Supporting Information for the article:

Recurrent HERV-H-Mediated 3q13.2-q13.31 Deletions Cause a Syndrome of Hypotonia and Motor, Language, and Cognitive Delays

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Clinical summaries

Patient 1 is an 8-year-old female born to non-consanguineous parents. Family history is significant for attention deficit-hyperactivity disorder (ADHD) in the father and a maternal family history of mild intellectual disability (ID). The pregnancy was complicated by mild preeclampsia and fetal echogenic bowel with some type of renal abnormality, possibly pyelectasis. Renal and abdominal ultrasounds at birth, however, were normal. She was initially evaluated at 6 months for hypotonia and motor delay, and her development has been monitored by regular clinical examinations thereafter. Metabolic studies, CK levels, and thyroid studies were all normal. She had gross motor delays but normal speech development, albeit with some stuttering. Speech therapy was given to improve her articulation. She was tested for possible silent seizures, but 24-hour EEG and MRI scan of the brain were normal at 3 years. By 8 years EEG showed slowing and spikes in the right temporal lobe. On physical examination at 3 years 10 months, her height, weight, and head circumference all exceeded the 95th percentile (with head circumference at +2.1 standard deviations, SD). Mild to moderate hypotonia was noted throughout, with patellar reflexes +1 bilaterally. Musculoskeletal features include joint laxity in the fingers, flat feet, and lumbar lordosis. Abnormal craniofacial features include bitemporal indentation, bilateral ptosis, down-slanting palpebral fissures, telecanthus, broad nasal bridge, slightly long philtrum, thickened maxillary alveolus, prominent full lower lip, and a broad, somewhat flat face (Figure 3A). She wears glasses for myopia, and her hearing is reportedly good. She is an anxious child and has a somewhat short attention span but does not have any definitive cognitive problems.

Patient 2 is a 3-year-old male born to unrelated parents following an uncomplicated pregnancy. Delivery was at 41 weeks by emergency C-section due to a nuchal cord; no resuscitation was required during his delivery. The neonatal period was complicated by difficulty latching and weight loss. Failure to achieve milestones became apparent in infancy. He rolled over at 8 months, sat at 1 year, stood at 20 months, and walked just after 30 months of age, signifying significant delays in motor development. Speech delays were also evident, with him speaking his first specific words at about 3 years. He has a history of strabismus. On physical examination at 3 years, his height is above the 95th percentile, while his weight and head circumference are in the 75th percentile. He has generalized hypotonia and requires ankle orthotics for walking. Craniofacial features include a dolicocephalic skull, palpable metopic ridge, down-slanting palpebral fissures, epicanthal folds, broad nasal bridge, small chin, and myopathic facies with prominent lower lip (Figure 3B-C). Other notable features include bilateral pes planus and tiny

inverted nipples. He has displayed some stereotypic behaviors and sensory issues. He is generally happy but becomes easily frustrated and can display some aggression.

Patient 3 is a 9-year-8-month-old female born to a 19-year-old mother and unrelated 22-year-old father. Family history is significant for an autism spectrum disorder in a younger sister, who does not have a 3q13 deletion. The prenatal ultrasound was normal, but the mother's pregnancy was complicated by fainting spells from 6 months onward, a motor vehicle accident at 7 months' gestation, anemia, and gestational diabetes at the end of the pregnancy. Delivery was by emergency C-section. Apgar scores were 2 and 9. Although the patient required brief resuscitation, she was breathing well at five minutes of age. As a baby she had marked hypotonia and was a slow eater. During the most recent examination, she still exhibits relatively low tone and requires ankle-foot orthotics to stabilize her gait. Before 2 years she experienced multiple stroke-like episodes, manifesting as facial asymmetry, clumsiness, and loss of skills. She also has a history of seizures, for which she has not required medication. Brain MRI showed agenesis of the corpus callosum, and several EEGs have been normal. Her clinical history also includes obstructive sleep apnea and disordered sleep, encopresis, constipation, and urinary retention. She was not fully toilet trained until 5.5 years of age. She is diagnosed with moderate developmental delay and high-functioning autism and is undergoing speech therapy. Her behavior previously included head banging and echolalia, which are now resolved. She used to play alone and do a lot of hand flapping, posturing, and self-stimulatory behaviors, though she is currently very social and outgoing. On physical examination at 9 years 8 months, her height and weight are in the 25^{th}-50^{th} percentile, while her head circumference is at the 50^{th}-75^{th} percentile. She exhibits significant scoliosis when bending over. Notable facial features include deep-set eyes, mild ptosis, down-slanting palpebral fissures, a relatively long nose with a bulbous, almost pear-shaped tip, dental crowding, and a broad alveolar ridge (Figure 3D-E). She wears glasses for strabismus and has hyperacusis.

Patient 4 is a 3-year-4-month-old male born to an unrelated couple. Family history is significant for maternal relatives with developmental delay and autism. During pregnancy, the mother was prescribed insulin and sertraline due to type 2 diabetes, depression, and anxiety; she also smoked one pack of cigarettes per day. Maternal blood and amniotic fluid AFP were slightly elevated, but acetylcholinesterase was absent from amniotic fluid, and fetal karyotype was normal. Delivery was by C-section due to oligohydramnios and fetal distress. The newborn required oxygen and dopamine during his stay in the NICU. Cardiac ultrasound revealed a patent ductus arteriosus, which spontaneously closed. A head CT, performed due to plagiocephaly at 11 months, was normal. Upon examination at 3

years 4 months, the patient presents with generalized hypotonia and marked development delay. He is unable to walk and talk and at 35 months of age had his cognitive development evaluated as equivalent to a 4-month-old. He also has a diagnosis of pervasive developmental delay. Craniofacial features include a small nose with depressed nasal bridge and bulbous tip, anteverted nares, hypertelorism, asymmetric palpebral fissures, downturned mouth, and a prominent upsweep of hair (Figure 3F). He also has a supernumerary left nipple. At 15 months, his head circumference was in the 75th percentile.

Patient 5 is a 16-month-old male born to a 33-year-old mother and 36-year-old father. He has three older siblings who are healthy and well. The mother reported no problems during pregnancy, though an ultrasound at 19 weeks yielded soft markers for a possible chromosome abnormality, while no specific structural anomalies were noted. Delivery was by C-section due to breech presentation and was complicated by a nuchal cord. He required surgical repair of left cryptorchidism. Clinical evaluation revealed a history of chronic constipation, mild generalized hypotonia, and global developmental delay. MRI showed a slight Chiari malformation. EEG and EMG were normal, as were a basic metabolic panel, CK, amino acids and acylcarnitines. There was no evidence of seizures; vision and hearing appeared normal as well. A muscle biopsy at 16 months showed nonspecific mild-to-moderate type 2 myofiber atrophy. On physical exam at 16 months, his length is at the 95th percentile, weight at the 20th percentile, and head circumference at the 99th percentile (+2.3 SD). He makes consonant sounds but has no words. Patellar reflexes were +1 bilaterally. Facial features include a prominent forehead, epicanthal folds, down-slanting palpebral fissures, a protruding full lower lip, slightly hidden upper eyelids, and a broad nasal bridge (Figure 3G-H).

Patient 6 is a 42-year-old woman with mild to moderate developmental delay and intellectual impairment. At birth, placental calcifications were noted, which may have been consistent with placental insufficiency. She was marginally small for dates. She developed a large angioma in her right shoulder, which had delayed involution. Motor milestones were delayed, with sitting acquired at 15 months and walking at 3 years. Although her language was delayed, with only the words “mama” and “dada” at age 3, she had rapid language acquisition thereafter. She had normal receptive language abilities. Macrocephaly was considered; however, she followed a normal curve throughout her childhood. She had nystagmus in the first few months of life. By school age, she had marked visual motor difficulty, social and emotional immaturity, and enuresis. Her verbal IQ was 94 while her performance IQ was 61. At age 14, her height and weight were in the 20th percentile. Neurological exam was consistent with cerebellar agenesis, BEAM testing with epileptic frontal focus. Physical examination

showed decreased tone throughout, with a normal Babinski sign, pes cavus, hyperextensibility, and dysdiadochokinesia. Evaluation at the age of 42 shows a height at the 10th-25th percentile, a head circumference at the 50th-75th percentile, and an obese habitus. Facial features include a ruddy complexion, absent eyebrows, thin hair, ptosis bilaterally, epicanthal folds, down-slanting palpebral fissures, horizontal nystagmus, sluggish and reactive pupils, inferior nares in a cleft pattern, microstomia with a report of small introitus, small teeth, large palatine tori, dorsocervical fat pad, and middle finger clinodactyly bilaterally.

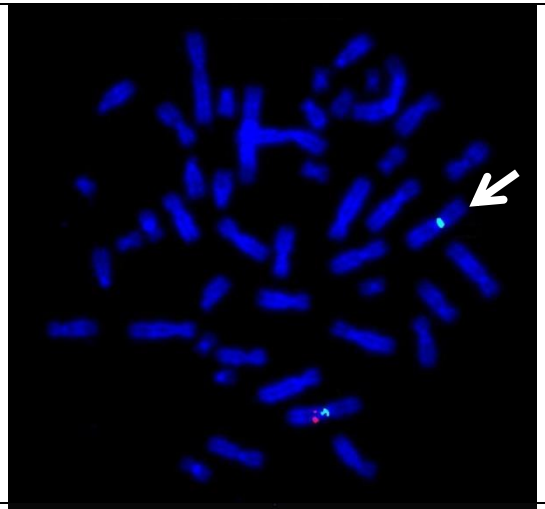
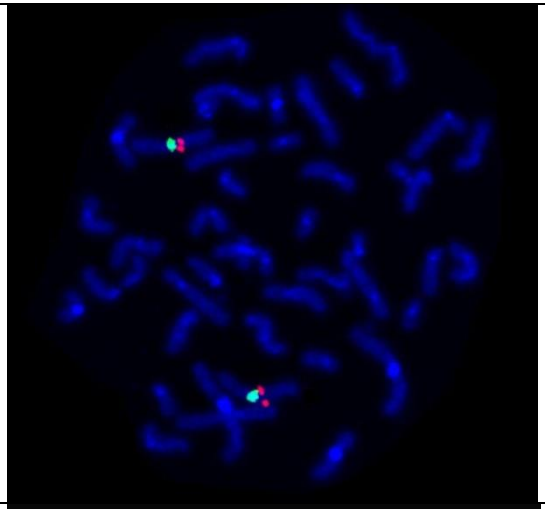
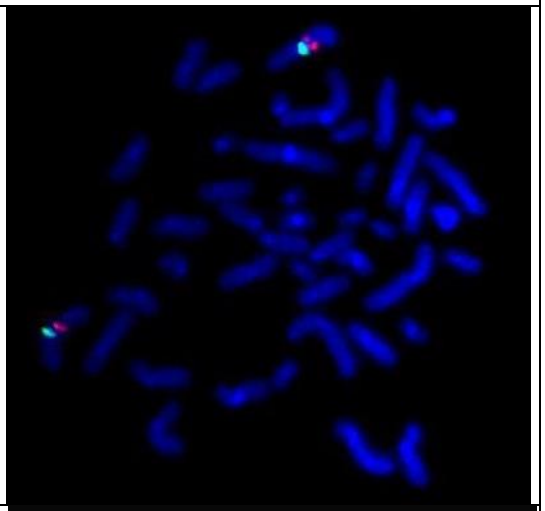
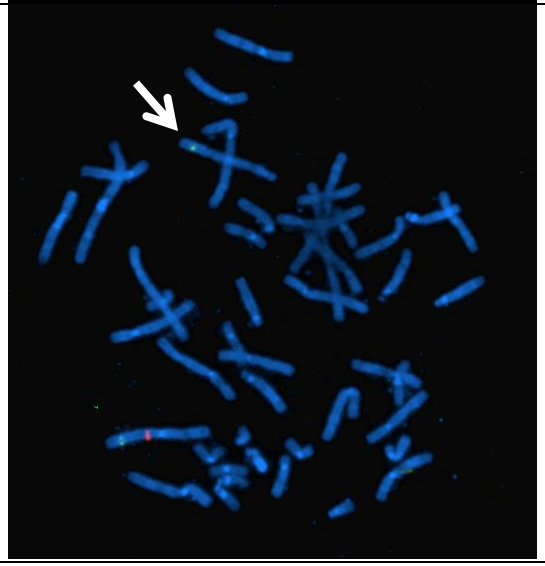
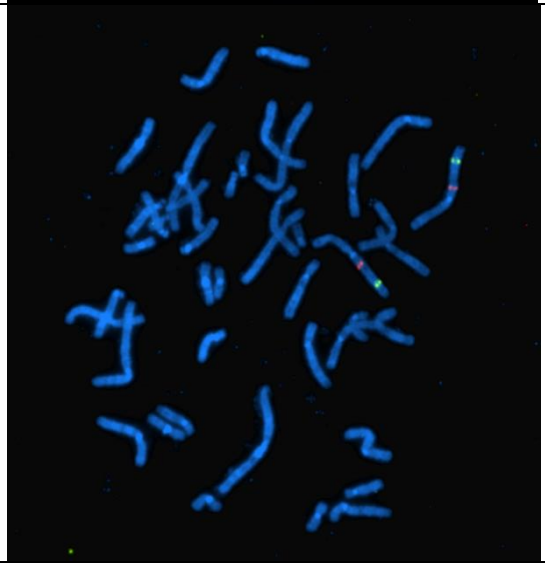
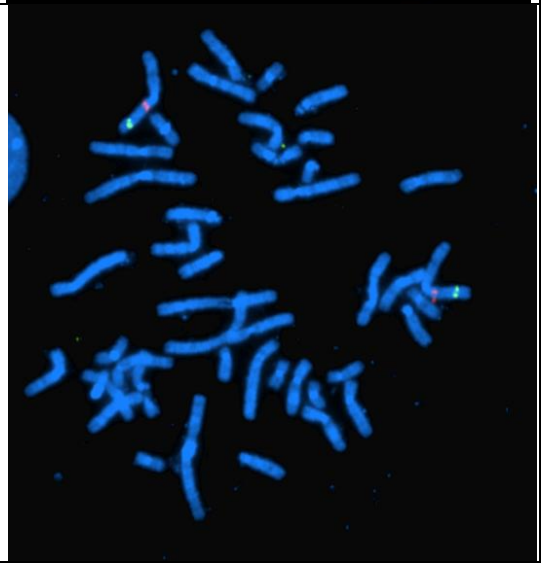
Patient 7 is a 16-year-old female. The only documented complication during pregnancy was oligohydramnios. She has ID, which included a delay in language and speech development. She is at the 50th percentile for height and weight, while her head circumference is at the 98th percentile. She has distinctive facial features including a rounded face, small mandible, high scalp hairline, flat zygomatic arches, absent eyebrows, deep-set eyes, mild eversion of the lateral lower eyelids, mild right ptosis, narrow nasal bridge with pointed nasal tip, short philtrum with thin upper lip and full lower lip, crowded teeth, normal palate and uvula, and normal ears. Additional anomalies include absent axillary hair, asymmetric shoulders with mild pectus excavatum, cervical kyphosis, ulnar deviation of the hands, hypothenar hypoplasia, 5th digit clinodactyly, normal nailbeds, and a gap between her first and second toes bilaterally. Her strength is normal, but she has decreased tone. She has normal reflexes and an abnormal gait with toe-ing in and a hitch in the left hip without a normal arm swing.

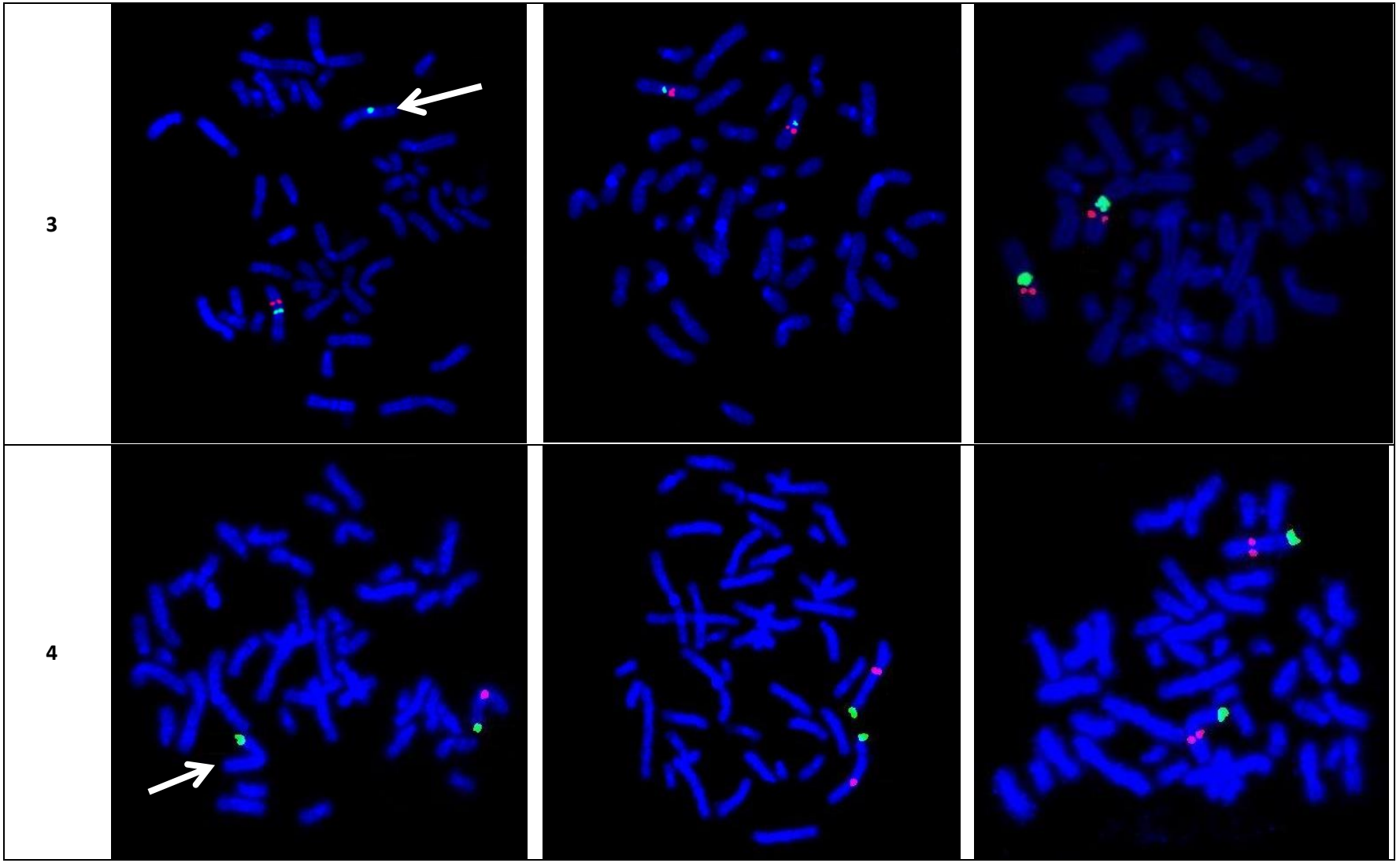
Patient 8 is a 10-year-old male born to a 32-year-old mother and an unrelated 33-year-old father. Family history is significant for a paternal second cousin with autism. The patient was delivered at term via spontaneous vaginal delivery; his Apgar scores were 7 and 9. He did not have any neonatal complications, and his newborn screen was normal. He had surgical repair of a left hydrocele. He did not have any nutritional problems, but his parents noticed that he had delays in all motor milestones. He was late in holding his head up without support, rolled over at 9 months, crawled at 15 months, and walked at 23 months. He started speaking after 3 years of age and continues to have speech delay. He functions approximately 2-3 grade levels below his age level. When he was about 20 months of age, he fell and hit the back of his head without loss of consciousness; however, a few hours after this incident he had multiple seizures. He has had at least one subsequent seizure episode. He has a history of sensory integration issues and constant hand washing and nocturnal enuresis continuing into school age. On physical exam at 4 years, his head size was 53.5 cm (95th percentile) with height and weight near the mean. At 10 years, his height is 149.4 cm (95th percentile), and his weight is 40.7 kg (75th-90th

percentile). He has particularly low muscle tone and strength and normal reflexes. He is farsighted. He has a prominent, broad forehead with frontal bossing and small, deep-set eyes with inner epicanthal folds, down-slanting palpebral fissures, mild hypotelorism, and a high palate (Figure 3I-J). His head CT is indicative of probable benign communicating hydrocephalus or benign enlargement of the subarachnoid spaces. Renal ultrasound showed absence of the left kidney and compensatory enlargement of the right kidney.

Patient 9 is a 5-year-old female born to unrelated parents. She was delivered vaginally at 29 weeks and was subsequently hospitalized for 10 days. The patient was referred to Genetics at 26 months for evaluation of developmental delay that was in excess of that explained by prematurity. Up until 9 months of age there was a 2-month global developmental delay, which by 2 years progressed to a 6-8 month delay. There was no developmental regression. She began rolling at 6 months, crawling at 8 months, walking between 16 months and 2 years, and running by 26 months. She has persistent, intermittent toe-walking. She began babbling at 16 months, with her first words at 2 years. At 27 months, she had 6 words but was not connecting them. At age 3, she was given the diagnoses of pervasive developmental disorder and mixed receptive and expressive language disorder. She did not meet full criteria for autism, and her hearing tested normally. Her family history is unremarkable. On physical exam at 27 months, her head size was 49.5 cm (90^{th} percentile), height was 91.4 cm (90^{th} peercentile), and weight was 14.6 kg (95^{th} percentile). She has a broad face with normally positioned eyes (inner canthal distance at 80^{th} percentile), epicanthal folds, esotropia, and large ears. Her nose is normally positioned with modest depression of the nasal bridge. She demonstrates modest retrognathia (Figure 3K-L). Neurologically, the patient has modest hypotonia. Deep tendon reflexes are present but not as brisk in the lower extremities as compared to the normal upper extremities. MRI of the brain at 3 years of age showed agenesis of the corpus callosum.

Supp. Figure S1. FISH experiments showing apparently *de novo* deletions

Patient	Patient's FISH	Mother's FISH	Father's FISH
1			
2			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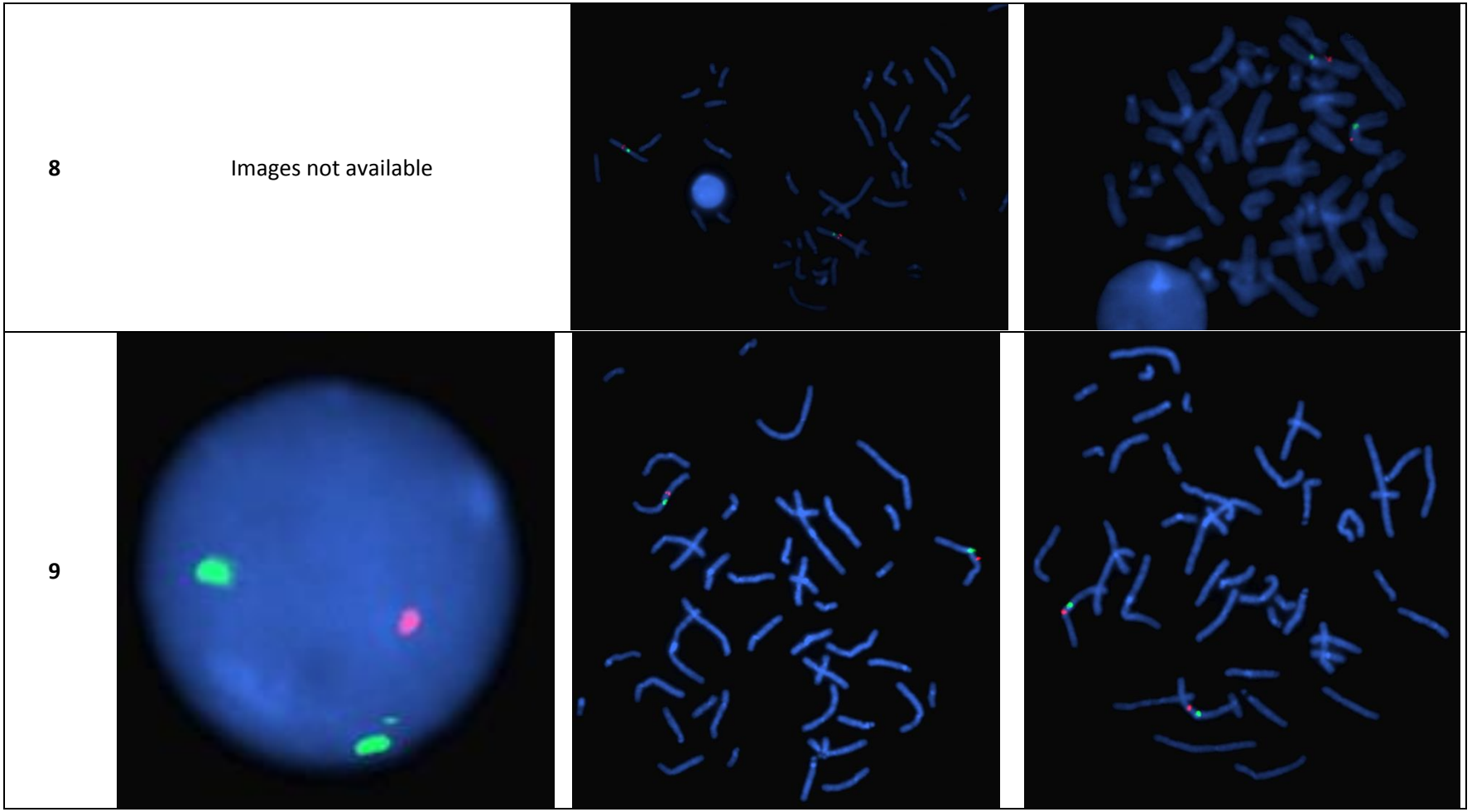

Supp. Figure S1. FISH experiments showing apparently *de novo* deletions. In all experiments, red probes are from the deleted region, and green probes are used as a control. The presence of a single red signal indicates deletion of 3q13 on one homologue in the probands (arrows). The presence of two red signals in the parents confirms an apparently *de novo* deletion in the probands. The probes used are as follows: RP11-184L10 (3q13.31, red, family of patients 1, 3); RP11-342J15 (red, family of patient 2); RP11-976O15 (3q13.2, red, family of patient 4); RP11-553L6 (red, family of patient 8); RP11-958M16 (red, family of patient 9); D3Z1 (chr3 centromere, green, family of patients 1, 3, 8, 9); RP11-306H5 (3p26.3, green, family of patient 4).

Supp. Table S1. Primer sequences used for breakpoint sequencing

Forward	GGTACCTAGAAACACCATGGTCTACCAGAA
Reverse	CTAGACCCCTCTGGCTCAAAGAAACAGATTAC
S1	AGGAATAGTAGAACAGCAGATGGAACACTGAG
S2	AGACATGAGGGCTAGGCTAAAACAGTAAGGTC
S3	TACTGTAGCAGGCGAGTGATAACAGGCTTT
S4	CACCAGAGTTGGGGAGTTTTAAGAGGTTTAG
S5	CAGGTGAGTTGAACAGTCCGATTTTCAGTG
S6	GGTCTAGGACTGTAAAGTGTCTCAGGGTTG
S7	GGGGAGGTGATAAAAAGATTATAGGGTGGAG
S8	GAGTAGAAGGAGGAATGGAGGGTGGAAGTT