missense substitution	g.42953387G>A	c.784C>T	r.(?)	p.(Arg262Trp)	De novo (32, 33); unknown (34)		Lines et al 2012 (32); Smigiel et al 2015 (33); this report (34)
35	exon 10	small deletion/duplication (stopgain)	g.[42953384_42953387del;42953369_42953380dup]	c.[784_787del;791_802dup]	r.(?)	p.(Arg262*)	Unknown		Lines et al 2012
36	exon 11	frameshift	g.42949875dup	c.933dupC	r.(?)	p.(Ser312Leufs*22)	De novo		Sarkar et al 2015
37a (proband), 37b (sibling), 37c (parent)	intron 11	splice site	g.42949813C>G	c.994+1G>C	r.spl?	p.?	Germline (inherited) (37a, 37b); unknown (37c)		Voigt et al 2013
38	intron 11	splice site	g.42949809C>T	c.994+5G>A	r.(spl?)	p.?	De novo		Need et al 2012
39	exon 12	splice site	g.42945661del	c.1052del	r.(?)	p.(Pro351Leufs*35)	De novo		this report
40	intron 13	splice site	g.42945170C>A	c.1149+5G>T	r.(spl?)	p.?	De novo		Lehalle et al 2014
41	exon 14	frameshift	g.42942404_42942411del	c.1172_1179del	r.(?)	p.(Ser391Thrfs*57)	De novo		Lines et al 2012
42, 43	exon 15	frameshift	g.42941138_42941139del	c.1297_1298del	r.(?)	p.(Met433Valfs*17)	De novo (42), unknown (43)		this report

ID	Exon / Intron	Mutation Class	gDNA [hg19; NC_000017.10]	Predicted cDNA [NM_004247.3]	mRNA Effect	Predicted Protein [NP_004238.3]	Segregation	Remarks	Reference
44	exon 15	missense substitution	g.42941130G>C	c.1306C>G	r.(?)	p.(Gln436Glu)	De novo	Originally reported as "c.1458C>G / p.Gln401Glu"	Luquetti et al 2013
45	exon 16	missense substitution	g.42940262A>G	c.1426T>C	r.(?)	p.(Cys476Arg)	Unknown		Lines et al 2012
46	exon 16	frameshift	g.42940253dup	c.1435dup	r.(?)	p.(Thr479Asnfs*2)	De novo		Lehalle et al 2014, Smigiel et al 2015
47a (proband), 47b (mother)	exon 16	missense substitution	g.42940192C>T	c.1496G>A	r.(?)	p.(Gly499Asp)	Germline (inherited) (47a); de novo (47b)		this report
48	intron 16	splice site	g.42940078T>C	c.1607+3A>G	r.(spl?)	p.?	Unknown		Lines et al 2012
49, 50	exon 17	nonsense	g.42937814G>A	c.1705C>T	r.(?)	p.(Arg569*)	De novo (49); unknown (50)		Gordon et al 2012 (49); this report (50)
51, 52, 53a, (proband), 53b (mother)	exon 18	nonsense	g.42937401G>A	c.1732C>T	r.(?)	p.(Arg578*)	De novo (51, 52); germline (inherited) (53a); unknown (53b)		Sarkar et al 2015 (51); this report (52, 53a, 53b)
54	exon 18	frameshift	g.42937373_42937374del	c.1759_1760del	r.(?)	p.(Val587Tyrfs*18)	Unknown		Lines et al 2012
55	exon 18	missense substitution	g.42937273C>G	c.1860G>C	r.(?)	p.(Lys620Asn)	De novo		Lehalle et al 2014
56	exon 18 / intron 18	splice site	g.[42937274T>A;42937269_42937270delinsCTC]	c.[1859A>T; 1860+3_1860+4delinsGAG]	r.1720_1860del	p.Ala574_Lys620del	De novo	Originally reported as "p.(Lys620Met)"; additional splice mutation subsequently confirmed by RT-PCR (Supp. Fig S2)	Smigiel et al 2015
57	intron 18	splice site	g.42936551T>C	c.1861-2A>G	r.spl?	p.?	Unknown		this report
58	exon 19	missense substitution	g.42936500A>C	c.1910T>G	r.(?)	p.(Leu637Arg)	De novo		Lines et al 2012
59	intron 19	splice site	g.42936447C>T	c.1962+1G>A	r.spl	p.?	Unknown		Lehalle et al 2014
60	intron 19	splice site	g.42934527T>A	c.1963-2A>T	r.1963_2045del	p.Val655Glufs*8	Unknown	Verified by RT-PCR (Supp. Fig S2)	this report
61	exon 20	frameshift	g.42934512delinsGGTGG	c.1976delinsCCACC	r.(?)	p.(Val659Alafs*7)	De novo		Lehalle et al 2014
62	exon 20	missense substitution	g.42934455G>T	c.2033C>A	r.(?)	p.(Thr678Lys)	De novo		this report
63	intron 20	splice site	g.42934442C>T	c.2045+1G>A	r.spl?	p.?	De novo		this report
64	intron 20	splice site	g.42934441A>C	c.2045+2T>G	r.spl?	p.?	Unknown		this report
65	exon 22	nonsense	g.42932028G>A	c.2155C>T	r.(?)	p.(Gln719*)	De novo		Lines et al 2012

ID	Exon / Intron	Mutation Class	gDNA [hg19; NC_000017.10]	Predicted cDNA [NM_004247.3]	mRNA Effect	Predicted Protein [NP_004238.3]	Segregation	Remarks	Reference
66	exon 22	nonsense	g.42931985C>T	c.2198G>A	r.(?)	p.(Trp733*)	Unknown		Gordon et al 2012
67	exon 22	frameshift	g.42931938dup	c.2245dup	r.(?)	p.(Thr749Asnfs*5)	Unknown		Lehalle et al 2014
68	exon 22	frameshift	g.42931930delG	c.2253delC	r.(?)	p.(Ser752Leufs*11)	Unknown		Vincent et al. 2015
69	intron 22	splice site	g.42931923C>T	c.2259+1G>A	r.spl?	p.?	De novo		Gordon et al 2012
70	exon 23	frameshift	g.42931688del	c.2296del	r.(?)	p.(Ile766Serfs*18)	Unknown		Lehalle et al 2014
71	exon 23	missense substitution	g.42931679C>G	c.2305G>C	r.(?)	p.(Gly769Arg)	Unknown		this report
72	exon 23	frameshift	g.42931649dup	c.2335dup	r.(?)	p.(Leu779Profs*3)	De novo		this report
73	intron 23	intronic (putatively novel splice donor)	g.42931571T>C	c.2347+66A>G	r.(spl?)	p.?	Germline (inherited)	Inherited from healthy father; pathogenicity unclear	Gordon et al 2012
74	intron 24	splice site	g.42930759C>A	c.2467-1G>T	r.spl?	p.?	Unknown		Lehalle et al 2014
75	exon 25	missense substitution	g.42930758C>T	c.2467G>A	r.(?)	p.(Ala823Thr)	De novo		Vincent et al. 2015
76	exon 25	missense substitution	g.42930740C>T	c.2485G>A	r.(?)	p.(Glu829Lys)	De novo		Luquetti et al 2013
77	exon 25	nonsense	g.42930732G>T	c.2493C>A	r.(?)	p.(Tyr831*)	De novo		Lines et al 2012
78, 79a (proband), 79b (sibling)	exon 25	nonsense	g.42930729G>C	c.2496C>G	r.(?)	p.(Tyr832*)	Unknown (78); Maternal germline mosaicism (proven by SNP testing) (79a, 79b; Supp. Fig S1)	Originally reported as “c.2495C>G”	Bernier et al 2012 (78); this report (79a, 79b)
80, 81, 82	intron 25	splice site	g.42929932del	c.2562-2del	r.spl?	p.?	De novo (80, 81); unknown (82)		Lehalle et al 2014
83, 84a (proband), 84b (mother)	intron 25	splice site	g.42929931_42929932del	c.2562-2_2562-1del	r.spl?	p.?	Unknown (83, 84b); germline (inherited) (84a)		Lehalle et al 2014
85	intron 25	splice site	g.42929931C>T	c.2562-1G>A	r.spl?	p.?	Unknown		Lehalle et al 2014
86	intron 25	splice site	g.42929931C>G	c.2562-1G>C	r.[=, 2467_2577 del, 2562_2577 del, 2562_2715 del]	p.[=, Ala823_Gln895del, Gly855Metfs*75, Gly855Leufs*29]	De novo		Voigt et al 2013

ID	Exon / Intron	Mutation Class	gDNA [hg19; NC_000017.10]	Predicted cDNA [NM_004247.3]	mRNA Effect	Predicted Protein [NP_004238.3]	Segregation	Remarks	Reference
87	exon 26	missense substitution	g.42929926G>A	c.2566C>T	r.(?)	p.(His856Tyr)	Unknown		Lehalle et al 2014
88	exon 26	frameshift	g.42929871_42929873delinsGACC	c.2619_2621delinsGGTC	r.(?)	p.(Phe874Valfs*11)	De novo		Gordon et al 2012
89a (proband), 89b (sibling)	exon 26	frameshift	g.42929870dup	c.2622dup	r.(?)	p.(Ile875Tyrfs*10)	Germline mosaicism (89a, 89b)	Two affected siblings; parental testing normal; paternity confirmed	Voigt et al 2013
90a (proband), 90b (mother)	exon 26	frameshift	g.42929791_42929794del	c.2698_2701del	r.(?)	p.(Val900Serfs*34)	Germline (inherited) (90a); unknown (90b)		this report
91	exon 26	nonsense	g.42929131G>A	c.2770C>T	r.(?)	p.(Gln924*)	Unknown		Lines et al 2012; Vincent et al. 2015
92	exon 27	missense substitution	g.42929088C>T	c.2813G>A	r.(?)	p.(Arg938His)	De novo		this report
93a (fetus), 93b (mother)	intron 27	splice site	g.42929077del	c.2823+1del	r.spl?	p.?	Germline (inherited) (93a); de novo (93b)		Gordon et al 2012, Lehalle et al 2014
94	intron 27	splice site	g.42929077C>A	c.2823+1G>T	r.spl?	p.?	Unknown		this report

For gDNA numbering:

Nucleotide numbering is from the first base of the chromosome 17 reference sequence [hg19] (NC_000017.10).

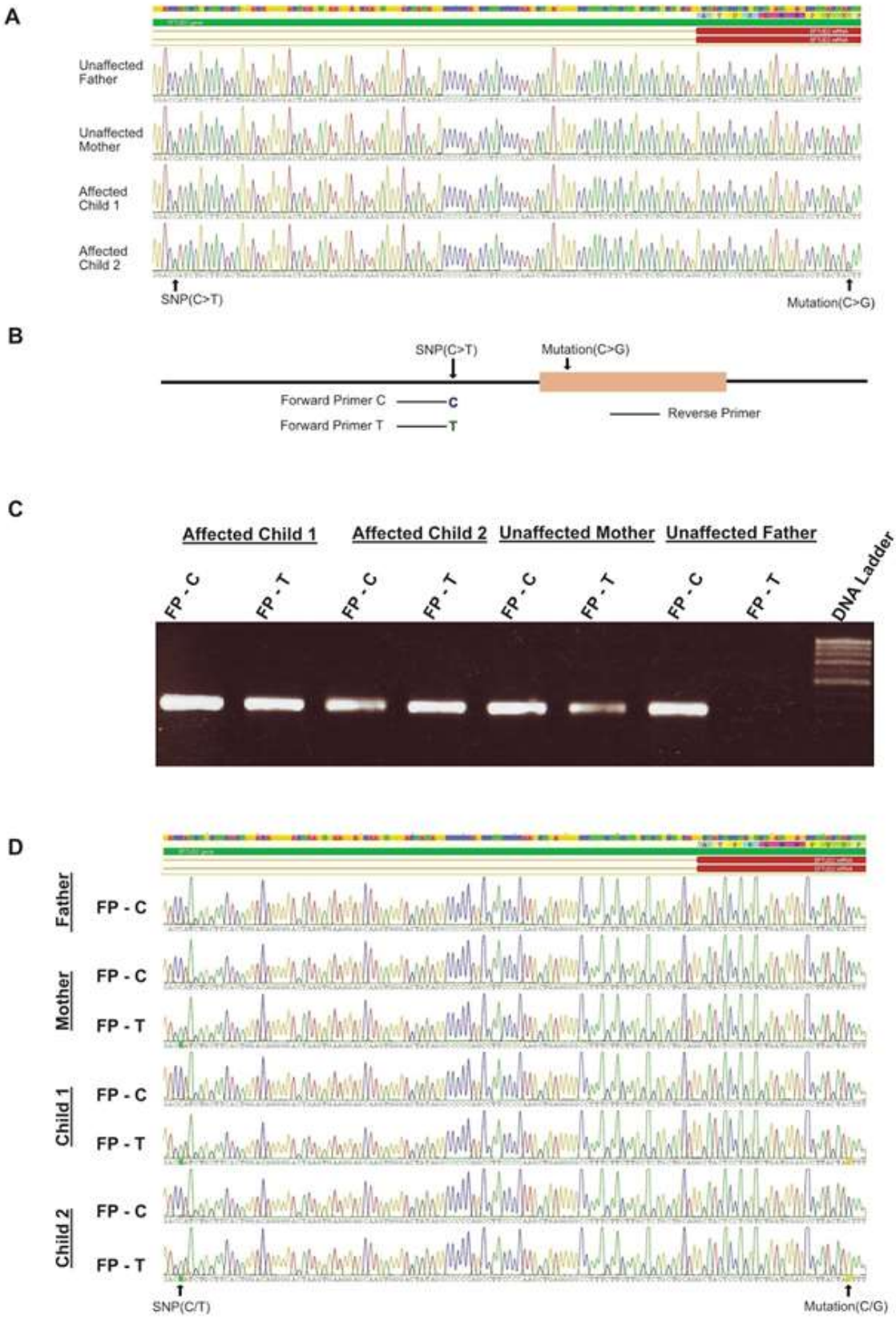
For cDNA numbering:

Nucleotide numbering uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.

Data confirming maternal gonadal mosaicism for c.2496C>G in probands 79a and 79b are presented in Supp. Figure S1.

RT-PCR data confirming splice mutations c.1963-2A>T, c.272-11_280del, and c.[1859A>T; 1860+3_1860+4delinsGAG] are presented in Supp. Figure S2.

The c.1058+3_1058+7del variant reported by Gordon et al (2012) has been omitted. As of September 2015, this variant is represented in the Exome Aggregation Consortium (ExAC) dataset (http://exac.broadinstitute.org/) with a frequency of 656/120582 (0.544%), including two homozygous individuals, and is therefore deemed non-pathogenic.



Supp. Figure S1

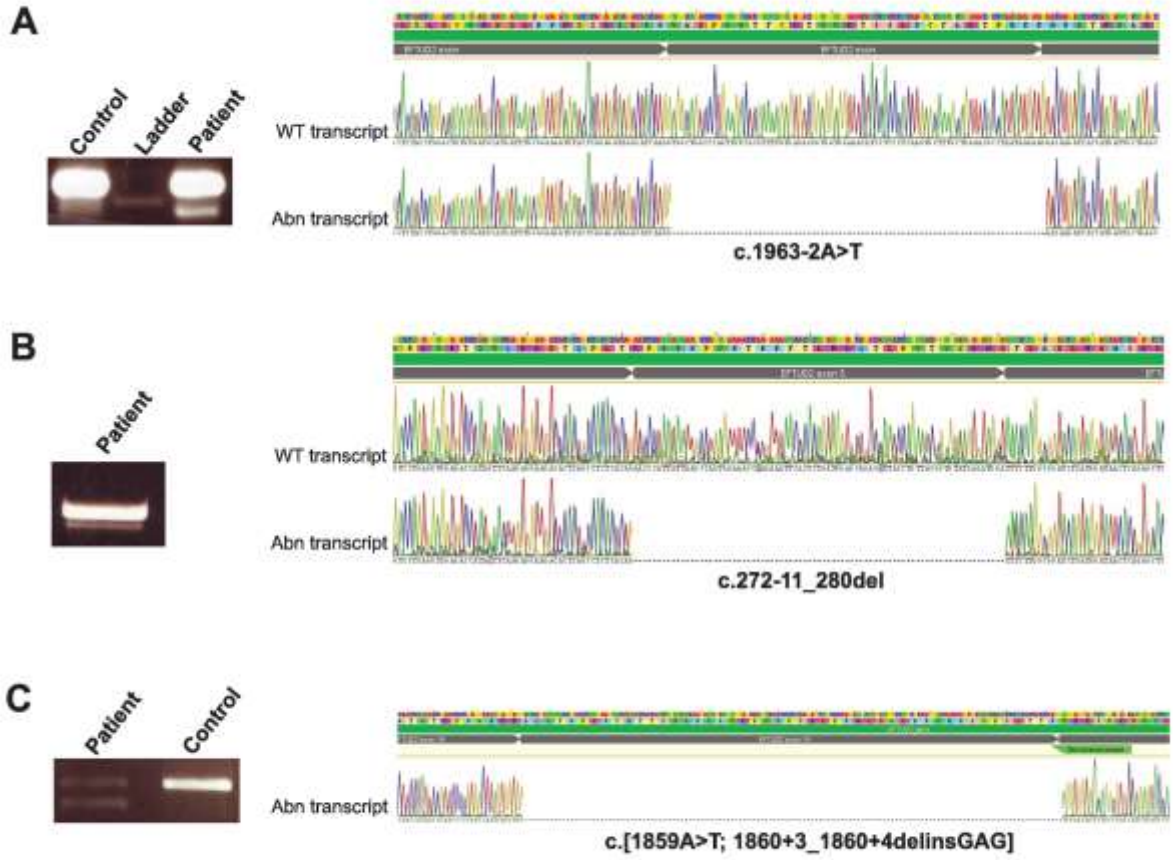
Supp. Figure S1. Confirmation of ovarian mosaicism in kindred 79.

Panel A: Genomic sequencing of intron 24 - exon25 boundary. C/T SNP rs78620114 (at left) is coamplifies with the mutation, c.2496C>G p.(Tyr832*). This SNP is heterogygous in both probands and in their unaffected mother, and homozygous (C;C) in their unaffected father. Both probands have therefore inherited their "T" allele from their unaffected mother, and the "C" allele from their unaffected father. Genomic primers for exon 25 are as previously reported in [Lines et al., 2012].

Panel B: Schematic of allele-specific PCR with alternate forward primers to selectively amplify either allele of rs78620114. The reverse primer (TGTAGGAGCCGAGGTGACTC) is common to all reactions. The forward primers, either 'FP-C' (AGAGAATGGGGCGGTAGGGGACC) or 'FP-T' (AGAGAATGGGGCGGTAGGGGACT) are selective for either the 'C' or 'T' allele of rs78620114, respectively. Position c.2496, the site of the probands' mutation, is coamplified with rs78620114.

Panel C: PCR amplification of leukocyte DNA was performed in the probands and both parents using standard protocols, according to the scheme in Panel B. As expected, FP-T fails to amplify in the probands' unaffected father, whose rs78620114 genotype is C/C.

Panel D: Sequencing of PCR products from Panel C. The 'T' allele of rs78620114 is in phase with the c.2496C>G p.(Tyr832*) mutation in both probands, indicating that the mutation occurs on the maternal allele. Maternal gonadal mosaicism is inferred.



Supp. Figure S2. Reverse transcriptase PCR (RT-PCR) validation of splice site mutations.

RT-PCR was performed on either lymphoblast or fibroblast RNA (as indicated below), products separated by agarose gel electrophoresis, and individual bands excised and sequenced, according to standard protocols. Chromatograms are shown aligned to cDNA reference (NM_004247.3)

Panel A: c.1963-2A>T / r.1963_2045del in individual 60. (Fibroblast RNA; primers: AATGAGGAGGCTCAGATTTTC, GAGCCTTGTCCACCTCAGAGG).

Panel B: c.272-11_280del / r.272_350del in individual 13. (Lymphoblast RNA; Primers: AGGCGAGAGCATCATGGATAC, CAGTATAGCACAGATCTTGGTC).

Panel C: c.[1859A>T; 1860+3_1860+4delinsGAG] / r.1720_1860del in individual 56. (Lymphoblast RNA; Primers: GACTGTGACCCTGATGGC, CATGGTGATCTTGTTCTTC).