

1 **Supplementary Information:**

2

3 **Case Reports**

4 Female JT had microcytic anaemia but was otherwise normal. She was
5 found to have an interstitial deletion of 267.4 kb that arose *de novo* on the maternal
6 chromosome and the breakpoint was mapped by FISH and Southern blotting before
7 being characterised at the sequence level by inverse PCR and found to have
8 occurred within an Alu repetitive element (Horsley et al., 2001).

9 Female OY had microcytic anaemia but was otherwise normal. Results
10 of fluorescence *in situ* hybridisation (FISH) show she has a deletion on the paternal
11 chromosome 16 which arose *de novo* and that the breakpoint lies within cosmid
12 419C1 (Supplementary Table 1), revealing a truncation of ~308 kb. The breakpoint
13 was refined by Southern blotting and identified by cloning and DNA sequencing of
14 telomere-anchored amplification products. DNA sequence analysis shows telomeric
15 repeats directly joined to the unique sequence (Figure 1). There is a 3 bp ambiguity,
16 suggesting a minimal telomerase-binding site contributed to the stabilisation of this
17 breakpoint by addition of a telomere. The last unique base is position 308,543 bp,
18 interrupting the second intron of *AXIN1* (Figure 2). There are no repeats within 300
19 bp either side of the break.

20 Female patient CS was initially referred at the age of 2 months because
21 of an abnormal head shape, which was shown by three dimensional computed
22 tomography scan of the skull to be associated with bicoronal craniosynostosis.
23 Screening known craniosynostosis-associated genes by DNA sequencing and for
24 copy number variation by MLPA revealed no pathogenic variants. However,
25 genome-wide microarray analysis (Agilent ISCA 60K oligoarray) revealed a ~290 kb
26 deletion, affecting 15 coding genes, between 36,766 bp and 328,247 bp on
27 chromosome 16, which was found to have been inherited from the patient's
28 unaffected father. CS was reported to have normal developmental milestones at the
29 age of 8 months and there were no concerns raised about her eating, sleeping or
30 any aspect of her behaviour. She attended normal preschool from the age of four
31 and on follow-up at the age of 5 years there were no reported concerns about her
32 development. Although the craniosynostosis in this patient is likely to be genetic in
33 origin, given the absence of this phenotype in all other cases with 16p13.3 deletions,
34 including her transmitting father, it is unlikely to be related to her chromosome 16

1 deletion; neither the patient nor her father have any abnormalities indicative of
2 broader ATR-16 syndrome.

3 Male BF was reported found to have α -thal trait and his deletion was
4 identified by MLPA and characterised by low coverage WGS to be between 166,680
5 bp and 342,681 bp (Heireman et al., 2019). This patient was one of six confirmed
6 deletion carriers in this family, one of whom (Patient 6) has symptoms consistent
7 with ATR-16 syndrome, however, this did not segregate with the deletion and the
8 authors conclude this to be unrelated to the chromosome 16 deletion as the
9 remaining carriers exhibited only α -thalassemia.

10 Female CV (patient F in Coelho et al., 2010) presented with microcytic
11 anaemia suspected of being α -thalassemia and was found by MLPA to have a
12 deletion of ~177 kb including the entire α -globin locus and extending beyond the
13 TMEM8A gene. This individual had no abnormalities beyond the α -thalassemia.

14 Individual AB was referred with a suspected haemoglobinopathy and
15 was found by MLPA to harbour a terminal chromosome 16p deletion of with a
16 maximum extent of 400 kb (Harteveld et al., 2005). This patient was not reported to
17 have any abnormalities beyond α -thalassemia.

18 Male LA presented with α -thalassemia with language delay and
19 hypotonia. However, he has no developmental delay or skeletal abnormalities. This
20 patient also has a brother who harbours the same deletion and has significant
21 developmental delay including speech delay, reduced attention span, behavioural
22 problems and seizures. Microarray analysis revealed this patient to have an
23 interstitial deletion of ~408 kb.

24 Individuals TY(MI) and TY(Mi) are father and daughter respectively and have
25 no abnormalities apart from α -thalassemia. FISH suggests the 16p breakpoint lies
26 within cosmid 338H10 (Supplementary Table 1), indicating a truncation of ~600 kb.
27 The deletion was refined by Southern blotting and identified by Sanger sequencing
28 of cloned telomere-anchored amplification products to be at 596,289 bp (Figure 1),
29 in the second intron of *RAB40C* (Figure 2). The presence of telomeric repeats
30 added directly to the breakpoint shows telomere mediated healing, however, there is
31 no overlap at the breakpoint junction. The break occurs at a position 16 bp within
32 the third iteration (of 6.5) of a 66 bp tandem repeat sequence. The repeat contains 2
33 ORFs, one similar to alpha collagen the other similar to AHA1, activator of heat
34 shock 90kDa protein ATPase homolog 2 (yeast). These repeats are not present
35 elsewhere in the genome. There is a long tandem repeat similar to MLT1E1 or

1 MLT1E2 located 221 bp centromeric of the breakpoint and 366 bp telomeric of the
2 break there is an LTR variant (L1ME3A). It is possible that the presence of these
3 repeated sequences contributed to the chromosomal leading to this deletion.

4 Female YA had speech delay (no words by the age of 2 years) and her
5 development was delayed by 6-12 months. She has some facial dysmorphism with
6 mild frontal bossing, prominent eyes, flat nasal bridge, low set ears and
7 macroglossia. She also had short stature, inverted nipples, supernumerary nipples
8 and a bifid uvula. YA showed evidence of microcytic anaemia and because of this
9 she was referred to our laboratory for genetic testing. FISH studies showed a
10 deletion at the tip of chromosome 16 with breakpoint at ~750 kb (between cosmid
11 clones C444G9 and C335H7 – Supplementary Table 1). The maternal
12 chromosomes appeared normal, however, as the father was lost to follow-up
13 parental origin could not be established. High-resolution microarray refined the
14 breakpoint to ~747,840 bp (Supplementary Figure 3) ~5 kb 5' of the *MSLN* gene in
15 an area containing L1MB7 and L1MB8 LINE repeats and AluSx and AluSq/x SINE
16 repeats that are likely to have contributed to the rearrangement.

17 Female patient BA was previously reported (Daniels et al., 2001) to have no
18 physical abnormalities but does have speech delay and a lack of spatial awareness
19 and visual memory. Cloning and DNA sequencing of telomere-anchored
20 amplification products shows the breakpoint to be at 762,272 bp (Figure 1)
21 disrupting the *MSLNL* gene by removing the last four exons. The break appears to
22 be have been healed by telomerase as TAACCC repeats are immediately adjacent
23 to the breakpoint and there is a potential 1 bp overlap. The break occurred at a
24 position 65 bp into an AluY repeat, which may have contributed to an instability in
25 this region.

26 Male patient GZ had surgery to repair pyloric stenosis shortly after birth and
27 had persistent microcytic anaemia, however, there was no developmental delay nor
28 any other abnormalities related to ATR-16 syndrome (Harteveld et al., 2007). MLPA
29 revealed this patient to have a terminal deletion with a maximum extent of 900,907
30 bp with the breakpoint disrupting the *LMF1* gene.

31 Individuals TN(Pe) and TN(AI) are brothers and TN(Pa) is their mother. All
32 three affected family members suffered from mild intellectual delay and
33 developmental delay (6 months to 1 year delayed speech and walking). This family
34 have been previously reported (Daniels et al., 2001), however, in this work we have
35 identified the breakpoint using monochromosomal somatic cell hybrid mapping

1 followed by DNA sequencing of telomere anchored amplification products to reveal
2 the lesion to be at 966,713 bp, (Figure 1). We also show that this deletion arose *de*
3 *nov*o on the maternal allele in Tn(Pa). The break interrupts the third exon of the
4 *LMF1* gene (Figure 2) with no repeats in the vicinity. The break appears to have
5 been healed by telomerase as there are TAACCC repeats joined with 3 bp of
6 overlap suggesting a minimal telomerase recognition site which facilitated the
7 telomere mediated healing (Figure 1). Whole genome sequencing of all three
8 affected individuals revealed no rare coding polymorphisms on the extant
9 chromosome 16 allele. However, all three affected individuals harboured a R12X
10 variant in *SMAD6*, a gene which has previously been associated with developmental
11 delay with variable penetrance.

12 The five individuals designated with the prefix F are from a Brazilian family
13 reported in 2008 who were found to harbour a deletion of ~931 kb identified by
14 MLPA (Bezerra et al., 2008). This deletion disrupted the *SOX8* gene and none of
15 the family members showed any of the broader phenotypic abnormalities of ATR-16
16 syndrome. This was the first reported case that showed haplo-insufficiency for
17 *SOX8* in humans doesn't cause phenotypic abnormalities.

18 Female patient GIB was initially presented with a mild hypochromic microcytic
19 anaemia and the presence of HbH inclusions (Gibson et al., 2008). Developmental
20 delay was diagnosed at the age of 2 years, however, she did not have any
21 dysmorphic features at this stage. By the age of 6 years craniofacial abnormalities
22 were reported including a high forehead, flattening of the maxilla and a high nasal
23 root and bridge together with up-slanting palpebral fissures. Intellectual abilities
24 were reported as being around the 4th centile. Together these features are all typical
25 of the broader ATR-16 phenotype. Microarray and FISH analyses showed a *de novo*
26 interstitial deletion of ~900 kb in the subtelomeric region of chromosome 16p13.3
27 likely to be responsible for causing the abnormalities present in patient Gib.

28 Patient SH(Pa) exhibited motor delay throughout childhood, he had an IQ of
29 48 when tested and has facial dysmorphism including micrognathia, maxillary
30 hypoplasia and a high, arched palate. He also has skeletal abnormalities including
31 clinodactyly, tapering fingers with narrow nails, a short 4th metacarpal, intoed gait, pes
32 planus and genu valgus. Other abnormalities include crypto-orchidism and a
33 hypoplastic scrotum. Monosomy for 16p13.3 was identified by FISH, with the break
34 being found to be between clones C349E11 and C344F5, (Supplementary Table 1) and
35 subsequently refined by Southern blotting and inverse PCR to obtain the breakpoint

1 sequence. This revealed the rearrangement to be an interstitial AluY mediated
2 1,098,548 bp deletion (between ~34037 bp to ~1132584 bp), the breakpoints of which
3 do not directly disrupt a gene (Figure 2). Because this deletion is mediated by Alu
4 repeats there is 38 bp of overlapping sequence, making it impossible to define the
5 precise breakpoint (Figure 1). However, minor variations in the repeat sequence allow
6 the last unique bases on either side of the deletion to be determined (asterisks in
7 Figure 1). The telomeric break removes the most distal gene on 16p13.3 (*POLR3K*)
8 and the proximal break occurs between the *C1QTNF8* and *CACNA1H* genes (Figure
9 3). Interestingly this breakpoint appears to be mediated by the same AluY element
10 associated with the interstitial deletion in JT (see Figure 2) suggesting this locus may
11 be particularly prone to rearrangement.

12 Male NL was diagnosed with α -thalassemia at the age of 9 years, there were
13 no signs of mental retardation, he followed a normal school education and had normal
14 developmental milestones. At present he is 19 years of age and studies law and plays
15 soccer in his local team. MLPA and subsequently microarray analysis (Phylipsen et
16 al., 2012) showed this patient to have a distal deletion of ~1.14 Mb with the breakpoint
17 falling between *C1QTNF8* and *CACNA1H* (Figure 2). This deletion is slightly larger
18 than that identified in family SH, surprisingly, however, it is not associated with any
19 abnormalities apart from α -thalassemia.

20 A large terminal deletion of at least 1 Mb was identified in case DO by Wilkie
21 and colleagues (Wilkie et al, 1990) using Southern blotting. This patient has
22 craniofacial abnormalities including hypertelorism, a prominent nasal bridge, a high
23 palate with crowded upper teeth and a small chin. Skeletal examination was normal
24 (apart from short stature) but she had a marked speech delay, having a very limited
25 vocabulary at the age of five years.

26 Male patient CJ had a birth weight of 3.2 kg and was initially referred for genetic
27 testing because of developmental delay, speech delay and slow cognitive, social and
28 motor development at 14 months of age. He exhibited a range of dysmorphic features
29 including a high, broad forehead, a long and flat midface, a high narrow palette,
30 retrognathia, micrognathia and downturned palpebral fissures. Skeletal abnormalities
31 included bilateral hallux valgus and bilateral clinodactyly of the 5th finger. He had a
32 systolic ejection heart murmur. Because CJ was found to have microcytic anaemia in
33 combination with these abnormalities a possible diagnosis of ATR-16 was pursued.
34 FISH studies revealed an interstitial deletion of the terminal region of chromosome 16
35 shown by absence of probes intervening the telomeric cosmid CRA36 and proximal

1 cosmid C399E4 (Supplementary Table 1). The deletion in this patient was refined by
2 high-resolution microarray, which revealed it to be between ~120,000 bp and
3 ~1,357,000 bp on chromosome 16 (Supplementary Figure 3) giving a total deletion
4 size of ~1.15 Mb (Figure 2).

5 Female patient MY had a birth weight of 2.7 kg, she initially fed poorly
6 although her health improved following surgical repair of an atrial septal defect.
7 MY's heart defect is unlikely to be linked to the ATR-16 syndrome as her sister also
8 had an atrial septal defect yet did not inherit monosomy for chromosome 16p13.3,
9 although this cannot be excluded. MY also has plagiocephaly and mild telecanthus
10 and hypertelorism. She had delayed speech and mild delay to her physical
11 development (the patient walked at 18 months of age). Results of FISH with 16p
12 cosmids suggest the breakpoint lies between cosmids 399E4 and 312E8
13 (Supplementary Table 1), indicating terminal deletion of approximately 1.4 Mb.
14 Southern blotting followed by cloning and DNA sequencing of telomere-anchored
15 amplification products showed the break occurred at 1,408,561 bp (Figure 1),
16 between two tandem AluSx SINE repeats, flanked by the *C16orf91* and *UNKL*
17 genes (Figure 2). There is a rearrangement at the breakpoint with complex deletion
18 of part of the AluSx repeat (Supplementary Figure 2). Telomeric sequence is joined
19 directly to the breakpoint with a 2 bp overlap, suggesting telomerase mediated
20 healing (Figure 1).

21 Female BAR was diagnosed with α -thalassemia by the identification of HbH
22 inclusions at the age of 7 years. She was found to have inherited the $-\alpha 3.7$ deletion
23 from her mother on one allele and MLPA and subsequently microarray analysis
24 revealed an interstitial deletion from ~0-23,949 bp to ~1,461,124 bp had arisen *de*
25 *novo* on the other allele (Figure 2 and Supp. Fig 3). BAR had no phenotypic
26 abnormalities beyond her α -thalassemia.

27 Male patient SCH was referred with at the age of 8 years with HbH disease to
28 a normal mother and a father a haematological profile with α^+ -thalassemia (Scheps
29 et al., 2016). In his neonatal period, he required hospitalisation for respiratory
30 distress and hypotonia. He also showed developmental delay and other dysmorphic
31 craniofacial features and abnormalities of the hands and lower limbs (Scheps et al.,
32 2016). FISH, SNP-array and MLPA revealed this patient to have an interstitial
33 deletion of ~1.2 Mb.

34 Male patient PV had developmental delay at the age of 2, at the age of 11 he
35 was noted to have mildly dysmorphic craniofacial features, his trunk showed pectus

1 excavatum and hyperlaxity of his joints was observed (Harteveld et al., 2007). MLPA
2 revealed this patient to have a terminal deletion with a maximum extent of ~1.7 Mb
3 (Harteveld et al., 2007).

4 Female patient FT was referred at the age of 30 years because of mild
5 microcytic hypochromic anaemia. In infancy it was noted she had a developmental
6 delay (Harteveld et al., 2007). At the age of 31 examination revealed craniofacial
7 abnormalities and abnormalities of the hands and feet including clinodactyly and
8 lateral deviation. MLPA revealed a large deletion of 16p13.3 with a maximum extent
9 of ~1.9 Mb.

10 Male patient BO had a small head, mild ptosis, a small mouth and a long
11 filtrum and bilateral talipes equinovarus (reviewed in Wilkie et al., 1990). The
12 breakpoint was refined by Wilkie and colleagues and subsequently identified as a
13 terminal deletion of ~1.9 Mb (Lamb et al., 1993).

14 Male patient HN was reported by Harteveld and colleagues (Harteveld et al., 2007).
15 At the age of 10 months he had delayed motor development and hypotonia. Speech
16 delay was reported at the age of 30 months and at 3 years he was found to have an
17 active language delay. He also had typical facial features associated with ATR-16
18 including downslanting palpebral fissures, mild hyperterlorism, abnormal ears. His
19 skeletal defects were mild with only a club foot (left) and a flat foot (right) reported.
20 MLPA revealed this patient to have a large deletion of a 1.9 Mb.

21 The phenotypic abnormalities of female patient IM have previously been
22 reported (Daniels et al., 2001; Fei et al., 1992; Felice et al., 1984). Briefly, she was
23 found to have microcytic anaemia at the age of 5 months, her growth was slow and
24 motor retardation was noted by the age of 1 year. IM was later reported to have to
25 suffer developmental delay (behaving like a 5 year old at the age of 8), however, her
26 language and adaptive development were considered normal. IM has mild facial
27 dysmorphism (tall forehead and macrodontia) and bilateral clubbed feet that were
28 surgically corrected in early childhood. Previous studies performed Southern blotting
29 (Fei et al., 1992) and FISH (Daniels et al., 2001) to refine the deletion, here we
30 conduct high-resolution microarrays that reveal a large terminal deletion of
31 chromosome 16 with the breakpoint at ~2,011,646 bp. The relatively mild
32 phenotypic abnormalities seen in this patient are surprising given the relatively large
33 deletion of chromosome 16 identified.

34 Female patient LIN had expressive speech delay and developmental delay
35 that has been described previously (Lindor et al., 1997). She has a degree of facial

1 dysmorphism including a tall, broad forehead and a broad nasal root and midface
2 retrusion. Previous FISH studies identified a terminal deletion of chromosome 16
3 with the breakpoint ~ 2 Mb from the telomere (Daniels et al., 2001). In this study we
4 further refined the deletion using Southern blotting and identified the junction
5 fragment by DNA sequencing of telomere anchored amplification products to be at
6 2,013,658 bp (Figure 1). The breakpoint is flanked by the genes *NPW* and
7 *SLC9A3R2* (Figure 2). There is a short region of subtelomeric sequence of
8 unspecified chromosomal origin between the break and the telomeric repeats
9 suggesting a recombination-based mechanism may have stabilised the
10 chromosomal breakage in this case (Figure 1). The lack of skeletal abnormalities
11 seen in LIN is surprising given that the deletion is over 2 Mb.

1 **Supplementary Methods**

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3 Genes and Assay Numbers Used in Quantification of Gene Expression

4 *POLR3K* (Hs00363121_m1), *C16orf33* (Hs00430677_m1), *C16orf35* custom assay
5 (Forward; CAACGCCCTCAGCTTTGG, Reverse; GCTGGTGAGGGTCATGTCATC, Probe;
6 CCCCAACCAGCAGC), *LUC7L* (Hs00216077_m1), *AXIN1* (Hs00394718_m1), *MRPL28*
7 (Hs00371771_m1), *TMEM8* (Hs00430491_m1), *NME4* (Hs00359037_m1), *DECR2*
8 (Hs00430406_m1), *Rab11FIP3* (Hs00608512_m1) *ACTB* (Hs99999903_m1). Two custom
9 assays were obtained from Eurogentec (www.uk.eurogentec.com): *MPG* (Forward; 5'-
10 GCATCTATTTCTCAAGCCCAAAG-3', Reverse; 5'-GGAGTTCTGTGCCATTAGGAAGTC-
11 3', Probe; 5'-AGTTCTTCGACCAGCCGGCAGTCC-3') and *C16orf9* (Forward; 5'-
12 GGCGGCCCGTTCAAG-3', Reverse; 5'-GAGCCCACAAGAAGCACA-3', Probe; 5'-
13 TCCCAGGGAACGCCGGTG-3'). Analysis was performed using the comparative C_T Method
14 ($\Delta\Delta C_T$) (Livak et al., 2001). Data in Figure 3A were obtained by amplification and Sanger
15 sequencing of informative polymorphisms from genomic DNA and cDNA.

16

17 **Microarray Analysis**

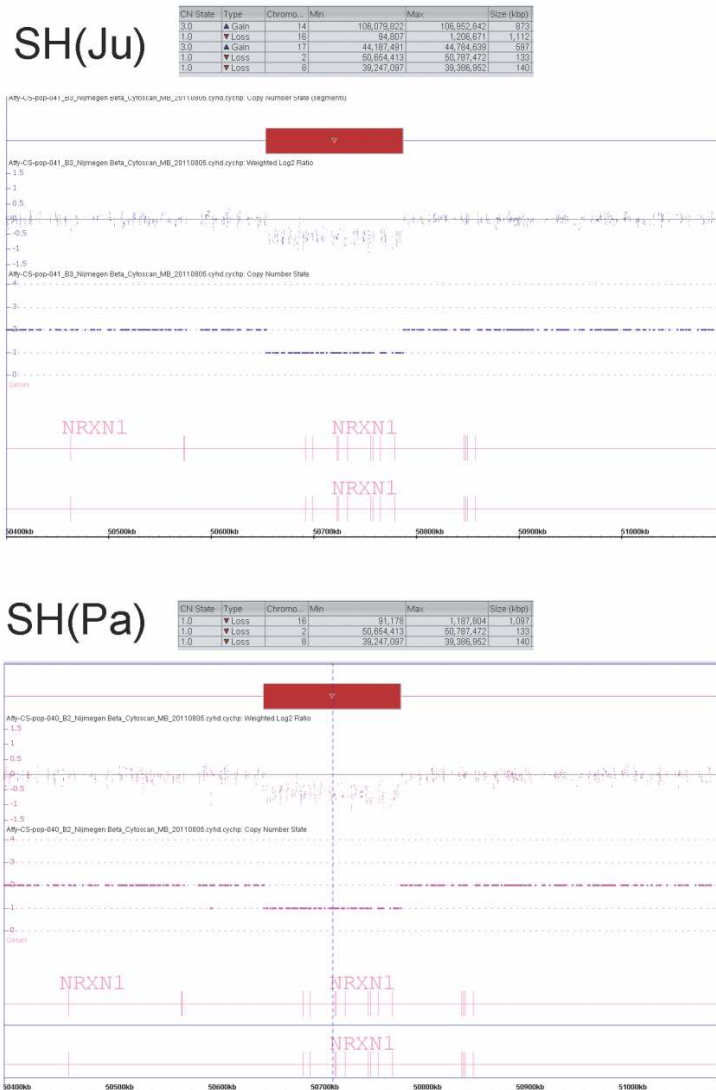
18 DNA from patient LA was tested using an 8 x 60K SurePrint G3 custom CGH + SNP
19 microarray (Agilent) and analysed using Agilent Cytogenomics software 4.0. Genomic DNA
20 from patients NL and BAR was analysed using a custom fine tiling array covering the alpha-
21 and beta-globin gene clusters and surrounding areas was used (Roche NimbleGen,
22 Madison, WI, USA). Array design was based on NCBI Build 36.1 (hg18) and used as
23 previously described (Phylipsen et al., 2012). Genomic DNA from patients SH(Ju) and
24 SH(Pa) was tested using CytoScan HD arrays (Affymetrix) and analysed using Karyoview
25 software. Microarray analysis was performed with genomic DNA from patients CJ, IM and
26 YA using the Sentrix Human CNV370 BeadChip (Illumina) and analysed using
27 GenomeStudio software. DNA from patient CS was analysed using the Agilent ISCA
28 (International Standards for Cytogenomic Arrays) 60K oligoarray.

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2 **Supplementary Figures**

Supplementary Figure 1

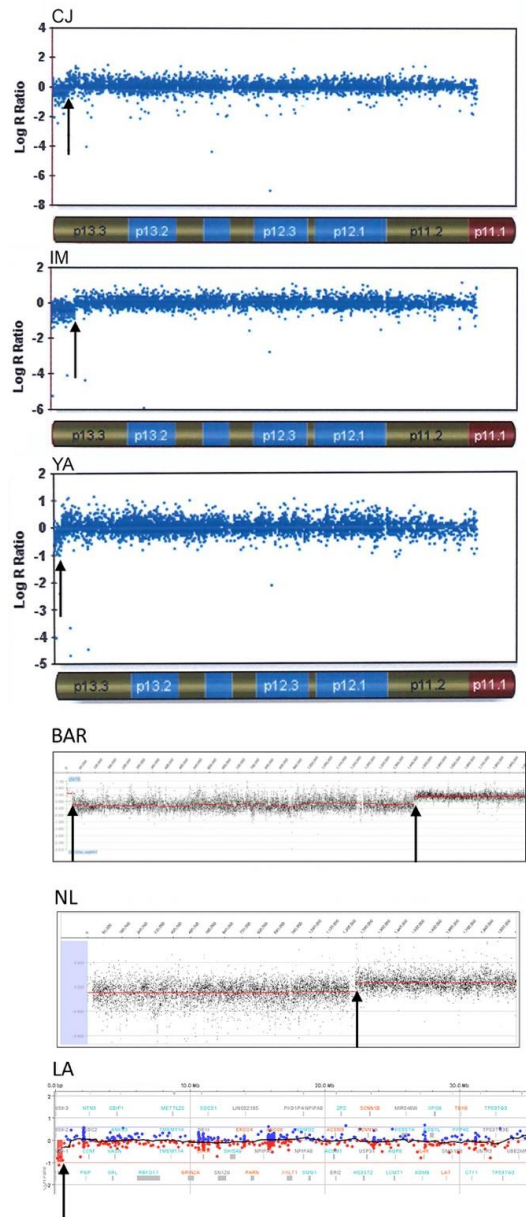


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4 **Supplementary Figure 1:** Copy number variations (CNVs) identified in SH(Ju) and
 5 SH(Pa). Tables show CNVs called by Affymetrix Cytoscan software and figures
 6 below show the log₂ ratio of probes and the copy number state for the chromosome
 7 2 deletion encompassing exons 5 to 13 of *NRXN1*. Both individuals also harbour
 8 deletions of chromosomes 8 and 16. The chromosome 8 deletion disrupts the
 9 pseudogene *ADAM5* and *tMDC* encoding uncharacterised protein
 10 ENSP00000328747. The chromosome 16 deletion underlies the ATR-16 syndrome
 11 in these patients.

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Supplementary Figure 3



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4 **Supplementary Figure 3:** Microarray data from patients CJ, IM, YA, BAR, NL and

5 LA showing the LogR ratios of individual probes, arrows indicate loci at which

6 analysis software identified the breakpoints.

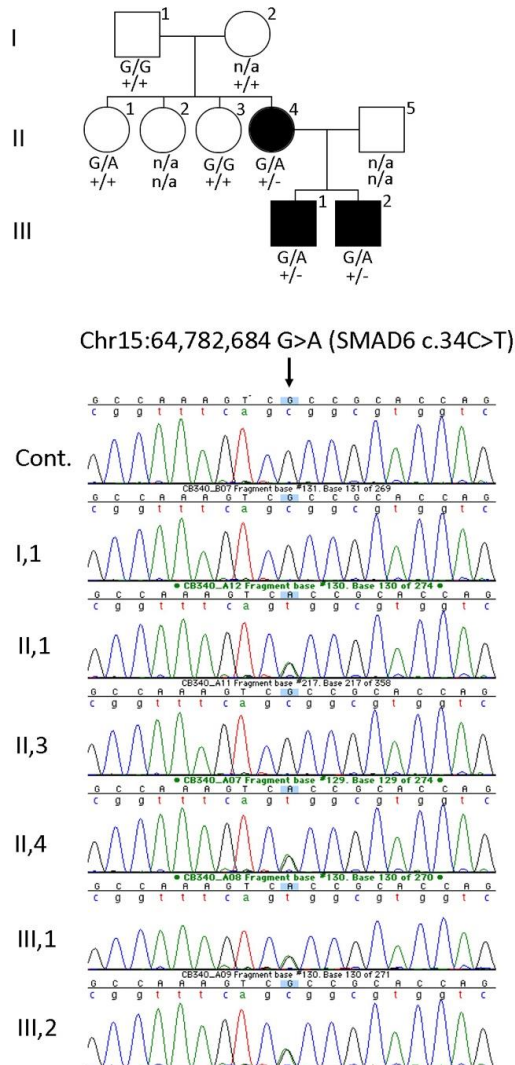
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2 **Supplementary Figure 4**

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4 **Supplementary Figure 4.** Identification of SMAD6 change in Family TN. Affected
 5 family members are shown with filled symbols and unaffected individuals are
 6 represented with open symbols. Below each symbol the genotype at
 7 chr15:64,782,684 is written on the upper line, G is reference and A is the variant
 8 leading to the R12X nonsense change in *SMAD6*. The lower line of text indicates
 9 the ATR-16 deletion in this family, + indicates an intact chromosome 16 and –
 10 indicates the deleted allele. Because two siblings carry the *SMAD6* variant this is
 11 likely to have been inherited from the grandmother (I,2). Coinheritance of these
 12 variants may have led to the relatively severe ATR-16 abnormalities present in this

- 1 family. The lower part of the figure shows chromatograms for a control individual
- 2 (Cont.) and family members as indicated.

Supplementary Table 1 FISH Probes

Probe	Coordinates	OY	TY	SH	MY	LIN	YA	CJ
Telomere Assay	na	N	nd	nd	N	nd	N	N
16p cosmid								
CRA36	52706-99963 bp	-						+
HSGG4	97541-111766 bp		-		-		-	
HSGG1	129137-172194 bp	-	-		-		-	-
Cos12	158777-203194 bp		-					-
C314G4	243192-285276 bp	-						
C419C1	265315-316860 bp	+/-	-					-
C338B10	297847-340834 bp	+						
C415C1	332201-381990 bp	+			-			
C356B8	485020-530746 bp	+						
C366D1	521427-565641 bp		-					
C407A10	557634-598294 bp		-					
C338H10	580029-610790 bp		+/-		-			
C398G5	594663-640361 bp	+	+					
C444G9	708336-751169 bp						-	
C335H7	737067-783265 bp						+	
C360B4	851308-895707 bp				-			
C313F9	1025418-1072203 bp	+						
C349E11	1068736-1111710 bp			-	-			-
C344F5	1122769-1160074 bp			+				
C320E12	1172104-1208298 bp							-
C399E4	1359308-1404844 bp				-			+
C312E8	1395863-1429931 bp				+			
C305C8	1461998-1505438 bp	+			+			+
C371H6	1755695-1799062 bp				+			
C439A6	1882600-1923854 bp	+						
910O19	1965888-2004708 bp					+/-		
1308N18	1996694-2036573 bp					+		
2171017	2038309-2078584 bp					+		

Presence of signals on both chromosome 16 homologues is indicated by "+", "-" indicates signal absent on one chromosome 16 homologue, "+/-" indicates weaker signal on one homologue compared to the other. The top row indicates patients who were analysed for subtelomeric rearrangements using a telomere assay (Knight et al., 1997 and Horsley et al., 2001): N, no rearrangement; nd, not done; na, not applicable.

Supplementary Table 2 Oligonucleotides used to clone breakpoints

Patient	Forward primer	Reverse primer
SH	5'-AGATACATGCTCCCAGTCTCA-3'	5'-CGTATATCTGGTCTCTATCTTC-3'
OY	5'-CAAAGCACGCATCCATAGGC-3'	5'-CCCTAACCCCTGACCCTAACCC-3'
LN	5'-GCAGAGGGAGAGCAGGTCTCAG-3'	5'-CCCTAACCCCTGACCCTAACCC-3'
MY	5'-CTGAAGGACTTGGCTGGTGGAT-3'	5'-CCTAACCCCTGACCCTAACCC-3'
BA	5'-GAGCAAAGTACACAAACTGGGTGAC-3'	5'-CCTAACCCCTGACCCTAACCC-3'
TN	5'-AACTGGCCTTGTCTGTGCCTTAAGCT-3'	5'-CCCTAACCCCTGACCCTAACCC-3'
TY	5'-CCTACCACCAGCAAGAACGGA-3'	5'-CCTAACCCCTGACCCTAACCC-3'

Supplementary Table 3 Deleterious Chromosome 16 Variants

Patients*	Gene#	Variant%	Identifier [^]	Freq ^{\$}	ANNOVAR [@]
TN(AI, Pa, Pe)	MRPL28	H27Y	rs3194151	11%	3/6
TN (AI,Pe)	PICQ	G523S	rs7187227	14%	3/6
TN(AI) YA	PICQ	T14A	rs2071979	~49%	2/6
TN(AI, Pa,Pe) YA	POLR3K	S24A	rs3194151	100%	2/6
TN(Pa)	PDIA	T286M	rs2685127	8%	3/6
TN(Pa)	CHTF	S63F	rs2277902	5%	1/6
(Pa)	PRR25	T92S	rs1005190	41%	1/6
YA	NPRL3	R158fs	rs35963490	33%	6/6
YA	RGS11	G499A	rs9806942	15%	3/6
YA	RHOT	R425C	rs3177338	33%	3/6
YA	RHOT	R245Q	rs1139897	33%	2/6
YA	RGS11	T728C	rs739999	31%	1/6
YA	WDR90	H899Q	rs45613635	34%	1/6

*Codes for patients harbouring each of the changes listed are shown in this column.

#Gene symbols are used. % Effects of variants on coding sequence. ^The unique identifier from dbSNP is listed for each variant. \$ Allele frequencies as a population average, data from dbSNP. @ The pathogenicity of each variant was given a custom deleterious score based on a six-point scale, (Fu et al., 2013) calculated using output from ANNOVAR (Wang et al., 2010).

Supplementary Table 4. Shared novel variants in 3 affected members of family TN

Chr#	Position (bp)	Gene	Variant [§]	ANNOVAR [%]	Associated abnormalities [^]
1	1336598	PRAMEF27	Q144R	1/6	na
1	26671545	CRYBG2	L524fs	6/6	na
2	71190373	ATP6V1B1	R331W	4/6	Renal tubular acidosis with progressive nerve deafness.
3	141163642	ZBTB38	K804N	5/6	Potential role in human height variation (nearby GWAS association).
4	17061677	CLCN3	A290S	4/6	Loss of hippocampus in mouse KO.
5	179068881	C5orf60	splicing	6/6	na
8	125052168	FER1L6	G837D	3/6	na
11	1018248	MUC6	T1518I	2/6	Gastric cancer
11	62293804	AHNAK	F2695L	2/6	Neuroblastoma
13	103718412	SLC10A2	K63fs	6/6	Chrohn's disease, bile malabsorbtion.
14	33014538	AKAP6	L227V	5/6	na
15	66995630	SMAD6	R12X	6/6	Aortic Valve Disease, Craniosynostosis, developmental delay.
19	59010556	SLC27A5	V483M	6/6	na
20	5170758	CDS2	P406A	4/6	na

[#]Chromosome. [§]The effect of each variant on the protein is shown. [%]The custom pathogenicity score attributed to each variant (see Supp Table 3 notes). [^]Annotation present in the OMIM database associated with each gene, na indicates no information in OMIM. The pathogenicity of each variant was given a custom deleterious score based on a six-point scale, (Fu et al., 2013) calculated using output from ANNOVAR (Wang et al., 2010).