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

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Abstract

We describe the molecular and clinical characterization of nine individuals with recurrent, 3.4-Mb, *de novo* deletions of 3q13.2q13.31 detected by chromosomal microarray analysis. All individuals have hypotonia and language and motor delays and also variably express mild to moderate cognitive delays (8/9), abnormal behavior (7/9), and autism spectrum disorders (3/9). Common facial features include down-slanting palpebral fissures with epicanthal folds, a slightly bulbous nose, and relative macrocephaly. Twenty-eight genes map to the deleted region, including four

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strong candidate genes, *DRD3*, *ZBTB20*, *GAP43*, and *BOC*, with important roles in neural and/or muscular development. Analysis of the breakpoint regions based on array data revealed directly oriented human endogenous retrovirus (HERV-H) elements ∼5kb in size and of >95% DNA sequence identity flanking the deletion. Subsequent DNA sequencing revealed different deletion breakpoints and suggested non-allelic homologous recombination (NAHR) between HERV-H elements as a mechanism of deletion formation, analogous to HERV-I-flanked and NAHR-mediated AZFa deletions. We propose that similar HERV elements may also mediate other recurrent deletion and duplication events on a genome-wide scale. Observation of rare recurrent chromosomal events such as these deletions helps to further the understanding of mechanisms behind naturally occurring variation in the human genome and its contribution to genetic disease.

Keywords

3q13; microdeletion; hypotonia; NAHR; recurrent; developmental delay; microarray; HERV-H

Introduction

Deletions of the proximal long arm of chromosome 3 are rare, with less than 30 cases reported in the literature to date. These deletions vary considerably in size, location, breakpoints, gene content, and associated clinical phenotypes. Various molecular or cytogenetic techniques have been used to characterize them [Arai et al., 1982; Jenkins et al., 1985; McMorrow et al., 1986; Okada et al., 1987; Fujita et al., 1992; Genuardi et al., 1994; Mackie Ogilvie et al., 1998; Hou, 2004; Kosaki et al., 2005; Lawson-Yuen et al., 2006; Sato et al., 2007; Simovich et al., 2008; Shimojima et al., 2009; Molin et al., 2012; Wisniowiecka-Kowalnik et al., 2013]. This variability, in addition to the relative case rarity, has made it difficult to make clear genotype-phenotype correlations.

Recently, a notable milestone was reached by Molin *et al*. [2012], who found a smallest region of overlapping deletion (SRO) of about 580 kb at 3q13.31 in 24 of 27 individuals reported with proximal 3q deletions. Features of this novel 3q13.31 microdeletion syndrome included global developmental delay, hypotonia, postnatal overgrowth, hypoplastic male genitals, and characteristic craniofacial features that included epicanthal folds, hypertelorism, down-slanting palpebral fissures, protruding lips with full lower lip and thin upper lip, and a high arched palate. The authors hypothesized that the observed global developmental delay may be caused by haploinsufficiency of *DRD3* (OMIM #126451) and *ZBTB20* (OMIM #606025), two genes within the SRO [Molin et al., 2012]. In this report, we present clinical and molecular findings for nine patients harboring recurrent 3.4-Mb deletions at 3q13.2q13.31 that completely encompass the previously reported SRO.

Recurrent copy-number variants (CNVs) are generally described as being of virtually identical size as a result of non-allelic homologous recombination (NAHR) between directly-oriented low-copy repeats (LCRs, also called segmental duplications) [Stankiewicz and Lupski, 2010]. These LCRs are defined as segments of the genome that are 1 kb or longer and at least 90% identical [Bailey et al., 2001], with those that mediate NAHR usually longer than 10 kb with over 95% DNA sequence identity [Sharp et al., 2005; Liu et

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al., 2012]. Although repetitive elements such as SINEs, LINEs and human endogenous retroviruses (HERVs) are not classically thought of as LCRs, some of them fulfill NAHR criteria and can mediate NAHR events [Beck et al., 2011]. Indeed, HERV-I (also named HERV15) repetitive elements ∼10kb in size and of 94% DNA sequence identity have been shown to mediate the recurrent ∼800-kb deletions at Yq11.2 in patients with complete germ cell aplasia (Sertoli Cell Only syndrome, OMIM 415000) [Kamp et al., 2000; Sun et al., 2000; Turner et al., 2008]. Likewise, HERV-H and L1MA4 elements have also been implicated in mediating recurrent deletions and translocations of autosomes [Rosenfeld et al., 2011; Hermetz et al., 2012; Lamb et al., 2012].

Here, we describe nine identically-sized deletions observed in patients with 3q13.31 microdeletion syndrome. Our molecular characterization of the breakpoints provides insight into the mechanisms of recurrent CNV formation and further supports the role of repetitive elements as substrates for NAHR. Moreover, the consistent genotype among these individuals allows for a clearer correlation of a phenotypic pattern with deletion of a specific set of genes.

Materials & Methods

Patient ascertainment

Patients 1-4 were ascertained by Signature Genomic Laboratories following referral for clinical microarray-based comparative genomic hybridization (aCGH) testing. Patients 5 and 6 were ascertained following referral for clinical genetic evaluation by Nemours Children's Clinic and Brigham and Women's Hospital, respectively. Patient 7 was ascertained by ARUP Laboratories following referral for clinical SNP microarray testing. Patients 8 and 9 were ascertained by Baylor College of Medicine (BCM) Medical Genetics Laboratories following referral for aCGH testing. Either de-identified clinical information was supplied by clinicians or informed consent was obtained to publish clinical information and images using an Institutional Review Board-approved protocol from Spokane or BCM.

Microarray analysis

Oligonucleotide-based aCGH analysis was performed on DNA from patient 1 using a 105K-feature, whole-genome microarray (SignatureChipOS version 1, custom-designed by Signature Genomics, Spokane, WA; manufactured by Agilent Technologies, Santa Clara, CA) as previously described [Ballif et al., 2008]. DNA from patients 2-5 was analyzed using a 135K-feature, whole-genome microarray (SignatureChipOS version 2, custom-designed by Signature Genomics; manufactured by Roche NimbleGen, Madison, WI) as previously described [Duker et al., 2010]. Results were analyzed and visualized using custom aCGH analysis and web-based data visualization software (Genoglyphix, Signature Genomics). DNA from patient 6 was studied using 44K-feature, whole-genome, oligonucleotide-based aCGH (GenomeDx, version 1.0, GeneDx, Gaithersburg, MD). DNA from patient 7 was analyzed using a 2.7 million-feature SNP array, consisting of ∼1.95 million non-polymorphic oligonucleotide probes for evaluating copy number and ∼750K SNP probes (Affymetrix CytoScan HD, Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. DNA from patients 8 and 9 was analyzed using 105K and 180K-feature

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oligonucleotide-based microarrays custom-designed by BCM Medical Genetics Labs, CMA v7.4 and 8.1 (exon-targeted), respectively, each manufactured by Agilent Technologies. Data were analyzed using custom web-based software as described previously [Cheung et al., 2005].

Fluorescence in situ hybridization

Copy-number abnormalities detected by microarray in patients 1-5 and 8-9 were visualized by metaphase or interphase fluorescence *in situ* hybridization (FISH) using one or more BAC clones located within the abnormal regions as previously described [Traylor et al., 2009]. When available, parental samples were also analyzed using FISH.

Long-range PCR and DNA sequencing

Long-range PCR primers were designed to sequence across a 3,137 bp region of high sequence identity harbored within two HERV-H elements to amplify the deletion-specific breakpoint fragment. Primer sequences are provided in the online supporting information (Supp. Table S1). Amplification was achieved using Takara *LA Taq* polymerase (Takara Bio, Otsu, Japan) according to the manufacturer's protocol. PCR products were treated with ExoSAP-IT (USB, Cleveland, OH, USA) to degrade unconsumed dNTPs and primers. Sanger sequencing with primers used for amplification was performed on the deletion-specific amplicon for each patient (Lone Star Labs, Houston, TX, USA). The breakpoint region for each patient was then determined by aligning the Sanger sequencing reads to the reference sequence using the Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA). Data are accessible at the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/, accession numbers AB811796 through AB811844).

Results

Molecular analysis

Microarray analysis showed all nine patients to have apparently identical, 3.4-Mb 3q13.2q13.31 deletions (Figure 1). No patients had any additional, clinically significant CNVs. Parental FISH showed the deletions to be apparently *de novo* in patients 1-4, 6, and 8-9 (Supp. Figure S1); additionally, the mother of patient 5 did not carry the deletion, while the father was not available for study.

Six of these recurrent deletions were identified among 51,000 routine postnatal clinical specimens submitted to Signature Genomics and BCM tested on oligonucleotide-based arrays (0.01%). No such deletions were identified among two adult control cohorts of 19,584 [a filtered set from Rosenfeld et al., 2013] and 3,181 [International Schizophrenia Consortium, 2008], among a pediatric control cohort of 2,026 [Shaikh et al., 2009], nor among 4,706 parents tested by oligonucleotide arrays at Signature or BCM. Additionally, no similar large deletions are present in the Database of Genomic Variants (http://projects.tcag.ca/variation/). The largest deletion among controls was in a single adult, overlapping 800kb of the proximal end of the region. Due to the rarity of the deletion, a case-control comparison does not reach significance (6/51,000 vs. 0/29,497, one-tailed p=0.06, Fisher exact test).

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Genomic sequences were downloaded from the UCSC genome browser (http://genome.ucsc.edu). Bioinformatic analyses of the shared deletion breakpoints revealed no LCRs; however, HERV-H elements were identified at both breakpoints. Each element is annotated in Repeat Masker (http://www.repeatmasker.org) as two long terminal repeat-7 (LTR7) sequences bracketing two central HERVH-int annotations totaling approximately 5.7 kb. These repeats lie in direct orientation with reference to each other and share three stretches of greater than 96% sequence identity spanning a total of approximately 4.8 kb. DNA for further testing was available for eight patients (1-5 and7-9). Long range PCR amplification of deletion-specific amplicons followed by Sanger sequencing analysis revealed that each patient has breakpoints occurring within a 1,340 base pair stretch of 97.6% sequence homology that is contained within both HERV-H elements (chr3:112,138,686-112,140,025 and chr3:115,514,625-115,515,964, hg19) (Figure 2).

Because of the high DNA sequence identity, exact determination of the breakpoints was not possible; however, single nucleotide and indel differences between the viral elements (*cis*-morphisms or paralogous sequence variants) enabled narrowing the regions containing the breakpoints to 9-121 bp segments (Table 1; Figure 2). Overall, six different breakpoint regions were identified. Patients 2 and 7 both had breakpoints that mapped between the informative *cis*-morphisms at 112,138,807 / 115,514,746 and 112,138,859 / 115,514,798. The remaining patients had unique breakpoint regions.

Clinical analysis

An overview of the clinical features of our nine patients is presented in Table 2, and detailed summaries are provided in the online supporting information. Neurologically, motor and language delays are observed in all patients. Cognitive delay is present in 8/9; patient 1 has gross motor delays, stuttering, anxiety, and short attention span but no definitive cognitive problems. Patient 3 displayed loss of skills at 2 years following stroke-like episodes. Seven patients exhibit abnormal behaviors, including stereotypic behaviors, sensory issues, and aggression. Patients 4 and 9 have diagnoses of pervasive developmental delay, and patient 3 has high-functioning autism, with improvements in her behavior with age. Hypotonia is found in all nine patients and is likely a major contributing factor to their prevalent motor delays. Brain abnormalities include agenesis of the corpus callosum (patients 3 & 9), Chiari malformation (patient 5), and probable benign communicating hydrocephalus (patient 8). Neurologic exam was suggestive of cerebellar dysfunction in patient 6, though brain imaging has not been performed. Patients 3 and 8 had multiple seizure episodes without need for medication, and EEG abnormalities were documented in patients 1 and 6. A characteristic craniofacial appearance is also present (Figure 3). Notably, epicanthal folds, down-slanting palpebral fissures, broad or depressed nasal bridge, bulbous nasal tip, and a prominent lower lip are present to varying degrees in many of our patients. All nine patients have a head circumference above the mean, with three demonstrating frank macrocephaly. Ocular abnormalities include myopia or hyperopia in three patients and strabismus or nystagmus in four other patients. Other congenital anomalies were uncommon, including hydrocele and unilateral renal agenesis (patient 8), unilateral cryptorchidism (patient 5), and spontaneously resolving patent ductus arteriosus (patient 4). Patient 5 also demonstrated nonspecific mild-to-moderate type 2 myofiber atrophy on muscle biopsy.

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Discussion

We report nine patients with a recurrent 3.4-Mb deletion at 3q13.2q13.31, all apparently occurring *de novo* when parents were tested. Our patients presented with a phenotypic spectrum, but a pattern could nevertheless be seen, including motor and language delays, hypotonia, variable cognitive impairment, abnormal behaviors, and dysmorphic features (Table 2, Figure 3). The large head sizes in our patients are in agreement with postnatal growth above the mean as a key characteristic of the 3q13.31 microdeletion syndrome [Molin et al., 2012]. In our cohort, this trend is most apparent with respect to head size, with a mean head circumference of +1.4 standard deviations (SD), while mean height is +0.8 SD, and mean weight is +0.6 SD. Two of the four patients with brain abnormalities had agenesis of the corpus callosum, a feature that has been previously reported in at least seven patients with 3q13 deletions [McMorrow et al., 1986; Genuardi et al., 1994; Mackie Ogilvie et al., 1998; Lawson-Yuen et al., 2006; O'Driscoll et al., 2010; Molin et al., 2012; Wisniowiecka-Kowalnik et al., 2013]. Genital anomalies were present in only two of the four male patients in this study, possibly indicating a lower frequency of the abnormal male genitalia that were previously identified as a key characteristic of the 3q13.31 microdeletion syndrome [Molin et al., 2012]. Genital anomaliesmay be more prevalent in patients whose 3q13 deletion breakpoints extend beyond the boundary of the recurrent deletion reported in this study, although a male with a smaller deletion within this region has been reported with micropenis and cryptorchidism [Molin et al., 2012].

Twenty-eight RefSeq genes have been mapped within the recurrently deleted region at 3q13.2q13.31 (Figure 1). At least five genes of these genes, *ZBTB20*, *DRD3*, *GAP43* (OMIM #162060), *BOC* (OMIM #608708), and *ZDHHC23* (Entrez Gene #254887) have been implicated in neurodevelopment or nervous system function, making them candidates for the neurodevelopmental phenotypes in these patients. Two of these genes, *ZBTB20* and *DRD3*, are within the currently defined SRO for the 3q13.31 microdeletion syndrome [Molin et al., 2012]. *Zbtb20* encodes a transcriptional repressor that is a cell fate determinant for hippocampal neurons [Nielsen et al., 2007; Xie et al., 2010]. Interestingly, Zbtb20 has been shown to regulate several genes whose haploinsufficiency results in overlapping neurodevelopmental phenotypes to this syndrome [Nielsen et al., 2013], including *Foxp2* [MacDermot et al., 2005], *Mef2c* [Le Meur et al., 2010], *Satb2* [Rosenfeld et al., 2009], and *Sox5* [Lamb et al., 2012]. Further support for the role of this gene in the microdeletion syndrome's pathogenesis is provided by its *de novo* disruption in a male with pervasive developmental delay due to a chromosome 3 inversion [Talkowski et al., 2012]. *DRD3* encodes a dopamine receptor present in the limbic system [Sokoloff et al., 1990]. Both heterozygous and homozygous knockout mice show hyperactivity [Accili et al., 1996], and homozygous knockout mice have deficits in spatial working memory [Glickstein et al., 2002]. *GAP43* encodes a phosphoprotein with roles in axonal growth, learning, and memory [Cammarota et al., 1997; Routtenberg et al., 2000; Pascale et al., 2004]. Heterozygous knockout mice demonstrate learning delays and autistic-like features, including resistance to change, stress-induced behavioral withdrawal, and anxiety [Zaccaria et al., 2010]. *BOC* encodes a co-receptor for SHH [Sanchez-Arrones et al., 2012], and targeted knockout in mice results in misguidance of commissural axons [Okada et al., 2006]. Finally, *ZDHHC23*

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encodes a palmitoyl transferase that modifies large conductance calcium-activated potassium channels [Tian et al., 2012], whose altered function can result in epileptic pathology [N'Gouemo, 2011], a feature seen in 4/9 of our patients.

Given their roles in neural development, haploinsufficiency of some of these genes may also contribute to the corpus callosum and cerebellar malformations in these and previously reported patients. Mice lacking *Gap43* display an absence of the corpus callosum, hippocampal commissure, and anterior commissure, due to failure of commissural axons to cross the midline during brain development [Shen et al., 2002]. Heterozygous mice exhibit features intermediate between those of wild type and *Gap43* knockout mice [Donovan et al., 2002; Shen et al., 2002]. Given its role in the SHH pathway, *BOC* is another attractive candidate for the corpus callosum abnormalities. Homozygous *Boc* knockout mice are viable and do not have holoprosencephaly (HPE)-related phenotypes, as the gene has overlapping roles with other Shh co-receptors [Allen et al., 2011; Zhang et al., 2011]. However, the gene has been shown to modify the HPE phenotype in mice lacking *Cdon*, which encodes another Shh co-receptor related to and a binding partner of Boc [Zhang et al., 2011]. Furthermore, one reported HPE-associated mutation impairs the ability of CDON to bind to BOC, although other HPE *CDON* mutations do not [Bae et al., 2011]. Finally, *Zbtb20* is also expressed in the cerebellum and corpus callosum [Mitchelmore et al., 2002]. Thus, it is possible that haploinsufficiencyof any, or a combination of, these genes may be involved in the brain abnormalities observed in patients with 3q13 deletions, though additional factors are likely required for manifestation, as penetrance is not complete for this phenotype.

The hypotonia and reduced reflexes present in these individuals are suggestive of neuromuscular dysfunction, and patient 5 showed myofiber atrophy. In addition to its role in the SHH pathway, BOC is also a cell adhesion molecule with roles in proper myogenesis [Kang et al., 2002], making it a candidate for these phenotypes. GAP43 may play important roles in skeletal muscle development and function [Guarnieri et al., 2013], and mice lacking the orthologue demonstrated muscle incoordination, reduced strength, and altered reflexes, with heterozygotes demonstrating moderate impairments [Metz and Schwab, 2004]. Finally, the dopamine receptor Drd3 helps regulate spinal reflexes [Clemens and Hochman, 2004], so its deletion may contribute to the depressed reflexes observed.

The deletion breakpoints of each of our patients were mapped within HERV-H elements. HERVs comprise approximately 8% of the reference human genome and are the remnants of viral infections in the germline cells of our distant ancestors [Lander et al., 2001; Paces et al., 2004]. The vast majority of HERVs contain single nucleotide variations, deletions or insertions that render them incapable of transposition or infection. Nonetheless, phylogenetic studies reveal HERVs undergo extensive inter-element recombination [Hughes and Coffin, 2001]. Thus, HERV elements scattered throughout the genome could provide a substrate for NAHR. Previous studies have identified HERV-H elements at the breakpoints of recurrent 1q41q42 deletions [Rosenfeld et al., 2011] and recurrent t(4;18) (q35.1;q22.3) translocations [Hermetz et al., 2012], each in two unrelated patients. Similarly, a single patient has been identified with a deletion at 8q13.3 involving *EYA1* apparently mediated by HERV-H elements [Sanchez-Valle et al., 2010].

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Evidence from cell culture suggests that NAHR events require stretches of extremely high homology or identity shared between two non-allelic loci known as minimal efficient processing segments (MEPSs) [Gu et al., 2008]. In human meiosis, minimum MEPS length was estimated at 300 to 500 base pairs [Reiter et al., 1998]. However, in a single sperm/cell assay, Lam and Jeffreys [2006] identified both meiotic and mitotic NAHR events between human alpha-globin genes mediated by matching fragments smaller than 50 bp. Interruption of the MEPSs with one or more base mismatches is known to deleteriously affect the efficiency of NAHR; in mice, the presence of as few as two nucleotide mismatches results in an ∼20 fold reduction in NAHR [Waldman and Liskay, 1988]. Based on the short length of uncertainty identified in our patients' breakpoints, when comparing to the reference haploid human genome, 300 bases of 100% identity are not present. Moreover, recent studies suggest that efficiency of NAHR is correlated with LCR length [Cooper et al., 2011; Liu et al., 2011; Dittwald et al., 2013]. However, the homologous regions of the HERV-H elements identified at the breakpoints in our patients totaled only 4.8 kb. Despite the apparent factors opposing NAHR between these HERV-H elements, we have identified nine such recurrent deletions.

Previous studies have identified evidence for NAHR events mediated by HERV elements [Kamp et al., 2000; Sun et al., 2000; Turner et al., 2008]. Sperm PCR analysis estimated the *de novo* mutation rate of the HERV-mediated AZFa deletion in Yq11.2 to be $\sim 2 \times 10^{-3}$ per generation, much higher than the average locus-specific CNV mutation rate throughout the genome. The observation that the deletions were present in the sperm, but not in blood, suggests that the rearrangements occurred during meiosis, strengthening the NAHR hypothesis [Turner et al., 2008].

Alternatively, the deletions identified in our patients might be best explained by errors in DNA replication, such as those proposed in fork stalling and template switching (FoSTeS) [Lee et al., 2007] or microhomology-mediated break-induced replication (MMBIR) [Hastings et al., 2009] mechanisms. Under such a model, the HERV-H sequences would provide the microhomology substrate that facilitates a template switch during DNA replication. However, to date, no recurrent CNVs mediated by FoSTeS or MMBIR have been described. Of note, the same sperm PCR analysis estimated the *de novo* deletion to duplication ratio at Yq11.2 to be 4.11 to 1, considerably higher than the ∼2 to 1 ratio observed at autosomal loci, suggesting a difference between these HERV-mediated events on the Y chromosome and other, more canonical NAHR events [Turner et al., 2008].

Our cohort confirms many of the features previously attributed to the deletion of the SRO within 3q13.31, including global developmental delay, hypotonia, and dysmorphic features. In addition, the recurrent deletions in our cohort are associated with a high prevalence of behavioral and brain abnormalities. The molecular characterization of our patients' breakpoints revealed that HERV-mediated structural rearrangements may be more common than previously thought. Additional studies will be required to elucidate the exact mechanism of formation of CNVs associated with retroviral elements and determine their importance to human disease.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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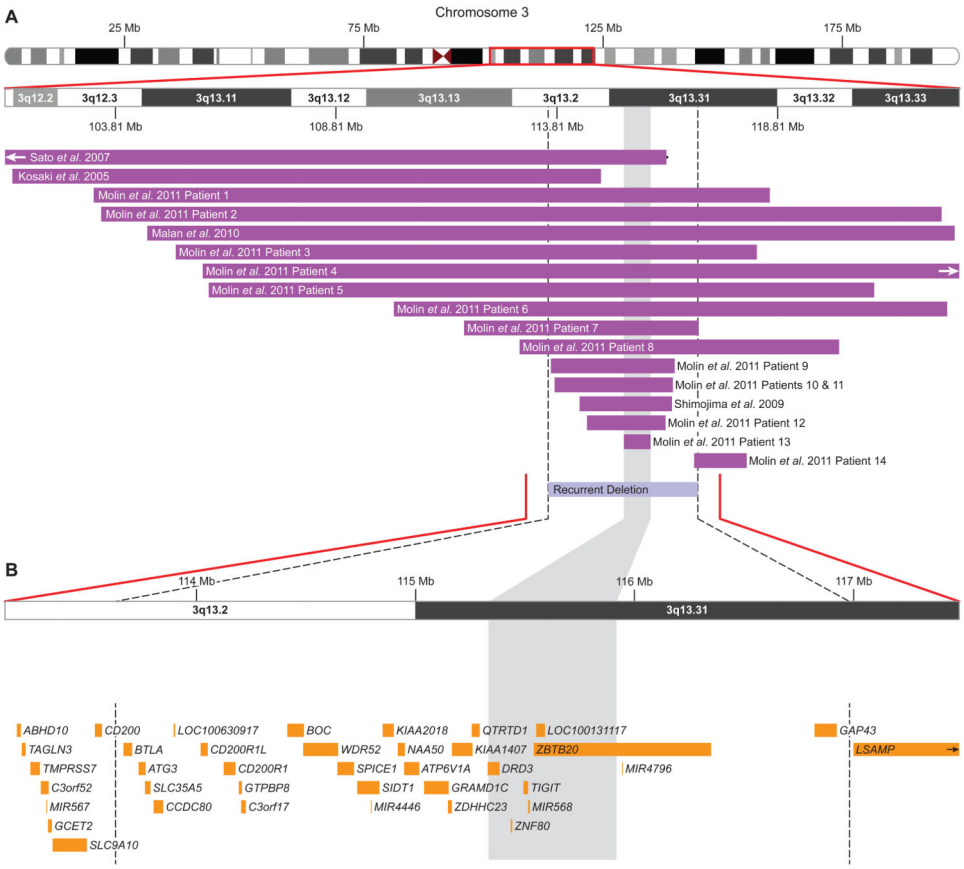

Figure 1. Microarray characterization of recurrent 3q13.2q13.31 deletions

(A) Schematic of molecularly characterized deletions in the literature overlapping the recurrently deleted region from this report. The purple bars represent the minimum size of the reported deletions, and, where available, horizontal black lines extend to show the maximum deletion size. The vertical dashed lines and light blue bar represent the recurrently deleted region. **(B)** Zoomed-in view of the recurrently deleted region with a representative microarray plot showing the deletion in patient 4, as characterized by a 135K whole-genome oligonucleotide-based array. Probes are ordered on the x-axis according to physical mapping positions, with the most proximal 3q13.2 probes to the left and the most distal 3q13.31 probes to the right. Values along the y-axis represent $\log_2$ ratios of patient:control signal intensities. Results are visualized using custom aCGH analysis software (Genoglyphix, Signature Genomics). Genes in the region are represented by yellow boxes. All coordinates shown are according to the hg19 build of the human genome, and the shaded gray region extending through the figure represents the SRO previously defined by Molin *et al* [2012].

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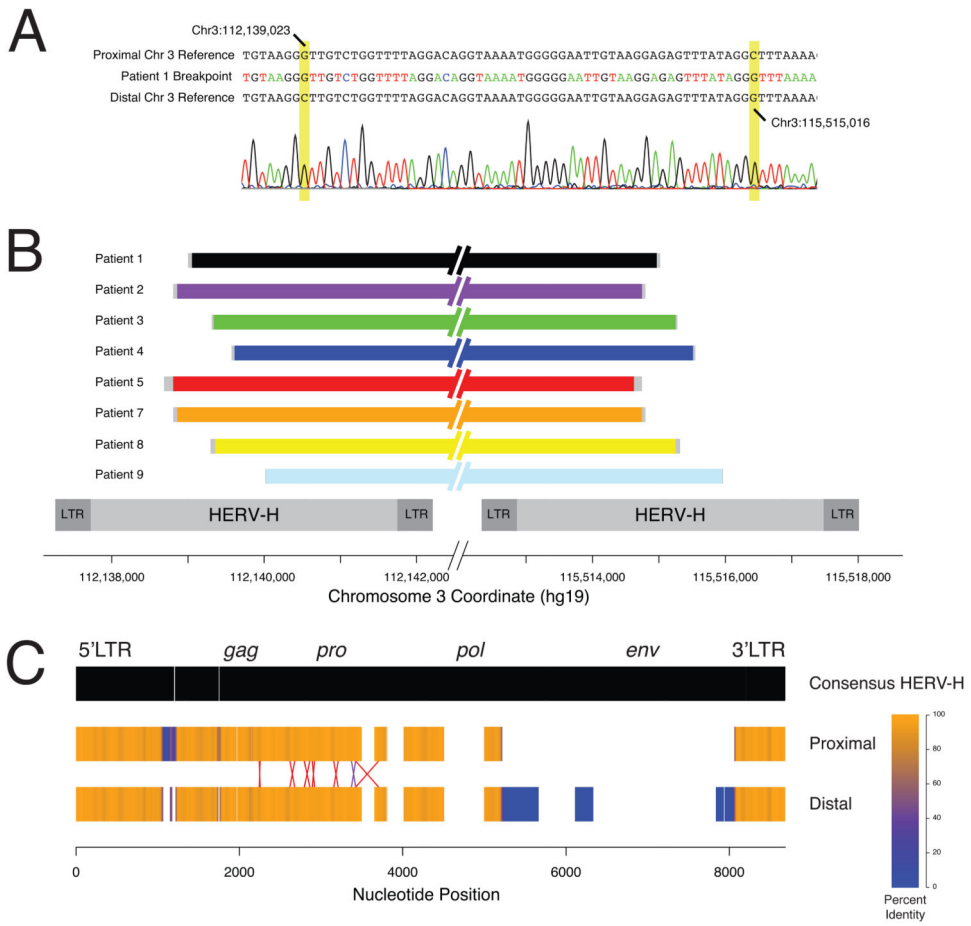

Figure 2. Breakpoint characterization of recurrent 3q13.2q13.31 deletions

(A) Representative Sanger sequencing trace of the breakpoint region of patient 1. The patient's breakpoint sequence is presented between the proximal and distal chromosome 3 reference sequences. The informative *cis*-morphisims that define the breakpoint uncertainty region are highlighted in yellow. **(B)** Schematic representation of the breakpoints of 8 patients that were available for further studies. The uncertainty region for each patient is depicted in grey. The structures of the HERV-H elements that contain the breakpoints of the patients are shown below as grey rectangles. All genomic coordinates are shown below in the GRCh37/hg19 assembly. **(C)** Structures of the 3q13.2q13.31 HERV elements compared to the consensus HERV-H sequence from RepBase. Gaps in the consensus represent insertions in the 3q13.2q13.31 HERVs. Gaps in the 3q13.2q13.31 HERVs represent deletions compared to the consensus. The color of the HERV elements denotes identity at that position when aligned with the other HERV over a 50 base pair window. Blue represents 0% sequence identity (*i.e*. caused by a 50 bp or larger insertion or deletion present in one 3q13.2q13.31 HERV but not the other) while orange represents perfect identity. The region of the cross-over in each patient is presented as an X with the size of the X representing the uncertainty bounded by informative *cis*-morphisms. The purple X represents the breakpoints in patients 2 and 7 that occur between the same two *cis*-morphisms.

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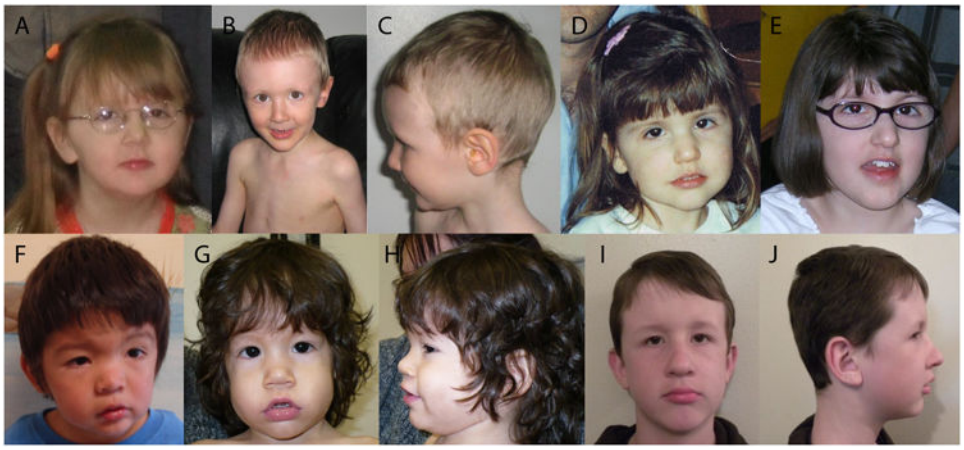

Figure 3. Facial features of individuals with 3q13.2q13.31 deletions

(A) Patient 1 at 5 years of age. **(B-C)** Patient 2 at 3 years of age. **(D-E)** Patient 3 at 2.5 years (D) and 10 years of age (E). **(F)** Patient 4 at 3 years of age. **(G-H)** Patient 5 at 16 months of age. **(I-J)** Patient 8 at 10 years of age. **(K-L)** Patient 9 at 5 years of age. Characteristic facial features include bulbous nasal tip, broad or depressed nasal bridge, prominent lower lip, and characteristically shaped eyes with mildly down-slanting palpebral fissures and mild epicanthal folds.

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Table 1

Breakpoint regions for 8 patients with recurrent 3q13.2q13.31 deletions

Patient	Proximal Min	Proximal Max	Distal Min	Distal Max	Uncertainty (bp)
1	112,139,023	112,139,077	115,514,961	115,515,016	55
2	112,138,807	112,138,859	115,514,746	115,514,798	52
3	112,139,311	112,139,338[a]	115,515,251	115,515,278[a]	27
4	112,139,553	112,139,611	115,515,494	115,515,552	58
5	112,138,686	112,138,807	115,514,625	115,514,746	121
7	112,138,807	112,138,859	115,514,746	115,514,798	52
8	112,139,369	112,139,435	115,515,310	115,515,376	66
9	112,139,977	112,139,986[a]	115,515,918	115,515,927[a]	9

[a]In these cases, the informative *cis*-morphisms are 1 bp deletions. All coordinates are provided in the GRCh37/hg19 assembly.

Hum Mutat. Author manuscript; available in PMC 2015 October 09.

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Table 2

Clinical features in individuals with recurrent 3q13.2q13.31 deletions

	Patient	1	2	3	4	5	6	7	8	9	Total	Previous reports[a]
	Inheritance	dn	dn	dn	dn	Not mat	dn	Unk	dn	dn	7/7 dn	3/3 dn
Demographics	Age	8y	3y	9y 8m	3y 4m	16m	42y	16y	10y	5y		10-13y
	Sex	F	M	F	M	M	F	F	M	F		2M, 1F
Growth	Height standard deviations	+1.7	+2.4	-0.4	WNL	+1.5	-0.9	0	+1.7	+0.7	1/9>+2	1/3 >+2
	Weight standard deviations	+2.2	+0.7	-0.4	WNL	-0.7	obese	0	+1.0	+1.2	2/9>+2	1/3 >+2
	OFC standard deviations	+2.1	+0.7	+0.7	+0.9	+2.3	+1.1	+2.1	+1.6	+1.1	3/9>+2	0/3 >+2
Neurological	Cognitive delay	-	+	+	+	+	+	+	+	+	8/9	3/3
	Motor delay	+	+	+	+	+	+	+	+	+	9/9	3/3
	Language delay/disability	+	+	+	+	+	+	+	+	+	9/9	2/3
	Brain abnormality	-	NA	+	-	+	NA	-	+	+	4/7	NS
	Seizures/EEG abnormalities	+	-	+	-	-	+	-	+	-	4/9	0/3
	Hypotonia	+	+	+	+	+	+	+	+	+	9/9	0/1
	Reduced reflexes	+	-	-	-	+	NS	-	-	+	3/8	NS
	Abnormal behaviors	+	+	+	+	-	+	-	+	+	7/9	1/3
	Autism spectrum disorder	-	-	+	+	-	-	-	-	+	3/9	0/3
	Enuresis	+	-	+	-	NA	+	-	+	-	4/8	NS
Musculoskeletal	Lordosis/scoliosis	+	-	+	-	-	-	+	-	-	3/9	0/3
	Foot abnormalities	+	+	-	-	-	+	+	-	-	4/9	0/3
Craniofacial	Absent eyebrows	-	-	-	-	-	+	+	-	-	2/9	0/2
	Epicanthal folds	-	+	-	+	+	+	-	+	+	6/9	1/2
	Down-slanting PF	+	+	+	-	+	+	-	+	-	6/9	0/3
	Ptosis	+	-	+	-	-	+	+	-	-	4/9	0/2
	Broad nasal bridge	+	+	-	+	+	-	-	-	+	5/9	1/2
	Bulbous nasal tip	-	-	+	+	+	-	-	+	-	4/9	1/2
	High/abnormal palate	+	-	-	-	-	+	-	+	-	3/9	NS
	Prominent lower lip	+	+	-	+	+	-	+	-	-	5/9	1/2
Ocular	Myopia/hyperopia	+	-	-	-	-	-	+	+	-	3/9	0/2
	Strabismus/nystagmus	-	+	+	-	-	+	-	-	+	4/9	1/2

Hum Mutat. Author manuscript; available in PMC 2015 October 09.

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	Patient	1	2	3	4	5	6	7	8	9	Total	Previous reports[a]
Urogenital	Genital abnormalities	-	-	-	-	+	-	-	+	-	2/4 M	1/2 M
	Renal abnormalities	-	-	-	-	-	-	-	+	-	1/9	0/3

dn, *de novo*; F, female; M, male; m, months; mat, maternal; NA, not applicable; NS, not specified; OFC, occipitofrontal circumference; PF, palpebral fissures; Unk, unknown; WNL, within normal limits; y, years

[a]Three patients reported by Molin *et al.* (cases 9-11) (14) were approximately of the same size and may represent this recurrent deletion.

Hum Mutat. Author manuscript; available in PMC 2015 October 09.