Supplemental information file Summary

This file includes some Methods and the results concerning parent-of-origin analysis, as well as four figures including:

Figure S1. Example of a graphic representation of array-comparative genomic hybridization.

Figure S2. Delimitation of breakpoints for deletions at interstitial 19p13.3 region.

Figure S3. Representative example of complex genomic rearrangements found at the interstitial region 19p13.3.

Figure S4. Molecular analysis of a macrocephaly patient by means of array-CGH.

The file also includes three tables including:

Table S1. Patient 14 and other Patients with interstitial 19p13.3 rearrangements reported in DECIPHER and ISCA consortium databases.

Table S2. Predicted G-quadruplex sequences in the 13 patients with genomic rearrangements at 19p13.3 included herein using QGRS software.

Table S3. Overview of sequence motifs at the delineated breakpoints for non-recurrent 19p13.3 deletions.

Methods:

1.- Additional laboratory studies:

CGH array

The aCGH experiments were applied using different array platforms depending on the Center and the time: at Center 1 (INGEMM, Spain) KaryoArray® v3.0 (8x60K Agilent-based; Agilent Technologies. Santa Clara, CA. USA; see Figure 1 Supplemental data) was used[23]. Samples 1-7, 9, 12 and 13 were additionally analyzed by a customized high-density array (8x60K Agilent-based, within chromosome region chr19:1477536-6653608; hg19, NCBI37) to refine breakpoints. At Center 2 (Signature

Genomics, USA) aCGH was done by one of the following formats: SignatureChip version 4.0, (Signature Genomics), SignatureChipWG (Signature Genomics), 12x135K-SignatureChipOS version 2.0, custom designed by Signature Genomics and manufactured by RocheNimblegen [Madison, WI, USA]), or 4x107K-SignatureChipOS version 4.0, custom designed by Signature Genomics and manufactured by Agilent, according to previously described methods[24-26]. Patient 8 was analyzed using Qchip_v3.0 (Q-Genomics laboratory, Barcelona, Spain).

Fluorescence *In Situ* Hybridization

FISH experiments were performed according to previously described methods[27], and using BAC clones: RP11-454N6 (Bluegnome, Cambridge, UK) in patients 1, 3, and 4, and RP11-577E16 (patients 5 and 13); CTD-2622I13 (patients 7, 9, and 11); CTD-2537J8 (patient 10) and RP11-615O9 (patient 12). When available, parental samples were assayed by FISH to rule out chromosomal rearrangements in this region (see an example for patient 4; Figure 1c Supplemental data).

Parent-of-Origin Analysis with Short Tandem Repeats

Parental testing was performed when parental DNA was available (patients 1-4) by segregation analysis of polymorphic microsatellite markers using fluorescently labeled primers (D19S1028; D19S591; D19S247; D19S424; D19S894; D19S216) in an ABI-3130XL genetic analyzer and Genemapper v4.0 software (http//www.appliedbiosystems.com). Determination of parent of origin for *de novo* deletions was done in patients 1-4, which showed that two were of maternal origin (patients 1 and 4) and one was of paternal origin (patient 3), while data from the parents of patient 2 were uninformative.

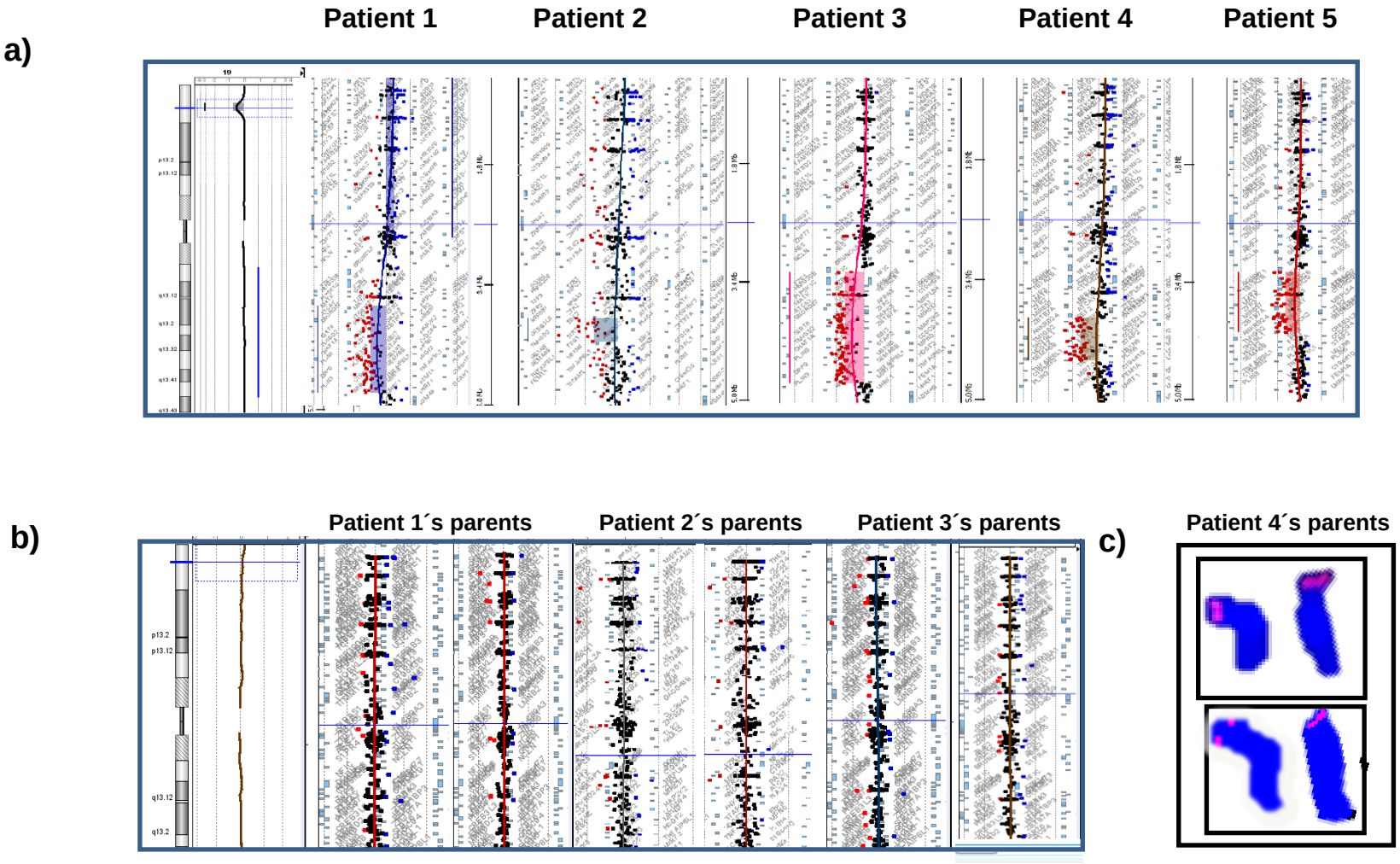


Figure S1. Example of a graphic representation of array-comparative genomic hybridization. a) Array-comparative genomic hybridization (aCGH) profiles from the three microdeletion patients (Patients 1–5). The array plots for Patients 1–5 show deletions at 19p13.3 chromosomal region ranging from a minimum of 0.306 Mb (Patient 2) to a maximum of 1.59 Mb (Patient 3). b) Array-comparative genomic hybridization (aCGH) profiles from the parents of patient 1 and 3, showing no imbalance in the region of interest. c) FISH using RP11-454N6 (chr19:4,086,722-4,246,199 hg19, browser NCBI37) on a metaphase cell from Patient-4´s parents, showing two signals in the region of interest.

Table S1. Other patients with interstitial 19p13.3 rearrangements reported in DECIPHER and ISCA public databases and Patient 14

Patients	Patient 14	DECIPHER 255689	DECIPHER 262609	DECIPHER 272700	DECIPHER 270854	DECIPHER 267980	DECIPHER 273505	DECIPHER 254007	DECIPHER 276706	DECIPHER 271675	DECIPHER 249482
Gender (M/F)	M	M	M	M	M	F	M		M	M	
Age (yr)/(at diagnosis)	1	1	8						3	10	
Type of genomic aberration	dup	del	del	del	Del dn	dup	del	dup	dup	del	del
Growth and development											
Psychomotor development delay	+	+	+	+	+, but Normal at birth				+	+	+
Intellectual disability	+	+	+	+	+				+	+	+
Speech delay	mild	NP			+						+
High or prominente forehead	+	+			+						
Face				++			+		mild	++	
Hypertelorism	-				+						
Downslanting palpebral fissures	-										
Short palpebral fissures	+										
Ptosis	-										+
Epicanthal Folds											+
Wide nasal bridge	+	+			+						
Depressed nose and root		+									
Short philtrum	+	Prom/deep									long
Thin upper lip											+
Ears abnormalities											
Machrocephaly/Microcephaly	Normoceph	Dolico/ Scafocephaly. Normoceph In size	Macroceph	Normoceph	Rel. Macroceph	Microceph	Macroceph at birth	Normoceph.	Normoceph .	Normo to Microceph	Normoceph
Neurology											
Hypotonia			+	+							+
Behaviour				Deficit of attention	autism				Poor social background		
MRI	N										
Hearing											
Others	mild brachydactyly, fifth finger clinodactyly, deep palmar creases. Genu varum and flat arches of feet.	hypospadias		Slender build	Fine/sparse hair		hydronephrosis				Abnorm feet
Cutis aplasia											
Obesity											
Proportionate short stature	+			+	+						
Urinary reflux		+					+				+
Gastroesophageal Reflux		+									
Feeding problems		+			+						
Overgrowth Synd. Testing	-									+	
Ophthalmologic abnormalities	-										+
Inguinal Hernias	-	+					+				
Sleeping disorders	-										
Heart disease	-	+	-								

Table S1. Other patients with interstitial 19p13.3 rearrangements reported in DECIPHER and ISCA public databases and Patient 14 (continued)

Patients	DECIPHER 259222	DECIPHER 266691	DECIPHER 267370	ISCA nssv578795	ISCA nssv582698	ISCA nssv578788	ISCA nssv577673	ISCA nssv578797	ISCA Nssv577674	DECIPHER 258067	DECIPHER 249485
Gender (M/F)	M	M	M							F	
Age (yr)/(at diagnosis)	12	8	25		13						
Type of genomic aberration	Del	dup	del	dup	del	dup	Del	Dup	Del	del	del
Growth and development											
Psychomotor development delay		+	+	+	+	+			+		+
Intellectual disability	+	+	+	+	+					mild	+
Speech delay											+
High or prominent forehead											Narrow
Face	+	+			+	++			++	+	
Hypertelorism										+	
Downslanting palpebral fissures	+	+									
Short palpebral fissures											
Ptosis											+
Epicanthal Folds											+
Wide nasal bridge											
Depressed nose and root											
Short philtrum		+									+
Thin upper lip											+
Ears abnormalities										+	
Macrocephaly/Microcephaly	Macroceph	Normoceph	Normoceph.	Microceph	Macroceph	Microceph	Macroceph	Rel. Microceph	Macroceph	Normoceph	Normo to Microceph
Neurology		spasticity									
Hypotonia											
Behaviour	hyperactive										Aggressive/ autistic
MRI											
Hearing											
Others	Fingers abnormal., recurrent infections	clinodactily	Diabetes, thyroid and somatotrope dysfunction			Intraut erine Growth retardation				Absent testis	
Cutis aplasia											
Obesity					+						
Proportionate short stature				+		+					+
Urinary reflux										+	+
Gastroesophageal Reflux										+	
Feeding problems										+	
Overgrowth Synd. testing					+						
Ophthalmologic abnormalities											
Inguinal Hernias											
Sleeping disorders											
Heart disease			+	+	-					+	

+, feature present; ++, severe feature, -, feature absent;

Table S2. Patient	Deletion/ duplication	QRS MAPPER	
01	19p13.3(3324026-4870882)x1dn	GGCCGGTGGCTCACACCGG	
02	19p13.3 (3873600-4183343) x1dn	(2) GGTGGCTTGGGGCTGGTGGCCTGGAGG GGCACACTGGAGAGCAAAGGTGG	
03*	19p13.3(3234036-4823723) x1dn	(9)distal GGGAAAGGCGTTCCAGGAAGAGTGCAGAGG GGCAGGACCACCACATCTGGTGTGTTGG GGAGGCCCTTGTGGCTGG GGAGGGGGAGAGAGGGAGG GGTTCTGTGTGTCTTGGAAGGACTTTGG GGTGGAGCCATGGAGACCTGTGG GGGGAGGGACAGGGTCTCTCAGGG GGGGAAACTAAGGCACAGAAGG GGCGCCTGGAGGTAGCTGG	Proximal GGGAGAAGGCGGCCGTCTGCAAGCTAAGG
04	19p13.3(3932785-4523183) x1dn	(2) GGTTTCACCATGCTGGCCAGGCTGG GGATTCGCTTCATGGAGCCAGGGAGCAGG	
05	19p13.3 (3279942-4168106) x1dn	GGGGTTTCACCATGTTGGTCAGG	
06	19p13.3(3789487 -3988737) x1dn 19q13.32(4612112-46387319) x1dn	(2) GGAGCCCCAGGGCTCCGGCAGGAGG GGAGGTGGAGG	
07	19p13.3 (3295068-4996928) x1dn	(2) GGGGAGGTGG GGTGAGGGGCCTTCCCATCCCAGGAGGGGG	
08*	19p13.3(2487767-4882351) x3dn		
09.1*	19p13.3(3190773-3448532) x1dn	(4) distal GGGGTGAGGCTGCTGGGCCTGGG GGCCCCGGGAAGAGCTGCGTCTGTGGGGG GGACGCTGGAGTCGGTCCTGG GGGAGGCTCCGGGGG	(5) proximal GGACGAGGGAAGGCAGAGCTGAGCTGG GGGCTCGGGTGACTCCCAGGTGATGGG GGGCCAGGAGCGAGGCCCAGG GGGTAAGAGGAGGCGGGGTGATGAGG GGCTTTGGGGAAGGTCCAGG
09.2*	19p13.3(3585930-4621011) x1dn	(7) distal GGTGTCGCCTTCCCGGGAGGCTCGG GGCCGGGGGAGGGGAGG GGCGGCGGCGG GGCCCGGGTGGGTGG GGCCCGGGAGGCCCGG GGTAAGGAGCCCGGACACCGG GGGGTGAGGGGTAGGGAGCTTGG	(3) proximal GGGGTCTTAGGGACCCCGG GGCTGGAGTGCAATAGTGTGGTCTCGG GGGGTTTCACCATGTTGGCCAGTCTGG
10*	19p13.3(2329320-3808325)x3dn	(6) distal GGCCCGTCTTGGGGGTCCAGCCTCGG GGCCGGCGGTGCCGGGCTCGG GGCTCTGTGGCCTTGAGCAGGCGG GGCGGAACGGGCACGG GGGGGTGCGGTGGGG GGCAGCATCCTGGAGCGGCCAGG	(3) proximal GGACAGGCACCGGGGTGG202 GGCTCTGTGCCGGGGTGG GGTGGGGGCTGAGCCCTCCCTTCCGG
11*	19p13.3 (3979568-4131259)x1unknown	(4) distal GGGCAGGTGTCCGGGGTGGGGCGTGGG GGGGATGCAGGCGTGG GGGGTAGGCGGCTCGGAACAGG GGGAAGGTGCTGGGAAACTGG	(7) proximal GGAAGGCTGAGGCTTGAGG GGGGTTCCAGGAAGACCTGGGCAACATAGG GGCGGGCTGGGCGCGG GGCTGAGATGGGCGGATCACCAGG GGTGGTGGTGG GGAGGCTGAGGCAGG GGCATGAACCCAGGAGGCGG
12	19p13.3 (3451211-4600362) x1dn	(2) GGTTCAGAGAGGCTTGTGTGTGGGGAGG GGCCCTCAGGCTTGGAGTTAGG	
13*	19p13.3(3413253-4194565)x1	(3) distal GGGATTACAGGTGTGTGCCACCACGGCTGG GGTTTCACCATGTTGGCCAGGCTGG GGGGCGTCTAATAGATGGGTAGG	(8) proximal GGAGGAGGAACTGGATTCATGG GGTATATGGGATAGCTGGGATCTAGG GGCCAAAAGGAGTAGTTGGGGCTCAGG GGCAACTAGGCATGGGG GGCATGGTGGGCAGGG GGAATAGCTGGGGATTCATGG GGCATTTGGAAGAGGAAGGG GGACCCTGTAGAGTATCTGGGGACATGG

Predicted G-quadruplex sequences in the 13 patients included herein using QGRS software. Analysis was performed within 200 bp at the breakpoint site in Sanger solved cases and in 500 bp at both distal and proximal breakpoints when customized 19p13.3 aCGH is used (with asterisk).

Table S3.

Motif name	Motif sequence	Number of sequences		Number of sequences Verdin et al, 2013			
		Observed breakpoint at 19p13.3 region (n=12)	Frequency 19p13.3 deletions (12)	Random control population (n=500)	Frequency random control population (500)	Observed FOXL2 breakpoint regions (n=48)	Frequency FOXL2 (48)
X-element E. coli	GCTGGTGG	1	0,08	1	0,002	1	0,021
Ade6-M26	ATGACGT		-		-	1	0,021
ARS consensus S. cerevisiae	WTTTATRTTTW		-	4	0,008	1	0,021
ARS consensus S. Pombe	WRTTTATTTAW		-	3	0,006	2	0,042
Consensus SAR 1	AATAAAYAAA		-	5	0,010	2	0,042
Consensus SAR 2	TTWTWTTWTT		-	46	0,092	7	0,146
Consensus SAR 3	WADAWAYAWW		-	109	0,218	14	0,292
Consensus SAR 4	TWWTDTTWWW		-	120	0,240	10	0,208
Deletion hotspot consensus	TGRRKM		-	385	0,770	31	0,646
DNA polymerase arrest site	WGGAG		-	285	0,570	24	0,500
DNA polymerase a frameshift hotspot 1	TCCCCC	2	0,17	32	0,064	1	0,021
DNA polymerase a frameshift hotspot 2	CTGGCG		-	7	0,014		-
DNA polymerase b frameshift hotspot 1	ACCCWR		-	138	0,276	17	0,354
DNA polymerase a/b frameshift hotspot 1	ACCCCA	1	0,08	42	0,084	7	0,146
DNA polymerase a/b frameshift hotspot 2	TGGNGT		-	142	0,284	15	0,313
D. Topoisomerase 2 consensus	GTNWAYATTNATNNR		-	2	0,004		-
Heptamer recombination signal	CACAGTG	1	0,08	23	0,046		-
Human hypervariable minisatelliets sequence 1	GGAGGTGGGCAGGARG		-		-		-
Human hypervariable minisatelliets sequence 2	AGAGGTGGGCAGGTGG		-		-		-
Human minisatelliets core sequence	GGGCAGGARG		-	1	0,002		-
Human replication origin consensus	WAWTTDDWWWDHWGWHMAWTTDHWGWHMAWTT		-		-		-
Human minisatellites conserved sequence/X-like element	GCWGGWGG		-	17	0,034		-
Ig heavy chain class switch repeat 1	GAGCT	1	0,08	116	0,232		-
Ig heavy chain class switch repeat 2	GGGCT	4	0,33	99	0,198		-
Ig heavy chain class switch repeat 3	GGGGT	4	0,33	87	0,174		-
Ig heavy chain class switch repeat 4	TGGGG	8	0,67	134	0,268		-
Ig heavy chain class switch repeat 5	TGAGC	3	0,25	130	0,260		-
LTR-IS motif	TGGAAATCCCC		-		-		-

Mariner transposon-like element	GAAAATGAAGCTATTTACCCAGGA		-		-		-
Murine MHC recombination hotspot	CAGRCAGR		-	25	0,050		-
Murine parvovirus recombination hotspot	CTWTTY		-	239	0,478		-
Nonamer recombination signal	ACAAAAACC		-	2	0,004		-
Pur-binding site	GGNNGAGGGAGARRRR		-		-		-
Recombination hotspot	CCNCCNTNNCCNC		-	10	0,020		-
Retrotransposon	TCATACACCACGCAGGGGTAGAGGACT		-		-		-
Translin-binding site 1	ATGCAG	2	0,17	34	0,068		-
Translin-binding site 2	GCCCWSSW		-	47	0,094		-
Vaccinia topoisomerase I consensus	YCCTT		-	257	0,514		-
Vaccinia topoisomerase II consensus	RNYNNCNNGYNGKTNYNY		-	2	0,004		-
XY32 homopurine-pyrimidine H-palindrome motif	AAGGGAGAARGGGTATAGGGRAAGAGGGAA		-		-		-

Overview of sequence motifs at the delineated breakpoints for non-recurrent 19p13.3 deletions. Analysis was performed within 100 bp at the breakpoint sites in Sanger solved cases; patients 1, 2, 4-7 and 12, using BLAST, SEQUENCHER, UCSC Genome browser and Repeat Masquer analysis softwares. Comparative data for FOXL2 locus were extracted partially from Verdin et al., 2013 (see reference 47).

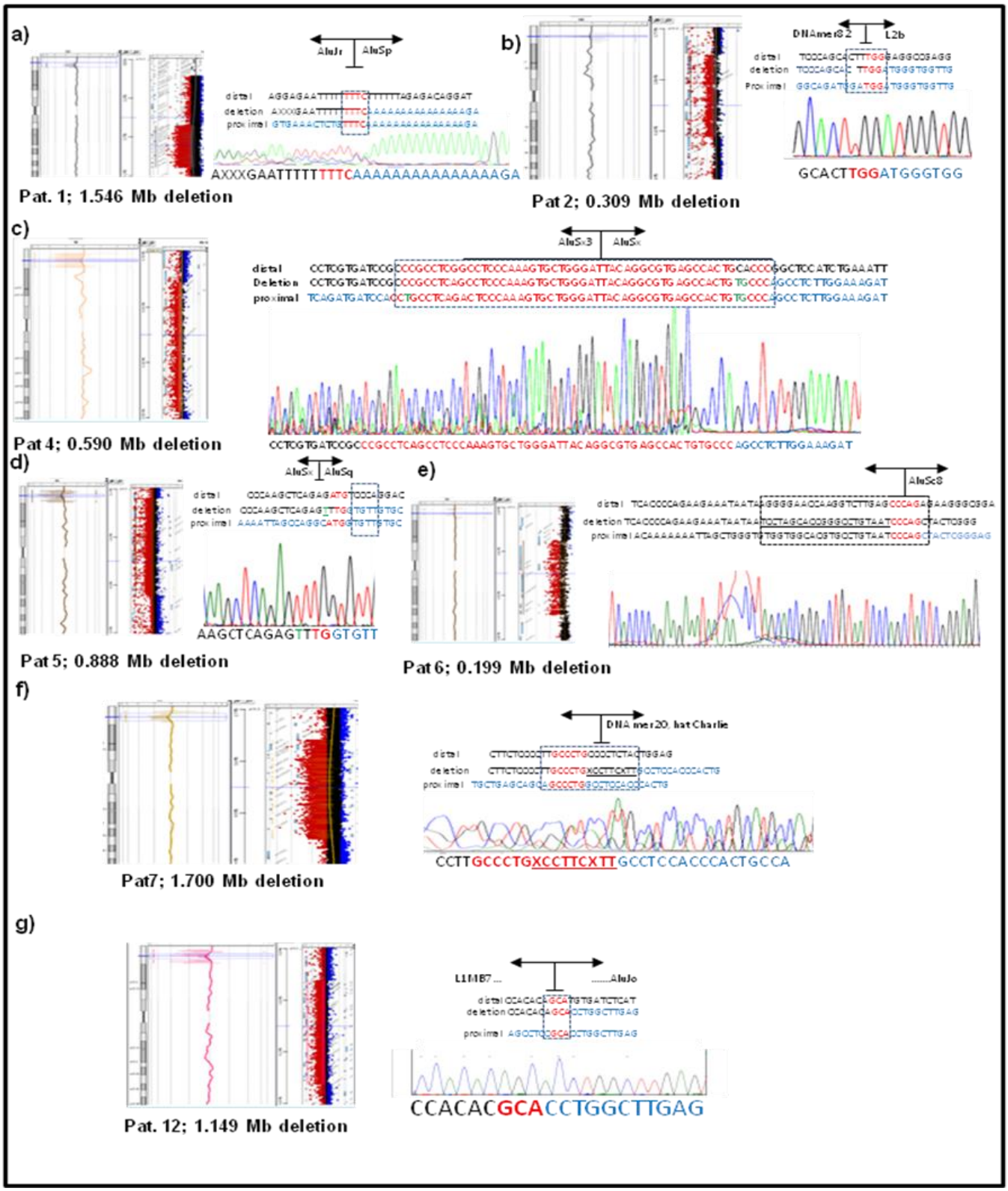
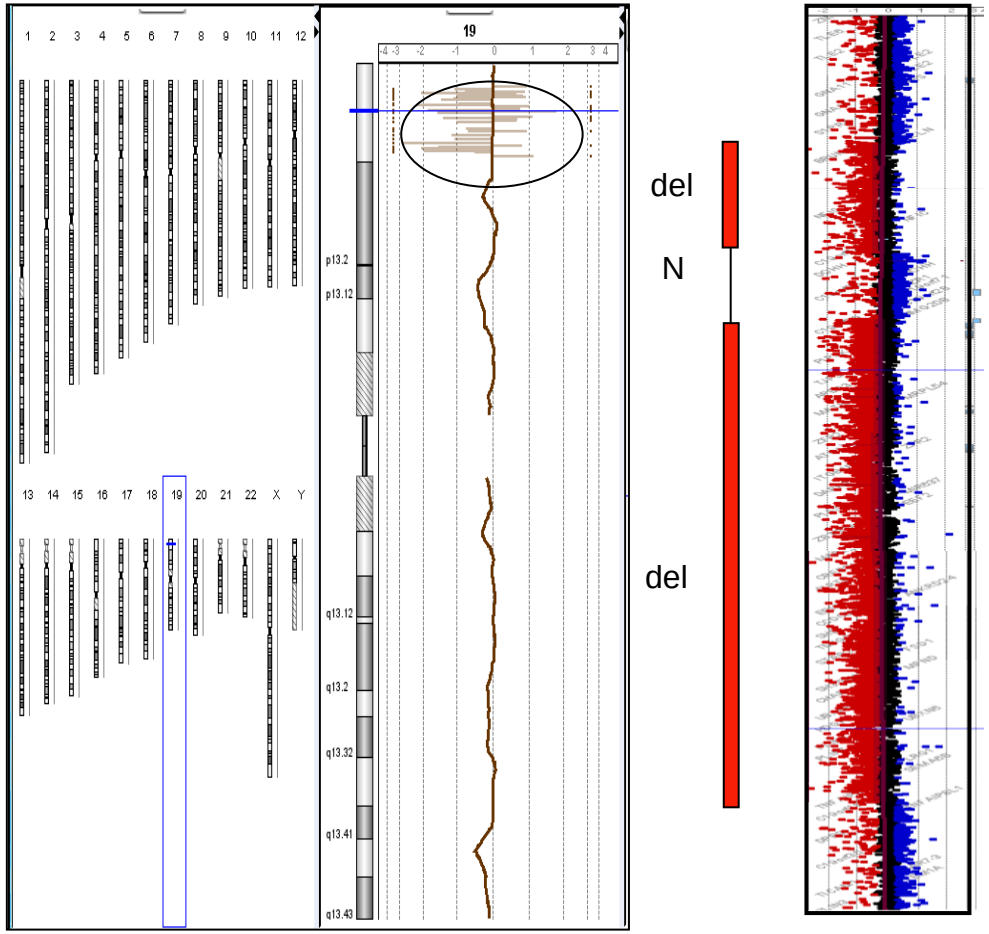


Figure S2. Delimitation of breakpoints for deletions at interstitial 19p13.3 region. Patient 1 (a), patient 2 (b), patient 4 (c), patient 5 (d), patient 6 (e), patient 7 (f) and patient 12 (g) using a 19p13.3 custom oligo aCGH (see above), long-range PCR and Sanger sequencing in an ABI 3070 XL. Whole junction fragments were analyzed with BLASTN application (http//www.ncbi.nlm.nih.gob/BLAST), University of California Santa Cruz Genome Browser and RepeatMasker application; http://www.repeatmasker.org/).

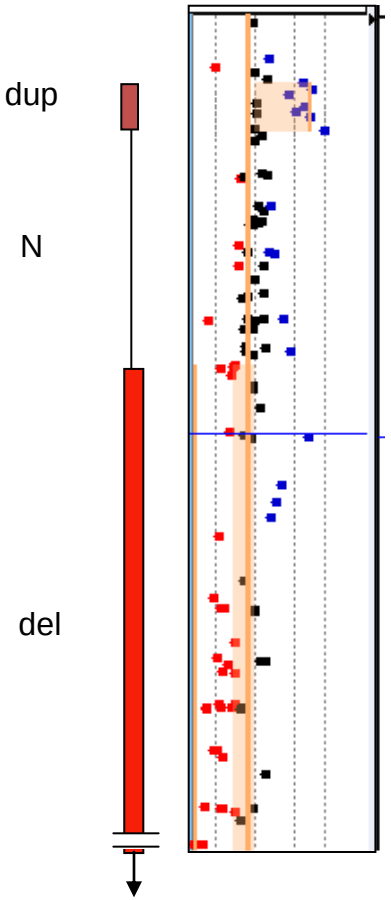
Figure S3. Representative examples of complex genomic rearrangements found at the interstitial region 19p13.3.



Patient 9

chr19.hg19:g.(3,184,457_3,190,773)_(3,448,532_3,454,848)del
ISCN: arr[hg19] 19p13.3 (3,190,773-3,448,532)x1/

chr19.hg19:g.(3,579,614_3,585,930)_(4,621,011_ 4,627,327)del
ISCN: arr[hg19] 19p13.3 (3,585,930-4,621,011)x1 dn



Patient 13

chr19.hg19:g.(3,397,174_3,403,490)_(3,405,207_3,411,524)dup
ISCN: arr[hg19] 19p13.3 (3,403,490-3,405,207)x3/

chr19.hg19:g.(3,406,937_3,413,253)_(4,195,610_4,201,926)del
ISCN: arr[hg19] 19p13.3 (3,413,253-4,195,610)x1

Figure S4. Molecular analysis of a macrocephaly patient by means of array-CGH.

Clinical features

No major dysmorphic features besides macrocephaly; OFC at first referral was >p97; height p50 and weight: p80 at 5 yrs. ASD, seizures. Only sounds, no words

Previous Genetic analysis.

FRAX study: negative; Karyotype: negative Subtelomeres-MLPA: negative; RGR-MLPA: negative; NDS1 analysis: negative; PTEN analysis: negative