Supplementary Material

Figure S1. IGV visualization of homozygous mutation c.53344_53347delAAGT from exome data. All reads show 4 bp deletion, sequence of wild type gene and exon annotation at bottom.

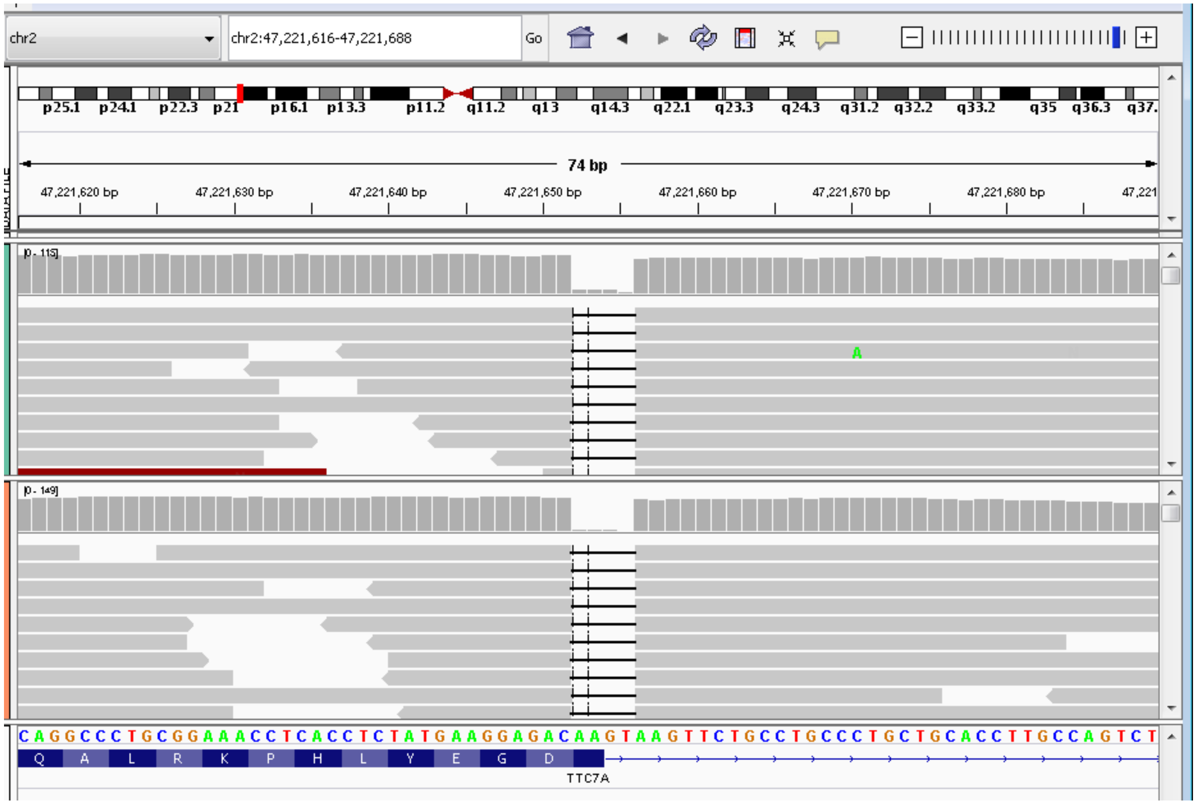


Figure S2. Agarose gel of RT-PCR reaction products from *TTC7A* encompassing exon 7. From left to right, source Hela cell RNA, 100bp size ladder (bottom band 200bp, top band 1000bp, source RNA extracted from human control amnyocytes (band is 780 bp and represents a WT spliced RNA that contains exons 4 to 12 including exon 7), source RNA of amniocytes of affected exome sequenced patient of pedigree 4 (band is 622 bp and represents an RNA that extends from exons 4 to 12 skipping exon 7 deleting 158 bp). See Materials and Methods for details on cells and RT-PCR protocol.

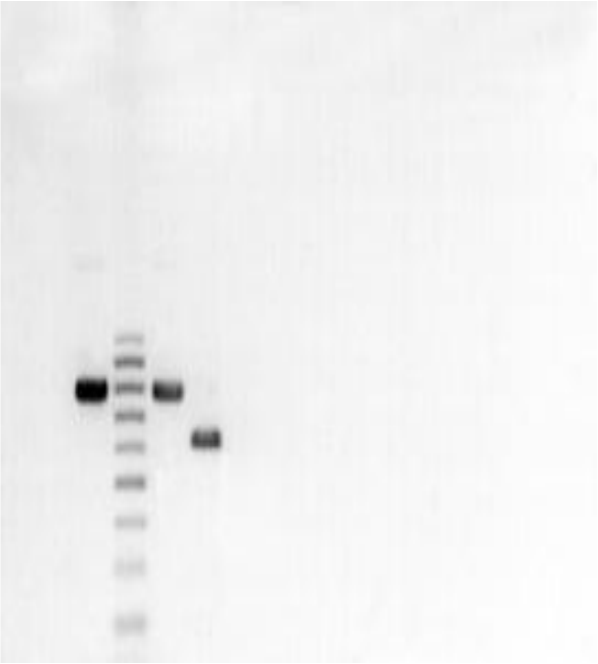
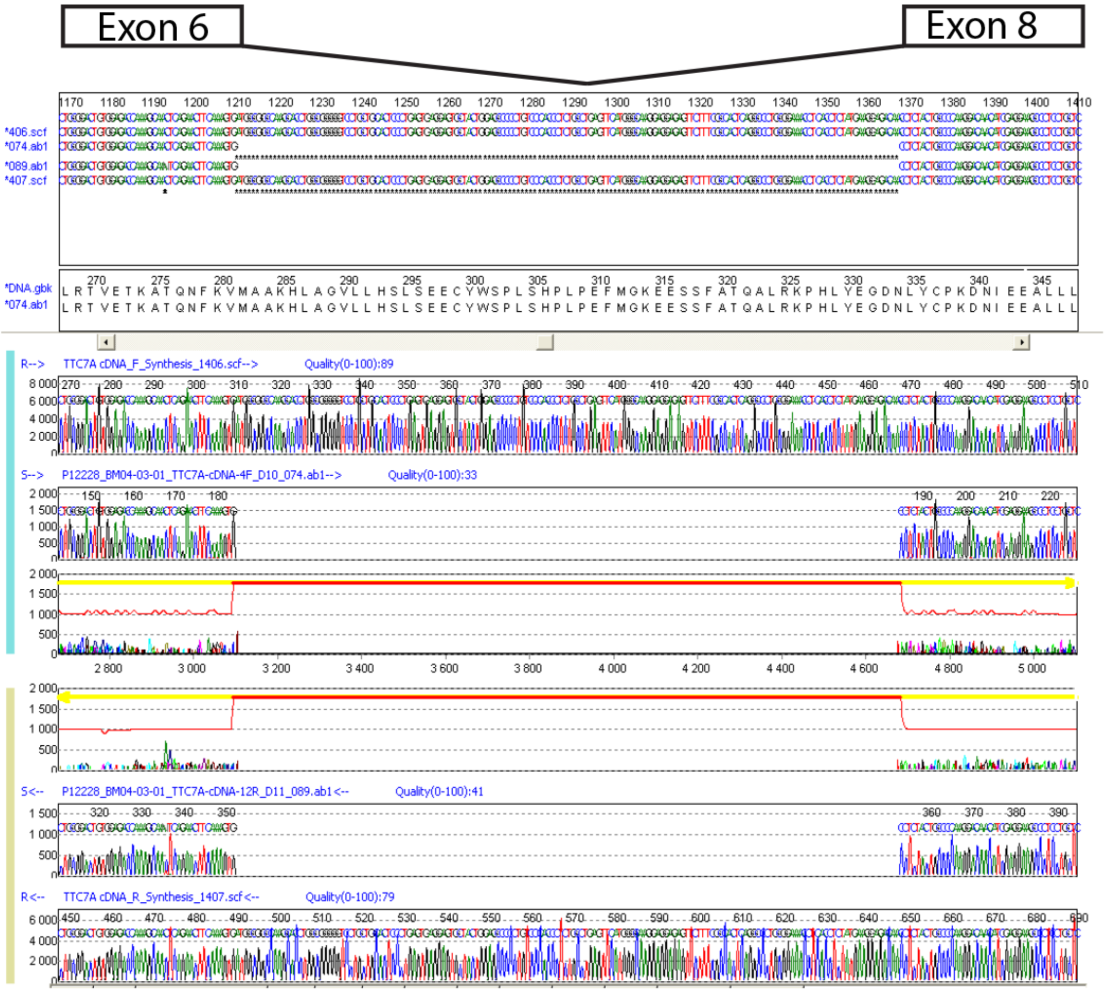


Figure S3. Sequence chromatograms of mutations in TTC7A and exon skipping products. A, homozygous mutation c.53344_53347delAAGT from sampled affected homozygous patient. B, sequence of RT-PCR products from sampled affected patient in family 4, aligned versus *TTC7A* reference cDNA to highlight exon 7 skipping. C, heterozygous mutation c.A133074G; p.L823P. Sequencing by standard PCR-Sanger fluorescent chemistry, visualized with Mutation surveyor. In each panel, top window shows nucleotide sequence of consensus from human genome project above mutant sequence, then amino acid sequence of consensus from human genome project above mutant sequence with exon number annotated. Lower window shows chromatograms for wild type sequence (virtual, generated from consensus genome project annotation), chromatogram from patient, and mutation calling by algorithm. Red arrows point to mutations in patient chromatogram.

A


*C7A.gbk
*269.scf
*018.ab1
53310 53315 53320 53325 53330 53335 53340 53345 53350 53355 53360
C C C T G C G G A A A C C T C A C C T C T A T G A A G G A G A C A A g t a a g t t c t g c c t g c c c t g c t g c a
C C C T G C G G A A A C C T C A C C T C T A T G A A G G A G A C A A G T A A G T T C T G C C T G C C C T G C T G C A
C C C T G C G G A A A C C T C A C C T C T A T G A A G G A G A C A A G T T C T G C C T G C C C T G C T G C A
* * * *
*C7A.gbk
*018.ab1
A L R K P H L Y E G D N
A L R K P H L Y E G D N
325
330
TTC7A_R_Synthesis_53269.scf <--
Quality(0-100):91
P12042_BM01-04_TTC7A-7Rint_H04_018.ab1 <--
Quality(0-100):35
4 650
4 700
4 750
4 800
4 850
4 900
4 950
5 000
5 050
5 100
5 150

B


Exon 6
Exon 8
*406.scf
*074.ab1
*089.ab1
*407.scf
*DNA.gbk
*074.ab1
L R T V E T K A T Q N F K V M A A K H L A G V L L H S L S E E C Y W S P L S H P L P E F M G K E E S S F A T Q A L R K P H L Y E G D N L Y C P K D N I E E A L L L
R--> TTC7A cDNA_F_Synthesis_1406.scf--> Quality(0-100):89
S--> P12228_BM04-03-01_TTC7A-cDNA-4F_D10_074.ab1--> Quality(0-100):33
S<-- P12228_BM04-03-01_TTC7A-cDNA-12R_D11_089.ab1<-- Quality(0-100):41
R<-- TTC7A cDNA_R_Synthesis_1407.scf<-- Quality(0-100):79

C

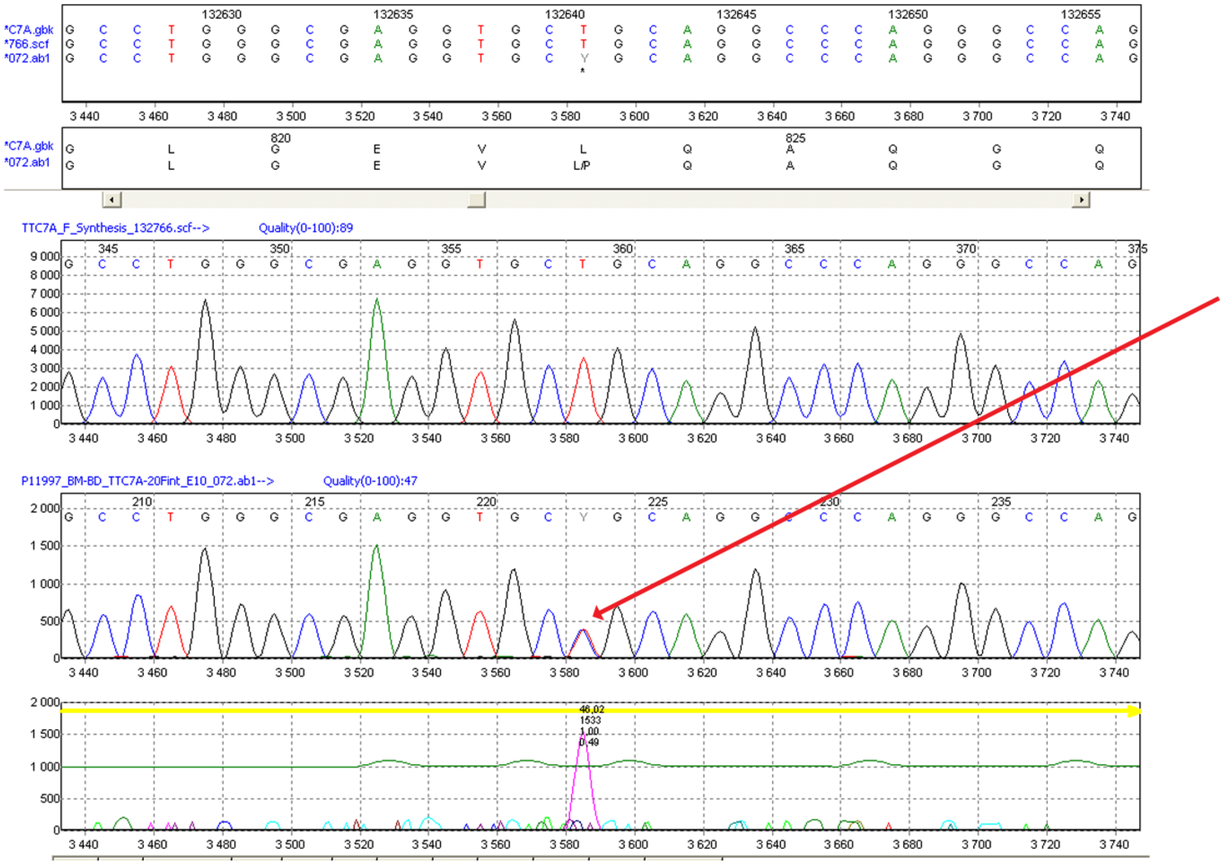

132630
132635
132640
132645
132650
132655
*C7A.gbk G C C T G G G C G A G G T G C T G C A G G C C C A G G G C C A G
*766.scf G C C T G G G C G A G G T G C T G C A G G C C C A G G G C C A G
*072.ab1 G C C T G G G C G A G G T G C Y G C A G G C C C A G G G C C A G
820
825
*C7A.gbk G L G E V L Q A Q G Q
*072.ab1 G L G E V L/P Q A Q G Q
TTC7A_F_Synthesis_132766.scf--> Quality(0-100):89
P11997_BM-BD_TTC7A-20Fint_E10_072.ab1--> Quality(0-100):47
46,02
1533
1,00
0,49

Figure S4. MutationSurveyor visualization of deconvoluted heterozygous mutation c.53344_53347delAAGT from Sanger sequencing of affected individual in pedigree 7. From top to bottom, panels are virtual wild type chromatogram, actual sequence chromatogram of heterozygous patient, deconvoluted non-deleted patient sequence, deconvoluted deleted patient sequence, deconvoluted correctly offset deleted patient sequence, mutation calling by software algorithm.

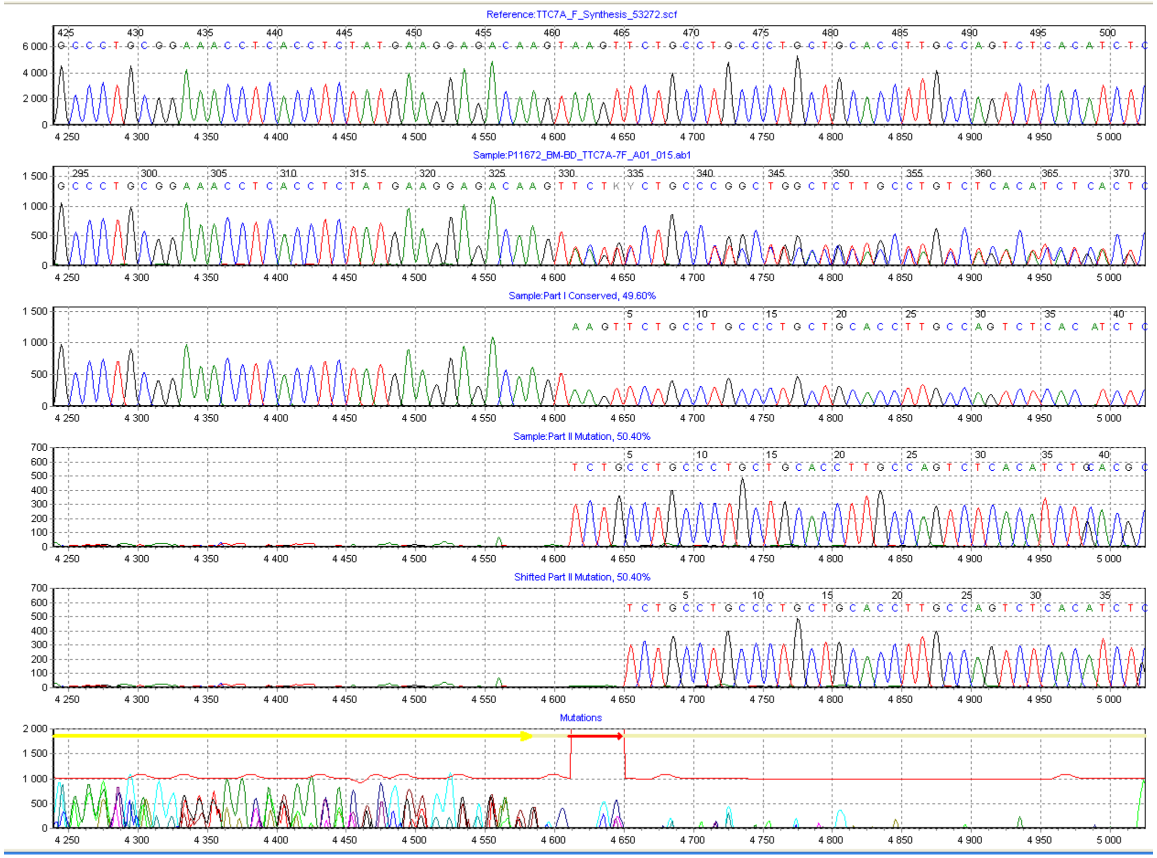


Table S1. Primer sequences for amplification and sequencing of TTC7A coding exons.

exon	PCR primers[1]		sequencing primer[1]	
1	TTC7A-exon1Fint	CTCCGCGCGGGATTAAAGT	idem	
	TTC7A-1R	GAGTCCAGCCAATGCACTGAT	idem	
			TTC7A-1Rint	TGCAGATACCACCATTTCACG
2	TTC7A-2F	CCGAGTCCTGGGAACTCTGTC	idem	
	TTC7A-2R	TGTTGCTCAGGTGACCAAATG	idem	
3	TTC7A-3F	GAGGAGGGGAGTTCTGAGCAA	idem	
	TTC7A-3R	ACCGGCATCCACCTTAGACAC	idem	
4	TTC7A-4F	CTAACACCACCGTCGCTTCAG	TTC7A-exon4Fint	TGCACCAGAGTGTCTGC
	TTC7A-4R	TGGGGGACAGAGAAGGTGACT	idem	
5	TTC7A-5F2	GCCCCTGAACAACTGGGACTGG	idem	
	TTC7A-5R2	GCACGTATGCACAGGCCAGAGA	idem	
6	TTC7A-6F	TCCTGGGCCTCTAAAGTCCTG	TTC7A-exon6Fint	CACTCCAGTTATCGAATGTGTTCA
	TTC7A-6R	TGGGAAAGGATGCTGAGATTG	idem	
7	TTC7A-7F	AGAGGGGTGGGAGCGCCAGT	idem	
	TTC7A-7R	ACCTGGACCCCAACACCTGCCT	TTC7A-exon7Rint	TCTAGTTCATCTGGCTCCTG
	TTC7A-ex7F-court	GGGTCCGAGTGCTTCCCTCT	idem	
	TTC7A-ex7R-court	GGGACAGAACCCTGCGTGAG	idem	
8	TTC7A-8F	GTGCCCATGAATTATGCAGGA	idem	
	TTC7A-8R	CCTCCAAATGAAGCCTTCGAC	idem	
9-10	TTC7A-9-10F	GGGCCTTTGTGGAGATAGGAA	TTC7A-exon10F	ATCACCCTGTGAGATCATTC
	TTC7A-9-10R	CCACAGACTCACACCAGCACA	TTC7A-exon9R	GATGGCAAAGAGCAAATG
11	TTC7A-11F	TTCTCTGCCCTGGACCTCTTC	idem	

	TTC7A-11R	CTCTCTGGAGGCCACAGTTCA	idem	
12	TTC7A-12F	GAGCACAAGGCTTCTGTTTCG	idem	
	TTC7A-12R	AGTCTGGGGGAAACAGTGGAA	idem	
13-14	TTC7A-13-14F	CTGCAGAGCTGTGTGCTTTGA	idem	
	TTC7A-13-14R	TGGAAGGCGAGGACACAAATA	idem	
15	TTC7A-15F	GGGCTCCCTTGAGTTGAGTGT	idem	
	TTC7A-15R	CCTGTCCTCACCCCTGTCTCT	idem	
16	TTC7A-16F	GCGAGTTAGGGAGGTGAGCAT	idem	
	TTC7A-16R	CGCTCTCATTCAAAAGCCTCA	idem	
17	TTC7A-17F	AGTGAGGCTGTCCCATTCTCC	TTC7A-exon17Fint	GTGAGGCTGTCCCATTC
	TTC7A-17R	AAGATCAGCCCCAGAAGTTGC	idem	
18	TTC7A-18F	CAGCTGTGGGTGAGAGGACAT	idem	
	TTC7A-18R	GCCAGAGGCGAGGACTCTGTA	idem	
19	TTC7A-19F	TGTGTCCCATGCTGTGATTTG	idem	
	TTC7A-19R	CCAGGGAACAGCAGTAGCAAG	idem	
20	TTC7A-20F	AGAGCTCCTGCAGTGGGTTTC	TTC7A-exon20Fint	AAGGCACAGTCACTTAACAG
	TTC7A-20R	CTGTGCTGAGAGTGGGGAGTG	TTC7A-exon20Rint	CATGGGTGAGGGTGAAG

1 All sequences are indicated in the 5' to 3' direction

Table S2. Primer sequences for reverse transcription, amplification and sequencing of TTC7A RNA encompassing exon 7.

TTC7A_cDNAex3F	aggcttttgtcatcaaaggcc	(mainly in exon 3 with few nucleotides overlapping the junction of exon 3-4)
TTC7A_cDNAex4F	ctgcaggaattggagaagacc	(mainly in exon 4 with few nucleotides overlapping the junction of exon 4-5)
TTC7A_cDNAex9R	ccacatctcgagttgccatg	(mainly in exon 9 with few nucleotides overlapping the junction of exon 8-9)
TTC7A_cDNAex12R	gtgctctgcttcctctagcc	(mainly in exon12 with few nucleotides overlapping the junction of exon 11-12)