SUPPLEMENTAL DATA

EXTENDED CLINICAL DESCRIPTION OF PATIENTS.

Cases 1 and 2 were the offspring of a consanguineous couple from Reunion Island that previously had a healthy son. Prenatal ultrasonography at 23 weeks of pregnancy of patient 1 showed shortness of all limbs, scalp edema, polydactyly, echogenic kidneys and low-set ears. MRI revealed frontal lobe cortical atrophy with external hydrocephalus. Biparietal diameter and echocardiography were normal. At birth anthropometric measures for weight, length and head circumference (HC) were -0.5 SD, -1.1 SD and -0.4 SD respectively. At this time micromelia, postaxial polydactyly of hands and feet, narrow chest and severe central and peripheral hypotonia were noted. Feeding difficulties were reported in the first weeks of life requiring forced-feeding and sparse, fine textured hair was observed at 9 months of age. Renal ultrasound showed normal renal corticomedullary differentiation and minor pyelectasis. The child was operated upon for bilateral inguinal hernia and he also had hypospadias. Importantly, no craniosynostosis was detected. The patient had several acute bronchiolitis episodes and at age 13 months developed severe pneumonia, eventually dying of cardiorespiratory arrest. For case 2 ultrasonography at 12 weeks of gestation showed hygroma colli associated with hexadactyly. A ventricular septal defect was also observed. This boy was born near term with birth weight +1.9 SD, length -0.3 SD and HC +2.3 SD. MRI showed scaphocephaly with partial cerebellar vermis hypoplasia. Kidneys and liver were normal. At birth, narrow chest, bilateral postaxial polydactyly of hands and feet, practically absent eyebrows and dysplastic nails were observed. X-rays showed short ribs, mesomelic shortening of the upper and lower limbs, hypoplastic distal phalanges, bilateral absence of ossification of middle and distal phalanges of 2^{nd} to 5^{th} toes and

bilateral bending of the proximal end of the ulna. The infant experienced bronchiolitis episodes and died at 5 months of age from severe respiratory distress leading to heart failure.

Case 3 is a 7 year-old male born to non-consanguineous parents from San Marino. He was identified with disproportionate short stature, bilateral postaxial polydactyly of hands and unilateral of feet and dysplastic nails and teeth. At 7 years of age weight, height, and HC were -3.9 SD, -3.8 SD, and -2.5 SD respectively. Dental abnormalities included small and absent teeth with enamel hypoplasia. He also had accessory labial frenulae, labiogingival frenulum hypertrophy, absence of upper mucobuccal fold, chronic renal dysfunction and hepatic fibrosis. Renal and liver abnormalities were not present at birth by renal ultrasound, but detected at 4-5 years of age. His older sister (case 4) had similar features including short stature, short limbs, bilateral postaxial polydactyly of hands and feet, chronic renal failure, multiple oral frenula, absence of upper mucobuccal fold and dental defects consisting in teeth fusion and hypodontia. At 8.5 years, her weight was: -3.0 SD, height:-2.9 SD, and HC: -3.4 SD. Ophthalmologic examination and 2-Dimensional color-Doppler echocardiography were normal in both sibs. They also had normal craniofacial features with no sagittal synostosis, normal hair and normal intellectual development.

Case 5 is a 5-months old Egyptian girl the second child of first cousin parents. On clinical examination she had disproportionate short stature with mesomelic shortening of limbs, narrow chest, postaxial polydactyly in both hands and feet, brachydactyly and mildly hypoplastic nails. Anthropometric measures for height: -4.1 SD and weight: -3.9 SD were below normal with normal HC: -1.3 SD and her upper-to-lower segment ratio

was 2.11, denoting short lower limbs. She also had dolichocephaly, high prominent forehead, sparse hair and eye brows, medial epicanthic folds, short upturned nose and short neck. Orodental manifestations included long philtrum, multiple labial frenulae, serrated alveolar ridge, absence of upper mucobuccal fold, short lingual frenulum and bifid tip of the tongue, narrow high arched palate and short uvula. Echocardiography was normal and abdominal ultrasound revealed hypoplastic left kidney.

Supplemental table S1. Pipeline used to analyze NGS-data.

Filter	Number of variants
Numbers of variants called	32,780
Not in dbSNP	2,668
Coverage of at least 10 reads (DP>=10)	2,140
Eliminated variants annotated as downstream/upstream/intergenic/within non coding genes	1,739
Variants with QUAL>100 (less than 0,01 probability that the variant does not exist)	1,534
Neither present in 1000G nor in ExAC with allele frequency higher than 1/10 000	774
Number of variants following a recessive model of inheritance (homozygous or compound heterozygous):	120
- variants inside ROH only shared by the two affected siblings of family 1	3
- missense, nonsense or splice site variants not in ROH	18
Number of candidate variants	21 (15 genes)

1000G: 1000 genomes database (http://www.1000genomes.org); ExAC: Exome Aggregation Consortium database (http://exac.broadinstitute.org); ROH: Regions of Homozigosity.

Supplemental table S2. Candidate variants after filtering.

Chromosome	Position	Ref	Alt	Zigosity	Gene	Protein Effect	UniProtKB	Polyphen2 Prediction	OMIM
1	169670709	A	T	Homozygous	*SELL*	L358S	P14151	PROB DAMAGING	
1	197889106	T	G	Homozygous	*LHX9*	M60R	Q9NQ69	BENIGN	
2	20182313	A	T	Homozygous	*WDR35*	-	-	N.P.	613610, 614091
2	31154981	C	G	Heterozygous	*GALNT14*	K337N	Q96FL9	PROB DAMAGING	
2	31178832	T	G	Heterozygous	*GALNT14*	K160Q	Q96FL9	BENIGN	
2	114017062	C	T	Homozygous	*PAX8*	-	-	N.P.	
2	122227895	T	C	Homozygous	*CLASP1*	-	-	N.P.	
3	9781443	C	T	Heterozygous	*BRPF1*	R454C	P55201	BENIGN	
3	9784653	A	C	Heterozygous	*BRPF1*	K670T	P55201	BENIGN	
3	182584081	A	G	Homozygous	*ATP11B*	K490R	Q9Y2G3	BENIGN	
5	52157303	G	A	Heterozygous	*ITGA1*	V69I	P56199	BENIGN	
5	52225518	T	G	Heterozygous	*ITGA1*	L920V	P56199	BENIGN	
9	128305386	T	C	Heterozygous	*MAPKAP1*	I304V	Q9BPZ7	BENIGN	
9	128347900	C	G	Heterozygous	*MAPKAP1*	R202T	Q9BPZ7	BENIGN	
9	130101926	T	C	Homozygous	*GARNL3*	L344P	Q5VVW2	PROB DAMAGING	
11	110561324	C	T	Homozygous	*ARHGAP20*	R45K	Q9P2F6	BENIGN	
12	106895192	G	T	Homozygous	*POLR3B*	G1026C	Q9NW08	PROB DAMAGING	614381
19	11304163	T	G	Heterozygous	*KANK2*	K198T	Q63ZY3	PROB DAMAGING	616099
19	11304385	A	G	Heterozygous	*KANK2*	V124A	Q63ZY3	PROB DAMAGING	616099
19	52538285	T	C	Heterozygous	*ZNF432*	K216R	O94892	POSS DAMAGING	
19	52538322	T	A	Heterozygous	*ZNF432*	N204Y	O94892	BENIGN	

Chromosome positions are referred to GRCh37/hg19 assembly. The reference (Ref) and alternative (Alt) allele are indicated. Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) was used to predict the protein effect of each variant. PRO: Probably; N.P.: not predicted; POSS: Possibly.

Supplemental table S3.Candidate variants inside the regions of homozygosity only shared by the two affected siblings of family 1.

Chromosome	Position	Reference allele	Alternative allele	Gene	Variant type
2	20182313	A	T	*WDR35*	intronic
2	114017062	C	T	*PAX8*	intronic
2	122227895	T	C	*CLASP1*	intronic

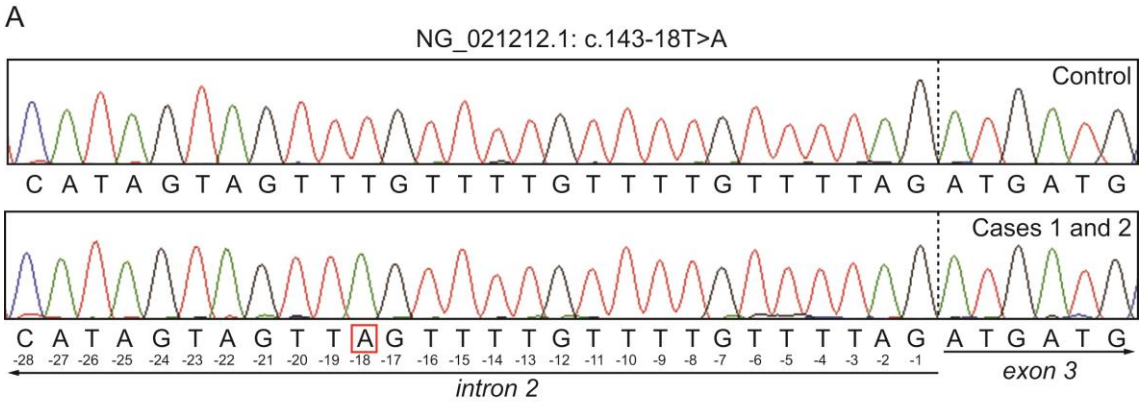
Chromosome positions are referred to GRCh37/hg19 assembly. Regions of homozigosity shared by the two affected siblings and not present in the unaffected brother of family 1: chromosome 2: 16000000-23100000; chromosome 2: 103000000-131000000; chromosome 4: 157000000-168000000 and chromosome 5: 0-13700000.

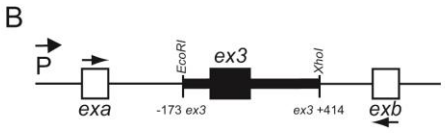
Supplemental table S4. Clinical features of the additional 10 EvC families tested for pathogenic variants in *WDR35*.

Fam.	Consanguinity	Gender	S/FA	Clinical findings	Radiological findings	*EVC*	*EVC2*
I	Unknown	M	FA	Hands polydactyly	Short scapula, short ribs, shortening of radius and ulna, hands polydactyly, narrow thorax, shortening of fibula and tibia	Negative	Negative
II	No	M	S	Bilateral hands postaxial polydactyly, unilateral postaxial polydactyly of feet, absence of CHD, gingival hypertrophy, labio-gingival adherence, genitourinary anomalies	IUGR, short limbs, polydactyly	Negative	Negative
III*	Unknown	F	S	Corpus callosum dysplasia, IUGR, atrial septal defect with single atrium		Negative	Negative
IV	N.A.	N.A.	N.A.	N.A.	N.A.	Negative	Negative
V	N.A.	N.A.	N.A.	Failure to thrive, bilateral hands and feet preaxial polydactyly, short limbs, frontal bossing, low-set and posteriorly rotated ears, micrognathism, cleft lip and palate, multiple mouth frenulae	Rhizomelic skeletal dysplasia, clubfeet, hypoplastic thorax, complex CHD, Dandy-Walker malformation	Negative	Negative
VI*	N.A.	N.A.	N.A.	Small stature, short limbs, bilateral hands polydactyly, unilateral polydactyly of the feet, ectodermal dysplasia: nail dysplasia, deciduous teeth, conical teeth, double cusp incisors, enamel hypoplasia, malocclusion, narrow thorax, atrioventricular canal defect	N.A.	Negative	Negative
VII	No	M	FA	See manuscript description (Family 2)	See manuscript description (Family 2)	Negative	Negative
VIII	N.A.	M	S	Bilateral hands and feet polydactyly, narrow thorax, absence of CHD, absence of oral manifestations, absence of ectodermal dysplasia	N.A.	Negative	Negative
IX	N.A.	N.A.	N.A.	N.A.	N.A.	Negative	Negative
X	N.A.	N.A.	N.A.	N.A.	N.A.	Negative	Negative

Fam.: Family; S/FA: sporadic/familial; CHD: congenital heart disease; IUGR: Intrauterine growth restriction; M: male; F: Female; N.A.: data not available. *: Cases excluded for pathogenic changes in *EVC* or *EVC2* reported in D'Asdia et al., *Eur J Hum Genet* (2013).

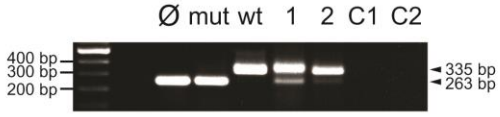
Supplemental figure S1





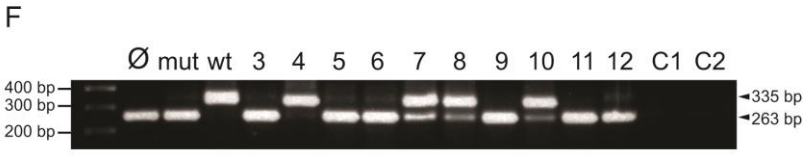
C

Lane	Nucleotide change
mut	c.143-18T>A (family 1 variant)
wt	c.143-18T (normal sequence)
1	c.143-18A>G
2	c.143-18delA



E

Lane	Nucleotide change
3	c.143-18T>A c.143-22A>G
4	c.143-18T>A c.143-21G>A
5	c.143-18T>A c.143-20T>C
6	c.143-18T>A c.143-19T>C
7	c.143-18T>A c.143-21_-19delGTT
8	c.143-18T>A c.143-17G>A
9	c.143-18T>A c.143-16T>C c.143-15T>C c.143-14T>C c.143-13T>C
10	c.143-18T>A c.143-17delG
11	c.143-18T>A c.143-16_-13delTTTT
12	c.143-13T>A



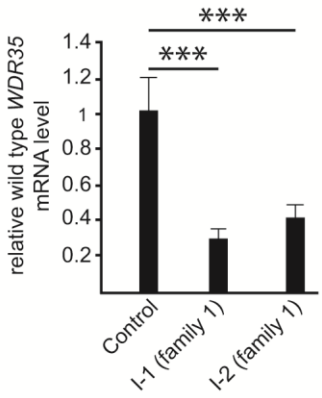
Supplemental figure S1. The *WDR35* pathogenic variant found in family 1 creates an intronic splicing silencer (ISS).

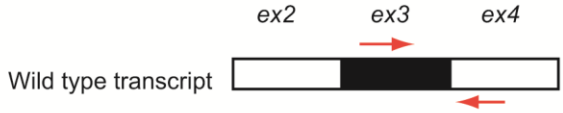
A. Genomic sequence chromatograms showing normal (control) and cases 1-2 sequence containing the c.143-18T>A homozygous variant (red squared nucleotide). Nucleotides positions of intron 2 are numbered. **B**. Graphical representation of the minigene

construct used to assess the effect on splicing of the c.143-18T>A variant. A genomic fragment corresponding to *WDR35* exon3 (*ex3*) and flanking intronic sequences (-173 exon3 to exon3+414) harboring the c.143-18T>A transversion or normal sequence was cloned into pSPL3 using the indicated restriction sites. Exa and exb are the plasmid artificial exons, arrows designate the location of RT-PCR primers and P the site of the promoter. **C-F**. RT-PCR results representative from at least three independent transfections using minigenes carrying either wild type sequence (wt), or the c.143-18T>A variant (mut), or the mutations described on tables C and E generated by directed mutagenesis. Of note, sequencing of RT-PCR products from COS-7 treated with the wt minigene showed preferential used of the normal 3'-splice site of exon 3, although to a lesser extent an alternative acceptor site located at position –23 to -21 was also utilized. Ø, C1 and C2 are RT-PCR results from cells transfected with the empty plasmid, or from non-transfected cells, or from a no cDNA template respectively.

Supplemental figure S2

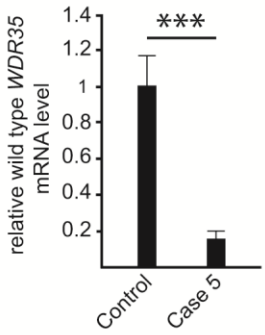
A

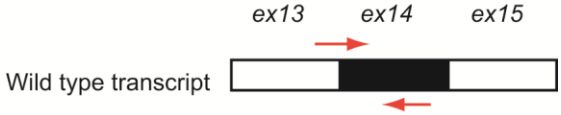




WDR35 wild type transcript family 1	
Forward	CCCCAGTAACCTTTCTATGAATCAG
Reverse	TGATACTGCTCATTCCATGTTACAAC

B





WDR35 wild type transcript case 5	
Forward	CGAAAAGAAGGGAGAGAAAGAATTTA
Reverse	GCACACCATCCATTGATCCA

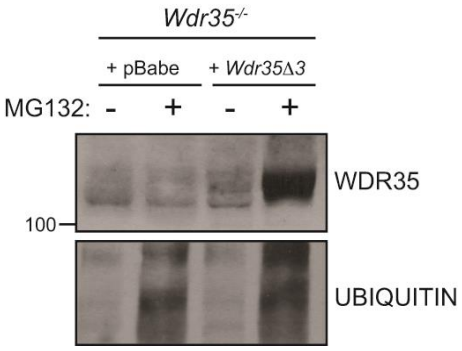
C

GUSB	
Forward	AAACGATTGCAGGGTTTCAC
Reverse	CTCTCGTCGGTGACTGTTCA

Supplemental figure S2. qRT-PCR analysis of the levels of *WDR35* wild type transcript in families 1 and 3 in whole-blood.

Relative quantification of wild type *WDR35* mRNA in the parents of family 1 (A) and case 5 (B) normalized against *GUSB* expression. Localization and sequence of the primer pairs used for each case are shown on the right. The forward primer in B spans the exon13-exon14 junction. Primers to amplify *GUSB* are in C. Graphs are the result of 2 independent experiments with each sample run in triplicates. *** $P<0.001$

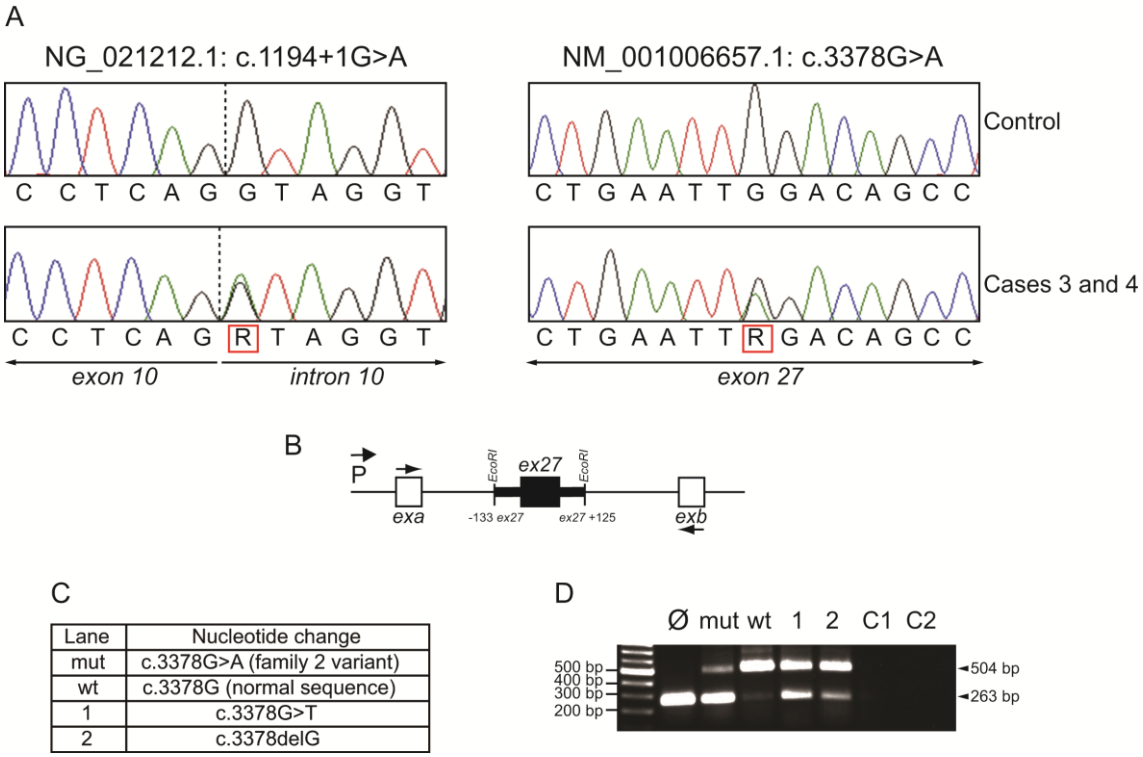
Supplemental Figure S3



Supplemental figure S3. In frame deletion of exon3 of *Wdr35* alters the stability of the resulting protein.

Wdr35$^{-/-}$cells retrotransduced with the empty vector (pBabe) or the *Wdr35Δ3:FLAG (Wdr35Δ3)* construct treated with MG132 (+) or its vehicle (-; DMSO) and analyzed by Western blotting (n=2). WDR35Δ3 protein levels increased after MG132 incubation suggesting that this protein is thermodynamically unstable. Anti-UBIQUITIN was used to confirm proteasome inhibition.

Supplemental figure S4



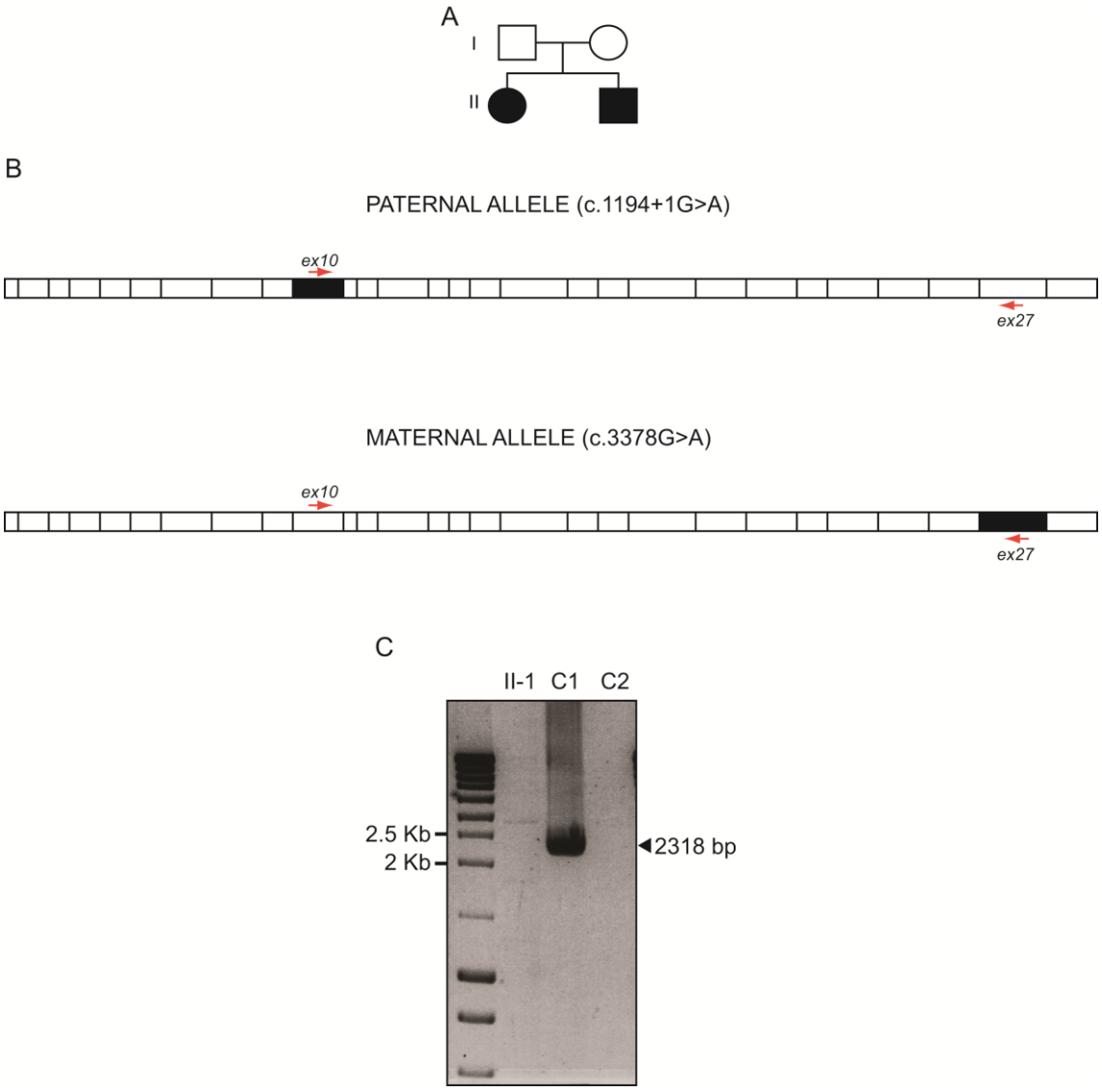
Lane	Nucleotide change
mut	c.3378G>A (family 2 variant)
wt	c.3378G (normal sequence)
1	c.3378G>T
2	c.3378delG

Supplemental figure S4. Characterization of the *WDR35* splicing variants identified in the patients of family 2.

A. Control and cases 3-4 genomic sequence chromatograms showing the two variants found in these cases in the heterozygous state. The paternal change is on the left and the maternal on the right. Altered nucleotides are squared in red. **B**. Graphical representation of the minigene construct used to test the c.3378G>A variant in COS-7. Exon 27 (*ex27*) and flanking intronic sequences were cloned into pSPL3 using the indicated restriction sites. Exa and exb designate the two pSPL3 artificial exons and the arrows indicate the position of RT-PCR primers. P is the promoter. **C-D** RT-PCR results from 2 independent transfections either with the wild type minigene (wt), with the c.3378G>A minigene (mut), or with minigenes carrying the mutations described in the table on the left that were generated by directed mutagenesis (C). Ø is the empty pSPL3 vector control, C1 is a control from untransfected COS-7 and C2 is a no cDNA

negative control. Note residual inclusion of exon 27 (504bp band) in the lane corresponding to the c.3378G>A variant (mut).

Supplemental figure S5

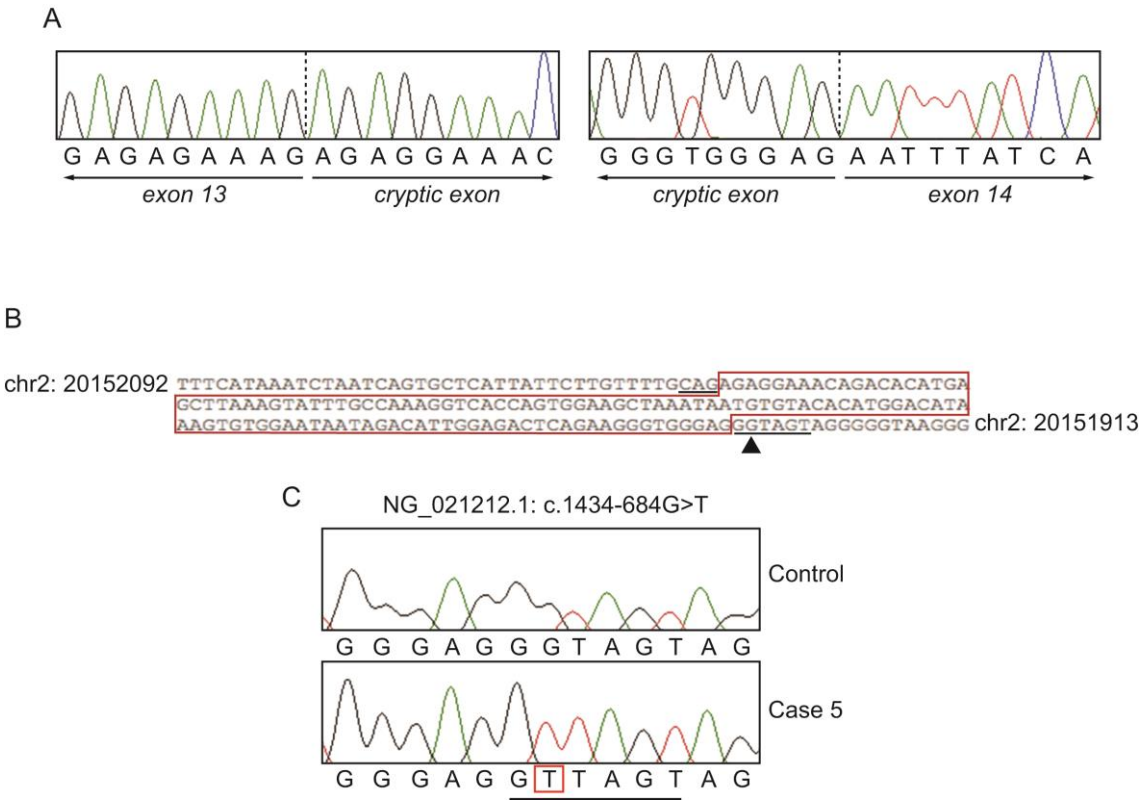


Supplemental figure S5. RT-PCR analysis showing absence of wild type *WDR35* transcript in patients from family 2.

A. Pedigree of family 2. **B.** Squematic representation of the paternal (top) and maternal (bottom) *WDR35* alleles of patients from family 2. Exons which are skipped in the paternal and maternal transcripts owing to the corresponding variant are shown with

black boxes. **C.** RT-PCR from case 4 (family 2, II-1) and an unrelated control (C1) using the combination of primers indicated in B (red arrows) and the same cDNA templates used in Figures 1K-L demonstrating amplification of the expected fragment in the control individual but not in the patient. C2 is a negative control with no template.

Supplemental figure S6



Supplemental figure S6. The c.1434-684G>T variant of family 3 activates a cryptic exon in *WDR35*.

A. Sequencing chromatograms corresponding to the longer RT-PCR product from case 5 (Figure 1Q) revealing incorporation of a new exon between exons 13 (left) and 14 (right) of the *WDR35* transcript. **B**. Normal sequence from *WDR35* intron 13 (chr2: 20152092-20151913; GRCh37/hg19 genome assembly). The cryptic exon is squared and the acceptor splice site underlined. The reference sequence which is converted into a donor splice site by the c.1434-684G>T change is also underlined. The arrowhead

designates the nucleotide position that is altered in the patient (c.1434-684G>T). **C**. Genomic sequence chromatograms from case 5 and a control individual. The nucleotide which is changed in case 5 is squared in red and the newly created donor splice site underlined.