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Recurrent Deletions and Reciprocal Duplications of 10q11.21q11.23 Including *CHAT* and *SLC18A3* are Likely Mediated by Complex Low-Copy Repeats

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Web Resources
Blast2 (http://blast.ncbi.nlm.nih.gov/Blast.cgi)
Database of Genomic Variants (http://projects.tcag.ca/variation/)
Human Genome Segmental Duplication Database (http:// projects.tcag.ca/humandup/)
Miropeats (http://genome.wustl.edu/software/miropeats)
Online Mendelian Inheritance in Man (http://www.omim.org)
Repeatmasker (http://www.repeatmasker.org)
UCSC genome browser (http://genome.ucsc.edu)

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Abstract

We report 24 unrelated individuals with deletions and 17 additional cases with duplications at 10q11.21q21.1 identified by chromosomal microarray analysis. The rearrangements range in size from 0.3 to 12 Mb. Nineteen of the deletions and eight duplications are flanked by large, directly oriented segmental duplications of >98% sequence identity, suggesting that nonallelic homologous recombination (NAHR) caused these genomic rearrangements. Nine individuals with deletions and five with duplications have additional copy number changes. Detailed clinical evaluation of 20 patients with deletions revealed variable clinical features, with developmental delay (DD) and/or intellectual disability (ID) as the only features common to a majority of individuals. We suggest that some of the other features present in more than one patient with deletion, including hypotonia, sleep apnea, chronic constipation, gastroesophageal and vesicoureteral refluxes, epilepsy, ataxia, dysphagia, nystagmus, and ptosis may result from deletion of the *CHAT* gene, encoding choline acetyltransferase, and the *SLC18A3* gene, mapping in the first intron of *CHAT* and encoding vesicular acetylcholine transporter. The phenotypic diversity and presence of the deletion in apparently normal carrier parents suggest that subjects carrying 10q11.21q11.23 deletions may exhibit variable phenotypic expressivity and incomplete penetrance influenced by additional genetic and nongenetic modifiers.

Keywords

CHAT; SLC18A3; genomic rearrangement; array CGH

Introduction

Segmental duplications comprise approximately 5% of the human genome [Bailey et al., 2002]. Misalignment of segmental duplications during meiosis can cause genomic instability through nonallelic homologous recombination (NAHR) [Lupski, 1998; Mefford and Eichler, 2009; Shaffer et al., 2001; Stankiewicz and Lupski, 2002]. In NAHR, improper crossing-over between nonallelic, yet homologous, segments (such as low-copy repeats [LCRs]) on sister chromatids or on homologous chromosomes produces microdeletions, microduplications, and inversions of the intervening genomic sequence, depending on the orientation of the segmental duplications [Koolen et al., 2006; Shaffer and Lupski, 2000; Sharp et al., 2006; Shaw-Smith et al., 2006; Stankiewicz and Lupski, 2002; Mefford and Eichler, 2009]. Chromosomal rearrangements associated with segmental duplications include deletions at 3q29 (MIM# 609425) [Willatt et al., 2005] and their reciprocal duplications (MIM# 611936) [Ballif et al., 2008a]; Williams-Beuren syndrome (MIM# 194050) and deletions at 7q11.23 and their reciprocal duplications (MIM# 609757) [Berg et al., 2007; Somerville et al., 2005]; Angelman (MIM# 105830) and Prader-Willi syndromes (MIM# 176270) and maternally and paternally derived deletions, respectively, of 15q11q13; deletions at 16p11.2p12.2 (MIM# 613604) and the reciprocal duplications [Ballif et al., 2007]; Smith-Magenis syndrome (MIM# 182290) and deletions at 17p11.2 and their reciprocal Potocki-Lupski syndrome duplications (MIM# 610883) [Potocki et al., 2007, 2000]; and duplication at 17p12 in Charcot-Marie-Tooth disease type 1A (MIM# 118220) and the reciprocal deletion causing hereditary neuropathy with liability to pressure palsies

(MIM# 162500) [reviewed in Stankiewicz and Lupski, 2002]. Such recurrent syndromes are termed “genomic disorders” and usually meet several criteria: the rearrangement has breakpoints in flanking segmental duplications, is usually de novo in affected individuals and rarely observed in controls, and patients with the same rearrangement have similar, consistent phenotypes [Lupski, 1998; Mefford and Eichler, 2009]. The underlying genomic architecture in each of the genomic disorders identified to date is similar: a stretch of unique sequence (50–10 Mb) flanked by large (>10 kb), highly homologous (>95%) segmental duplications that provide the substrate for NAHR.

To date, interstitial deletions involving 10q11.2 have been reported in over 10 patients with variable abnormal phenotypes, individuals with a normal phenotype, and two prenatal cases, one with a normal and the other with an abnormal phenotype [Bisgaard et al., 2007; Fewtrell et al., 1994; Fryns et al., 1991; Ghai et al., 2011; Holden and MacDonald, 1985; Kirchhoff et al., 2005; Lobo et al., 1992; Shapiro et al., 1985; Zenger-Hain et al., 1993]. The only clinical feature common to a majority of subjects was intellectual disability (ID)/developmental delay (DD); whereas epilepsy, ataxia, clinodactyly, and bowel obstruction were all reported in one subject. However, because all but three of these individuals were identified by standard G-banded chromosome analysis, the precise deletion intervals are unknown.

Here, we report the clinical and molecular characterization of 24 individuals with deletion at 10q11.21q21.1 and 17 individuals with duplications of the same region.

Materials and Methods

Patient Ascertainment

Individuals with 10q11.21q21.1 deletions and duplications reported here were identified after referral for chromosomal microarray analysis to clinical laboratories, including Signature Genomic Laboratories (SGL) (patients 8–14, 17, 18, 24, 30–38), Baylor College of Medicine (BCM) (patients 1–6, 19–23, 25–29), Washington University School of Medicine (patients 7, 39–41), Children's Hospital of Philadelphia (patient 15), and Service de Génétique Médicale CHUV, Lausanne, Switzerland (patient 16). Common indications for study included DD, ID, dysmorphic features, and congenital anomalies. We reevaluated DNA samples by sequencing for patients 1–7, 11, 15, 16, and 18–20 after obtaining informed consent via protocols approved by Institutional Review Board (IRB) for Human Subject Research at BCM and IRB, Spokane.

Initial Microarray Analysis

Deletions and duplications were initially ascertained through microarray-based comparative genomic hybridization (aCGH) using either BAC-based whole-genome arrays (SignatureChipWG v1.0.1 [designed and manufactured by SGL; patients 9–10, 13–14, 24, 29, 31–32]) or oligonucleotide-based whole-genome arrays (44K BCM V6 [patients 1–3], 105K BCM V7 [patients 4–6, 21, 22], 180K BCM V8 [patients 19–20, 22, 23, 25–28], 105K SignatureChip OS v1.0 [SGL, patient 8], 135K SignatureChip OS v2.0 [SGL, patients 11–12, 17–18, 24, 33–38], or 244K off-the-shelf array [Agilent Technologies, Santa Clara, CA; patient 16]). The deletion in patients 7 and 15 and duplications in patients 39–41 were initially identified using the genome-wide human single nucleotide polymorphism (SNP) array 6.0 (Affymetrix, Santa Clara, CA). DNA samples from both parents of patient 16 were tested using Agilent oligoNT 180K (Agilent Technologies, Santa Clara CA, USA). The BCM oligonucleotide arrays and the SignatureChip OS v 1.0 are custom designed by BCM Medical Genetics Laboratory (MGL) [Lu et al., 2007; Ou et al., 2008] and Signature Genomics, respectively, and manufactured by Agilent Technologies. The SignatureChip OS v2.0 is custom designed by SGL and manufactured by Roche NimbleGen (Madison, WI).

Studies were performed according to the manufacturer's instructions or previously described methods [Ballif et al., 2008a,b; Cheung et al., 2005; Duker et al., 2010].

High-Resolution Microarray Analysis to Refine the Breakpoints

Patients whose deletions were initially ascertained on BAC array had subsequent higher resolution studies done on whole-genome oligonucleotide arrays (105K SignatureChip OS v1.0, patient 9, or 135K SignatureChip OS v2.0, patients 10, 13, 14, and 27). These arrays have coverage of the unique sequence in the region (proximal to LCR 10q11.2A, between LCR 10q11.2A and B, between LCR 10q11.2B and C, between LCR 10q11.2C and D, between LCR 10q11.2D and E, between LCR10q11.2E and F, and distal to LCR10q11.2F).

Custom region-specific high-resolution 72K oligonucleotide arrays (Roche NimbleGen; median probe spacing 193 bp) were designed to determine the more precise location of the breakpoints, and DNA samples from patients 2–6 were reanalyzed on this array. Whole-genome high-resolution oligonucleotide microarray CGH analysis was also performed with the NimbleGen arrays 385K (patient 1) and 2.1M (patients 2, 3, and 4), in accordance with the manufacturer's instructions.

Fluorescence in situ Hybridization (FISH) Analysis

FISH was performed using BAC clones RP11-70E21 (patients 1– 4, 6, 20, 22, 25, 26, and 28), RP11-563N6 (patients 5, 19, and 22), RP11-541M12 (patients 7, 40, and 41), RP11-635L5 (patients 8, 9, 24, 29, and 35), RP11-100M24 (patients 10, 11, 33, and 36), RP11-140C5 (patients 12, 17, and 18), RP11-1005F22 (patients 13, 14, 30, 31, and 38), RP11-165A4 (patient 27), RP11-10N24 (patient 32), RP11-219K22 (patient 34), and RP11-168P8 (patient 37) from the 10q11.21q21.1 region to confirm and visualize the abnormalities using standard methodology.

DNA Sequencing of CHAT

Overlapping amplicons covering the entire coding region of 17 exons from all isoforms of *CHAT* (MIM# 118490) were amplified and sequenced in patients 1–7, 9, 11, 15, 16, 18, and 19 by conventional Sanger di-deoxynucleotide sequencing (Lone Star Labs, Houston, TX). DNA sequences were analyzed by comparison with reference sequence (NM_001451.2) with the use of Sequencher V4.8 (GeneCodes, Ann Arbor, MI). Individual primer sequences and polymerase chain reaction (PCR) conditions are available on request.

Bioinformatics and In Silico Sequence Analysis

Genomic sequences of 1 Mb in size for the region between 45 and 57 Mb (10q11.21q21.1) were downloaded from the UCSC genome browser (Build hg18, UCSC genome browser, March 2006) and masked using Repeatmasker. The repeat-masked sequences were then analyzed using Blast2, and the sequences with >90% identity and alignments of more than 400 bp were aligned according to the coordinates and their orientation.

To assess the chromosome architecture causing deletions and duplications in the 10q11.2 region, we evaluated the presence of LCRs using the ICAass (v 2.5) algorithm. The graphical display was performed using Miropeats (v 2.01) (The Genome Institute at Washington University, St Louis, MO) [Parsons, 1995a, b]. The program was run using two thresholds of 1,000 and 5,000 bp. For ease of computation, the 8.0 Mb interval was analyzed in two nonover-lapping blocks of 3.9 Mb each. The hg18 coordinates for these blocks are chr10: 45,300,000–49,200,000 and chr10: 49,300,000–53,200,000.

Results

Molecular Analysis

We identified 24 unrelated individuals with microdeletions at 10q11.21q11.23 by chromosomal microarray analysis. The deletions range in size from ~1.9 to ~10.9 Mb (Tables 1 and 2; Figs. 1–3).

Twenty deletions were visualized by FISH, and parental testing in 15 subjects showed that the deletions were inherited from the mother in six patients (3, 5, 6, 9, 11, and 20), from the father in seven patients (1, 2, 4, 7, 10, 14, and 24), and were apparently de novo in patients 15 and 16 (Tables 1 and 2). Parental samples for the remaining nine patients (8, 12, 13, 17–19, and 21–23) were unavailable.

We have also identified 17 individuals with reciprocal microduplications involving 10q11.21q21.1, ranging in size from ~0.3 to ~12 Mb (Table 3; Fig. 3). Parental testing showed that the duplications were inherited from the mother in six patients and from the father in three patients. Parental samples for the other eight patients were unavailable for testing (Table 3).

Smallest Region of Overlap

A comparison of the approximately 1.9 Mb smallest region of deletion overlap in the 24 deletions reported here (between 48.9 and 50.8 Mb) (Fig. 3) to locations of benign copy number variants (CNVs) in the Database of Genomic Variants revealed the presence of a small, unique sequence overlap of approximately 1.5 Mb (between 49.2 and 50.6 Mb; LCR10q11.2C to LCR10q11.2D) that has not been reported to be deleted in control individuals.

DNA Sequencing of CHAT

To determine whether the second allele of *CHAT* has a point mutation that could be unmasked by the 10q11.2 deletion, we sequenced all coding exons of *CHAT* in a cohort of 13 subjects (patients 1–7, 9, 11, 15, 16, 18, and 19) with heterozygous deletions of 10q11.2 that included *CHAT*. We did not identify any change in 221 amplicons analyzed.

Computational Analysis of the 10q11.21q11.23 Region

Using the hg18 build of the UCSC genome browser [Bailey et al., 2001] and the hg17 build of the Human Genome Segmental Duplication Database, we identified a complex arrangement of six segmental duplication clusters in the 10q11.21q11.23 region, labeled LCR 10q11.2A-LCR10q11.2F (Fig. 3). These segmental duplications range in size from 32 to 427 kb and have a complex evolutionary structure [Deloukas et al., 2004]. More than half of the analyzed 6.5 Mb region in 10q11.21q11.23 is occupied by these LCR clusters and seven genomic gaps. We identified 18 paralogous pairs of directly oriented LCR subunits between LCR10q11.2A–C and LCR10q11.2D–F (Fig. 3; Supp. Table S1). Sixteen similar sized approximately 6 Mb deletions (patients 2, 4–12, 15, 17–18, 20, 23, and 24) and five duplications (patients 26, 28, 33, 40, and 41) are flanked by LCR10q11.2A and LCR10q11.2E that harbor subunits approximately 130 kb in size with greater than 98.2% DNA sequence identity and in direct orientation with respect to each other. Rearrangements in six individuals might have been caused also by NAHR between LCR10q11.2C and LCR10q11.2D (patients 16 and 21) and LCR10q11.2A–B and LCR10q11.2D–E (patients 22, 29, and 32), and by LCR10q11.2B and LCR10q11.2E (patient 35). Five deletions (patients 1, 3, 13, 14, and 19) and nine duplications (25, 27, 30, 31, 34, 36–38, and 39) are not flanked by directly oriented LCR subunits and represent atypical breakpoints (Fig. 3; Supp. Table S1).

Using Miropeats program, we have identified the paralogous LCR subunits in the proximal and distal LCR10q11.2 that can serve as a substrate to NAHR (Figs. 3 and 4; Supp. Table S1). Those directly oriented overlap with the defined breakpoint regions and can serve as the substrates for NAHR (Fig. 3).

Identification of Additional Copy Number Changes in Patients

Nine of the 24 individuals with deletions have secondary copy number alterations. Three of the additional copy number changes in patients 3, 7, and 20 were inherited from phenotypically normal mothers. Patient 5 has an approximately 12 Mb additional, de novo deletion in 3q13, which likely confounds the clinical phenotype associated with the 10q deletion. The parental origins of the additional copy number changes in patients 9, 12, 19, and 22 are unknown. Patient 16 had mosaic trisomy 2 confined to the placenta identified by aCGH and confirmed by FISH in 15% of cells (Table 1). Uniparental disomy (UPD) testing was not performed.

Deletion and Duplication Frequency in Affected and Control Populations

We compared the frequency of the 10q11.21q11.23 rearrangements among our study population to the frequency in controls to determine whether the deletion predisposes individuals to an abnormal phenotype. No complete deletions or duplications of the unique sequence between LCR 10q11.2C and LCR 10q11.2D were found in six control groups consisting of 2,792 individuals [Kirov et al., 2009], 2,493 individuals [Itsara et al., 2009], 2,026 individuals [Shaikh et al., 2009], 1,152 individuals [Zogopoulos et al., 2007], 450 individuals [Conrad et al., 2010], and 270 individuals [Redon et al. 2006], although several small CNVs within this region were reported in two of the control populations [Itsara et al., 2009; Shaikh et al., 2009]. CNVs overlapping the proximal LCRs are also more frequent in these control populations, such as that seen in patient 14 (Fig. 2). Combining these populations yields a frequency of 0/9,183 LCR10q11.2C to LCR10q11.2D deletions in healthy controls.

To determine the frequency of the rearrangements in our study populations, we have combined the frequency of the deletions and duplications (involving the entire 1.9 Mb region between LCR10q11.2C and D), respectively, detected at BCM (11/25,354 and 4/25,354) with the frequency in Signature Genomics' patient database (10/32,821 and 8/32,821). Thus, the combined frequencies of deletions and duplications in our study populations are 21/58,175 and 12/58,175, respectively. The differences in deletion and duplication prevalences between the affected and the control populations are statistically significant ($P = 0.046$, Fisher Exact test) for deletions and not significant ($P = 0.17$, Fisher Exact test) for duplications, when considering the number of deletion and duplication alleles in diploid individuals or the number of individuals harboring deletion and duplication events.

Clinical Analysis

Clinical characterization of 20 of 24 individuals with microdeletions at 10q11.21q11.23 revealed variable clinical features (Table 4). The only clinical features common to a majority of individuals were ID and DD. Other clinical features identified in two or more individuals include failure to thrive, growth retardation, or short stature (42%, 8/19), chronic constipation (37%, 7/19), hypotonia (32%, 6/19), gatroesophageal reflux (GERD, 32%, 6/19), sleep apnea (26%, 5/19), cleft or high palate (26%, 5/19), epilepsy (21%, 4/19), autism spectrum disorders (ASDs; 27%, 4/15), microcephaly (19%, 3/16), corpus callosum abnormalities (18%, 2/11), attention deficit hyperactivity disorder (ADHD; 16%, 3/19), ataxia or disco-ordination (16%, 3/19), micrognathia (16%, 3/19), vesicoureteral reflux (16%, 3/19), severe eczema (16%, 3/19), scoliosis (11%, 2/19), significant congenital heart

defects (11%, 2/19), microphthalmia (11%, 2/19), dysphagia (11%, 2/19), short halluces (11%, 2/19), nystagmus (11%, 2/19), and ptosis (11%, 2/19). Interestingly, patient 1's brother with the same deletion had constipation, and Decipher patient 248902 (deletion of chr10: 49,130,990–50,638,651, including *CHAT*) presented with hypotonia, epilepsy, and sleep apnea.

Although detailed clinical information was not obtained for the duplication patients, the indication provided for testing for most patients included DD/ID. The next most common manifestations included ASDs (patients 25, 26, 28, and 31) and epilepsy (patients 28, 33, 37, and 38) (Table 3).

Discussion

The conventional wisdom surrounding genomic disorders posits that they fit several criteria: the deletions/duplications are large, highly penetrant, de novo in the majority of individuals, and associated with a uniform constellation of clinical features [Mefford and Eichler, 2009]. Smith-Magenis syndrome, Prader-Willi syndrome, and Williams-Beuren syndrome are examples of such “classic” genomic disorders. In contrast to these “classic” genomic disorders, many of the more recently described recurrent genomic lesions identified in large case–control studies demonstrate apparently diverse phenotypes and are frequently inherited while showing reduced penetrance [Ensenauer et al., 2003; Hannes et al., 2008; Klopocki et al., 2007; Mefford et al., 2008; Sharp et al., 2008., Ullmann et al., 2007; Yobb et al., 2005]. These studies suggest the phenotypic effects of such copy number changes are pleiotropic and imply the existence of shared biologic pathways among multiple neurodevelop-mental conditions, which may explain the variability of neurological manifestations within some families. However, even classical deletion syndromes can present with diverse phenotypes [Shah et al., 2008].

In the present study, the only consistent clinical features present in the majority of individuals with deletions or duplications were DD and ID (Tables 1–4). However, these clinical features probably reflect an ascertainment bias, because individuals are often referred for chromosomal microarray testing for general indications such as ID/DD.

Comparison with previously reported individuals with deletions encompassing 10q11.2 [Bisgaard et al., 2007; Fewtrell et al., 1994; Fryns et al., 1991; Ghai et al., 2011; Holden and MacDonald, 1985; Kirchhoff et al., 2005; Lobo et al., 1992; Puliti et al., 1993; Shapiro et al., 1985; Zenger-Hain et al., 1993] is uninformative because most of these deletions were identified by standard cytogenetic analysis and are substantially larger than the deletions reported here. Only two of the 15 deletions in our study for which parental samples were available were de novo. In several recently described syndromes including 8p23 duplication [Barber et al., 2008], 1q21.1 microdeletion (MIM# 612474) [Mefford et al., 2008], 15q13.3 microdeletion (MIM# 612001) [Sharp et al., 2008], and 16p12.1 microdeletion (MIM# 136570) [Girirajan et al., 2010], deletions have been found in control populations as well as in unaffected family members. Recurrent microdeletions of 16p12.1 have been identified in individuals referred for genetic testing for idiopathic ID and congenital anomalies and appear to be enriched in such individuals compared to clinically normal controls. Almost all the 16p12.1 microdeletions identified have been inherited from a carrier parent; carrier parents for the 16p12.1 microdeletion are more likely to exhibit learning disability, bipolar disorder or depression, and epilepsy than noncarrier parents. The presence of varying degrees of learning disability in the adult family members suggests that some transmitted abnormalities are pathological and have an underappreciated contribution to the phenotype [Girirajan et al., 2010]. Similarly, 10q11.21q11.23 deletions are found in 10 apparently normal parents (patients 2–7, 9–10, 14, and 20) and one grandparent (patient 7); however,

none have been reported in 9,183 controls. Two carrier parents (patients 1 and 11) and two carrier siblings (patients 1 and 11) are affected. The frequency of these deletions is significantly enriched in cases versus controls.

The variable expressivity resulting from haploinsufficiency of genes in the deletion region and its inheritance from apparently healthy carrier parents suggests additional modifiers, genetic and nongenetic, may influence the pathogenicity of the 10q11.21q11.23 microdeletions. Several explanations have been proposed for the variable expressivity and clinical heterogeneity in some genomic disorders. First, atypical or variable-sized copy number changes may account for the variable phenotypes in some apparently recurrent lesions. In our study population, the deletion sizes varied from 1.9 to 10.9 Mb, although there was substantial clustering of deletion intervals. In addition, five of the deletions and nine of the duplications were not flanked by directly oriented LCRs and thus are unlikely to be caused by NAHR. Atypical breakpoints have been reported for other recurrent rearrangements mediated by segmental duplications: for example, some of the rarer rearrangements of 17p11.2 associated with Smith-Magenis syndrome do not have breakpoints flanked by the typical paired segmental duplications and are not associated with known genomic architectural features [Stankiewicz et al., 2003], and some of the breakpoints in the recently identified 16p11.2p12.2 microdeletion syndrome are not flanked by segmental duplications [Ballif et al., 2007].

A “two-hit” model has also recently been proposed to account for phenotypic variability; it was first used to describe the recurrent deletion 16p12.1 [Girirajan et al., 2010]. In that study, 25% of probands carried a “second hit”—a 40-fold increase for two or more copy number changes over the general population. Furthermore, the clinical features in probands with two hits were different from those with just the second hit. Further analysis of other genomic disorders has shown clustering of two hits in copy number changes with variable phenotypes compared to syndromic lesions [Girirajan and Eichler, 2010]. One hit may be sufficient to reach a threshold that results in mild neurodevelopmental deficits, whereas a second hit is necessary for the development of a more severe neurological phenotype, including ID/DD, ASDs, or schizophrenia [Girirajan and Eichler, 2010]. Patients 5, 7, 9, 16, and 19 have additional large copy number changes, including ~12 Mb deletion 3q13.12q13.32, ~4.8 Mb duplication 12q23.1q23.2, ~11.4 Mb duplication 6p25.3p22.1, confined placental mosaicism for trisomy 2, and ~1.4 Mb duplication 15q21.2q21.3, respectively, that might have contributed additional features to their abnormal phenotypes not observed in other patients lacking additional copy number changes. In patient 19, one cannot exclude the possibility of genomic imprinting in this region also contributing to the phenotype.

A comparison of the approximately 1.9 Mb smallest region of overlap in the 24 deletions reported here to locations of benign CNVs revealed the presence of a small, unique sequence overlap of approximately 1.5 Mb (between 49.2 and 50.6 Mb, LCR 10q11.2 from C to D), where complete deletions have not been reported in control populations. This critical interval encompasses 11 RefSeq genes: *MAPK8* (MIM# 601158), *ARHGAP22* (MIM# 610585), *WDFY4* (MIM# 613316), *LRRC18*, *FAM170B*, *DRGX* (MIM# 606701), *ERCC6* (MIM# 609413), *PGBD3*, *SLC18A3* (MIM# 600336), *CHAT*, and *OGDHL* (Fig. 1). Some of these 11 genes are known to contribute to human disease. Recessive mutations in *ERCC6* have been reported in patients with Cockayne syndrome type B (CSB, MIM# 133540) [Falik-Zaccai et al., 2008; Ghai et al., 2011]. Interestingly, two genes, *CHAT*, encoding choline acetyltransferase and the single exon *SLC18A3* that maps to the first intron of *CHAT* and encodes vesicular acetylcholine transporter, are involved in cholinergic neurotransmission [Harold et al., 2003]. Point mutations in *CHAT* have been found in patients with autosomal recessive myasthenic syndrome with episodic apnea (CMS-EA;

MIM# 254210) [Ohno et al., 2001]. Of note, a few of our patients manifest some features typical for CMS-EA such as hypotonia, sleep apnea, dysphagia, and ptosis. Usually, haploinsufficiency for enzymes is not deleterious; however, exceptions have been described [Bademci et al., 2010]. We suggest that the above features as well as chronic constipation, gastroesophageal and vesicoureteral refluxes, epilepsy, ataxia, dysphagia, and nystagmus present in some of our patients with heterozygous deletions at 10q11.2, could have been exacerbated by haploinsufficiency of *SLC18A3* (cis-genetics effect) and may be mitigated with acetylcholinesterase inhibitors. In support of this notion, a 50–100% increase of the choline transporter 1 (*Slc5a7*) mRNA was found in the heterozygous $Chat^{+/-}$ mice and was proposed to compensate for the reduced Chat activity and restore the acetylocholine amount [Brandon et al., 2004]. Alternatively, the abnormal phenotype in patients with a heterozygous deletion of a gene responsible for an autosomal recessive trait can result from unmasking of a recessive mutation or functional polymorphism of the remaining allele [Kurotaki et al., 2005]. However, we did not identify any change in 221 amplicons sequenced in the second allele of *CHAT* in 13 patients with heterozygous deletions of 10q11.2.

Notably, none of the deletions presented here harbor the *RET* proto-oncogene that maps 2.5 Mb centromeric to the proximal LCR10q11.2 LCR cluster. Mutations in *RET* have been reported in patients with multiple endocrine neoplasia, type IIA (MEN2A; MIM# 171400), MEN, type IIB (MEN2B; MIM# 162300), Hirschsprung disease (HSCR; aganglionic megacolon; MIM# 142623), and medullary thyroid carcinoma (MTC; MIM# 155240). Interestingly, Puliti et al. [1993] described a patient with a cytogenetically visible deletion 10q11.2q21.2 and a variant of HSCR.

As expected from the NAHR mechanism, we also identified patients with the reciprocal microduplications. None of them were referred for microarray testing for any of the specific clinical features that we suggest may be due to haploinsufficiency of the *CHAT* and *SLC18A3* genes, although detailed clinical descriptions were not available to us.

We report novel deletions and duplications at 10q11.21q11.23 that are likely caused by NAHR between LCRs. Our findings challenge the traditionally used paradigm in the diagnostic setting that aberrations inherited from a phenotypically normal parent are usually without clinical consequences. Large copy number alterations such as those described in this report are associated with unpredictable and variable phenotypic outcomes and pose diagnostic and counseling difficulties. Careful consideration of additional factors that may influence variable phenotype should be considered. Larger studies are needed to obtain a better understanding of this complex genomic region and its associated pathology. Further analysis of the 10q11.21q11.23 deletion in a well-phenotyped family might reveal that the deletion has recognizable phenotypic consequences, although the effect in some individuals may be more subtle depending on genetic and/or nongenetic modifiers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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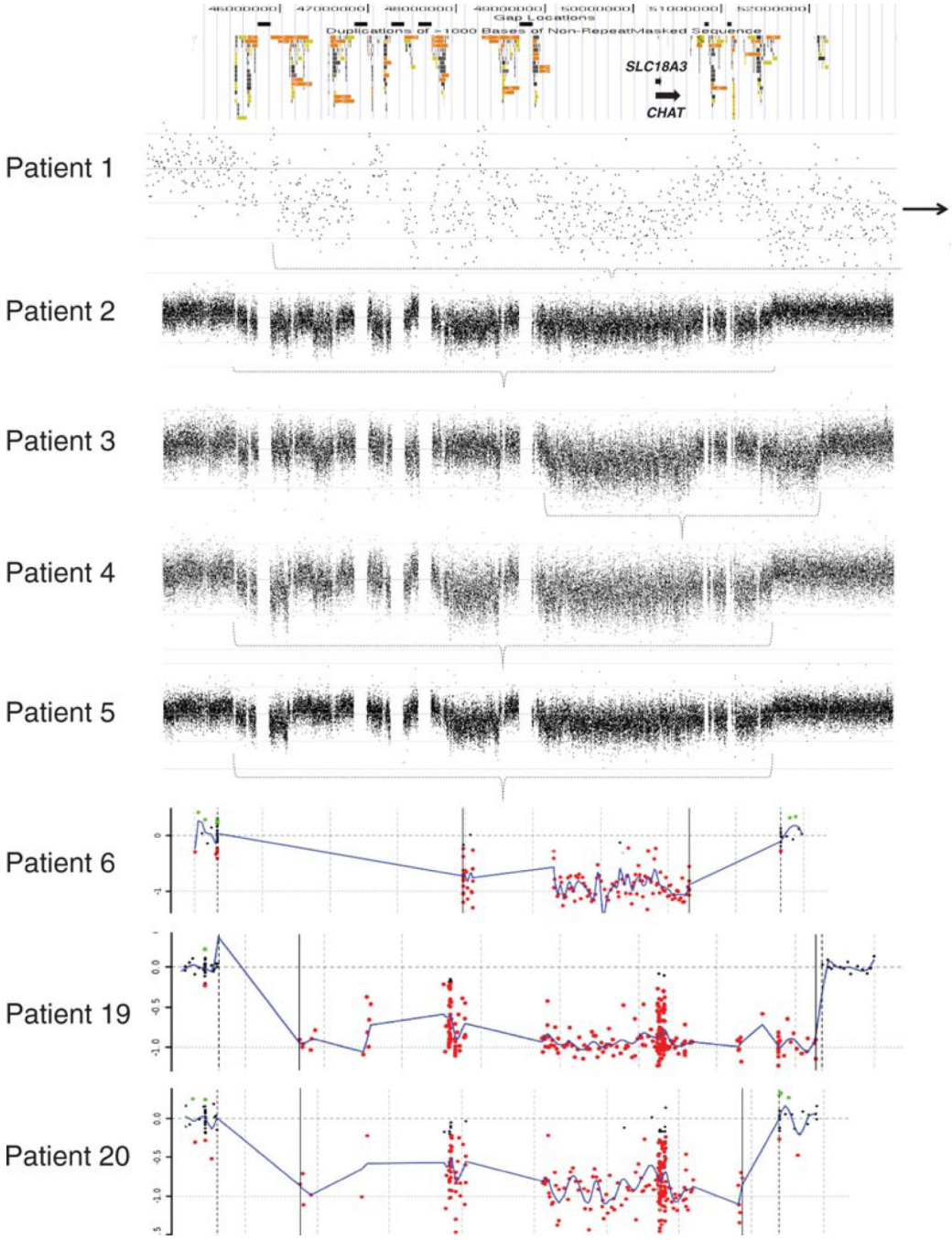

Figure 1.

Chromosomal microarray results for eight samples analyzed by BCM, using RocheNimbleGen oligonucleotide-based microarrays. At the top is a schematic of the genomic architecture of the region. Black boxes represent sequence gaps. Colored boxes represent segmental duplications. For each plot, probes are arranged on the X-axis according to physical mapping positions, with the most proximal 10q11.21 probes to the left and the most distal 10q11.23 probes to the right. Values along the Y-axis represent $\log_2$ ratios of patient:control signal intensities. Deletion intervals are represented by a dotted bracket (patients 1–5) or vertical, solid black lines (patients 6, 19–20).

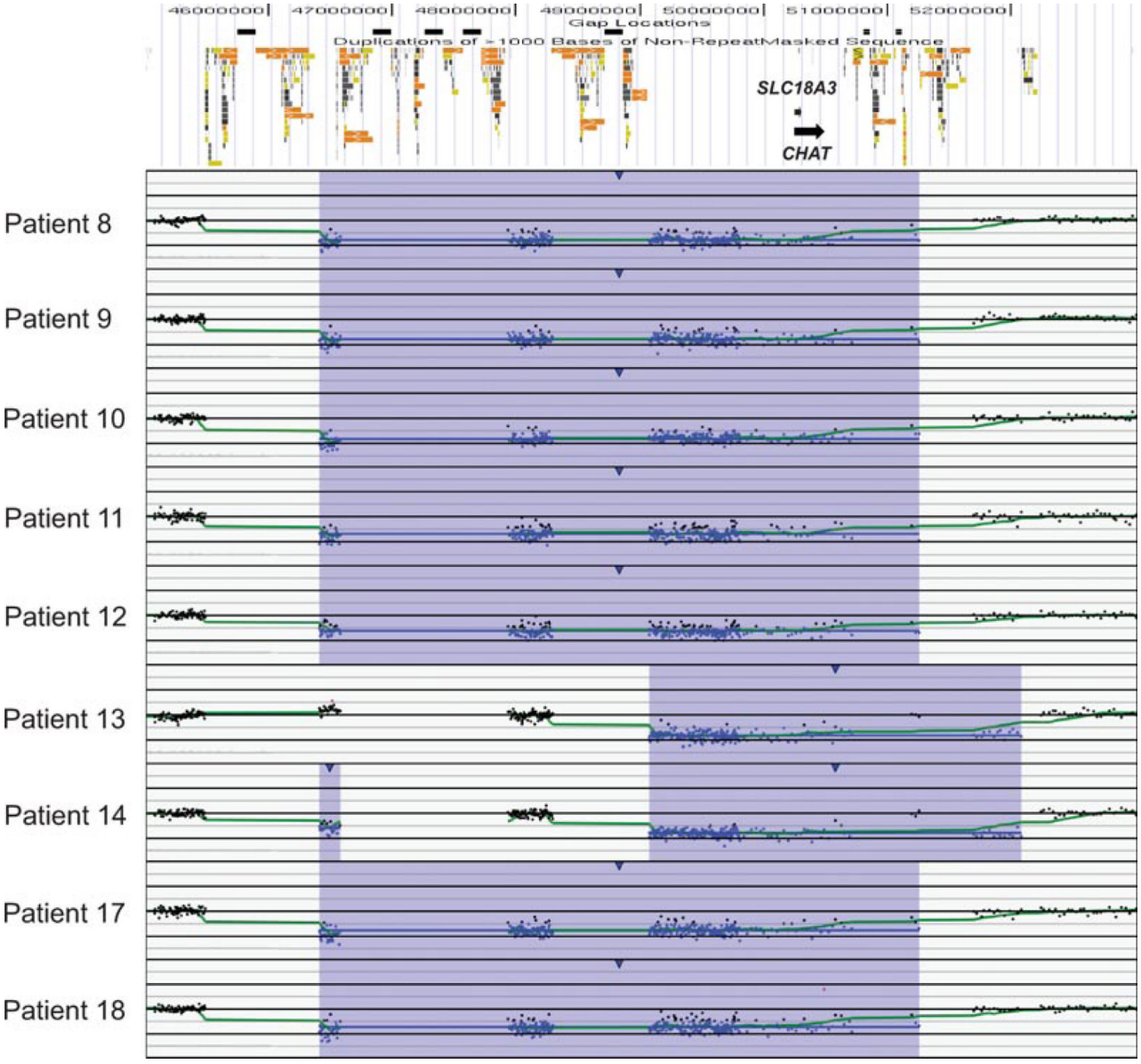

Figure 2.

Chromosomal microarray results for patient samples analyzed by SignatureChip oligonucleotide-based microarray. At the top is a schematic of the genomic architecture of the region. Black boxes represent sequence gaps. Colored boxes represent segmental duplications. For each plot, probes are arranged on the X-axis according to physical mapping positions, with the most proximal 10q11.22 probes to the left and the most distal 10q11.23 probes to the right. Values along the Y-axis represent $\log_2$ ratios of patient:control signal intensities.

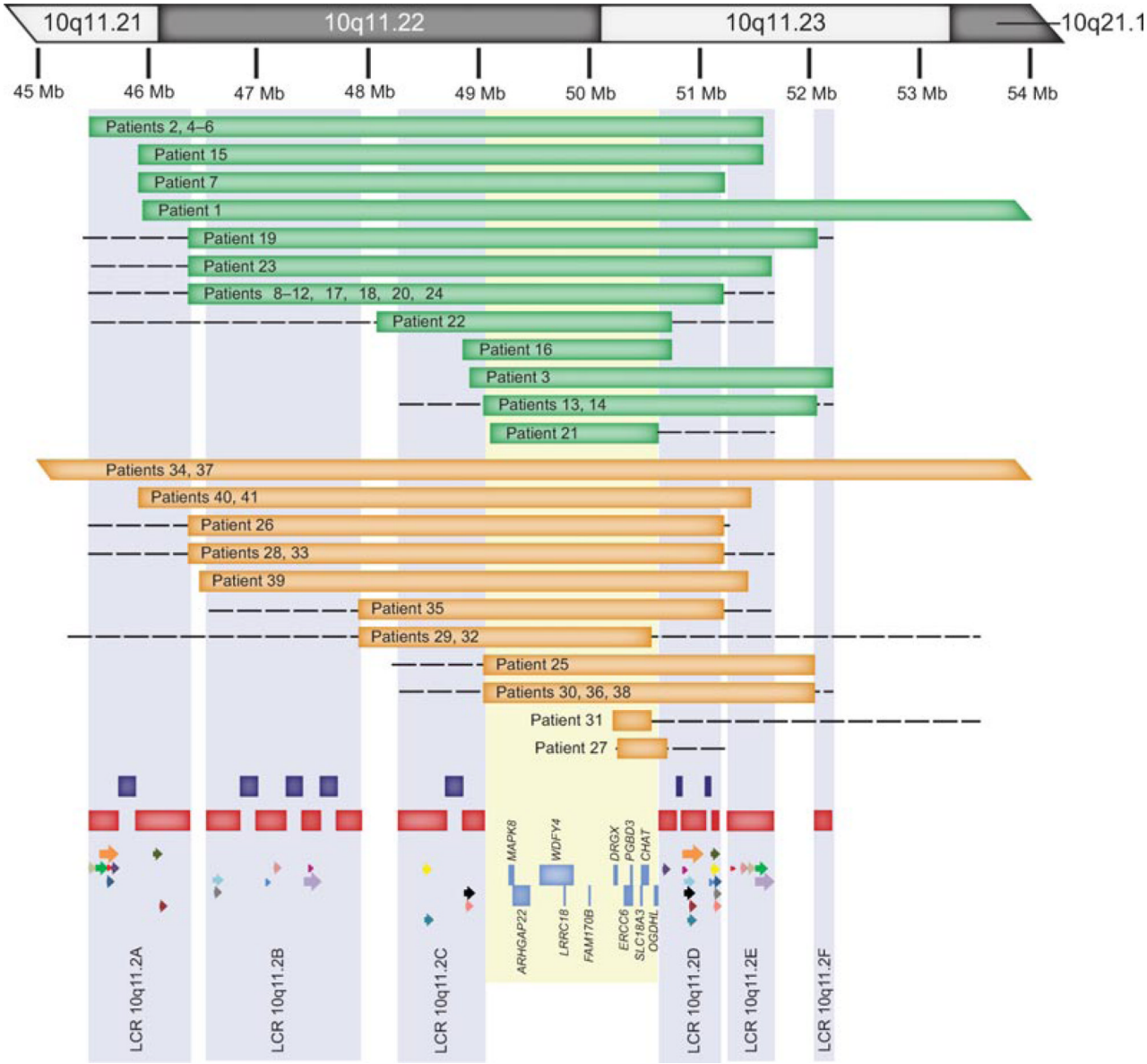

Figure 3.

Summary of chromosomal microarray analysis of individuals with deletions and duplications 10q11.21q21.1. At the top of the figure is a partial idiogram showing chromosome bands 10q11.21q21.1 with genomic coordinates corresponding to the hg18 build of the human genome. Green bars represent the deletions and the orange bars the duplications. Red bars represent segmental duplications. Only the directly oriented paralogous LCR subunits (18) between LCR10q11.2s A–C and D–F, that have a potential for NAHR, are shown for illustrative purposes as the colored arrows. Each of the 18 paralogous pairs is shown in different color (Supp. Table S1). Note that NAHR might have mediated rearrangements in patients 2, 4–12, 15, 17–18, 20, 23, 24, 26, 28, 33, 40, and 41 (between LCR10q11.2A and LCR10q11.2E), 16 and 21 (between LCR10q11.2C and LCR10q11.2D), 22, 29, and 32 (between LCR10q11.2A–B and LCR10q11.2D–E), and 35 (between LCR10q11.2B and LCR10q11.2E). Dark blue bars depict seven genomic gaps. Semitransparent light blue shading represents the collapsed LCR clusters A–F.

Semitransparent yellow shading represents the smallest region of unique sequence shared by all deletion patients.

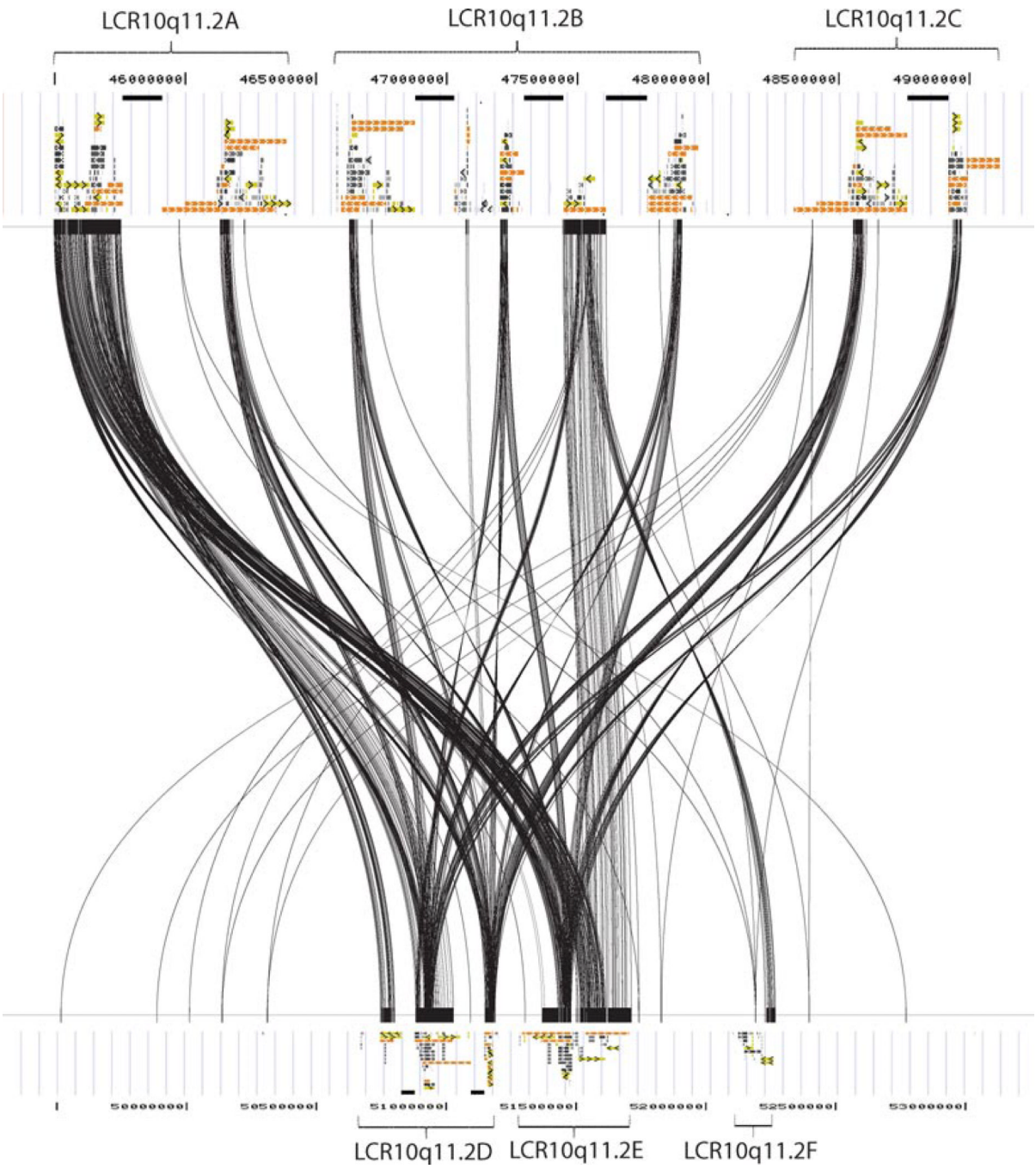

Figure 4.

DNA sequence homology between the proximal (chr10: 45,300,000–49,200,000) and distal (chr10: 49,300,000–53,200,000) LCR10q11.2 clusters for the paralogous subunits larger than 1 kb in size (hg18) using Miropeats program analysis. The upper and bottom panels depict the UCSC segmental duplication track.

Table 1

Summary of the Results of Microarray Studies of 20 Unrelated Individuals with Microdeletions in 10q11.21q11.23

Patient	Coordinates (hg18)	Size (Mb)	Parental origin	Microarray platform	FISH verification	Possible mechanism of formation	Additional copy number changes
1	45,979,000–56,907,000	~10.9	Paternal	72K (NimbleGen)	RP11-70E21	?	-
2	45,512,000–51,585,000	~6	Paternal	72K (NimbleGen)	RP11-70E21	NAHR	-
3	48,941,000–52,218,000	~3.3	Maternal	72K (NimbleGen)	RP11-70E21	?	arr 13q22.3(77,299,714–77,471,991)×3mat
4	45,480,000–51,585,000	~6	Paternal	72K (NimbleGen)	RP11-70E21	NAHR	-
5	45,612,000–51,585,000	~6	Maternal	72K (NimbleGen)	RP11-563N6	NAHR	arr 3q13.12q13.32(108,208,150–120,229,969)×1dn
6	45,612,000–51,585,000	~6	Maternal	72K (NimbleGen)	RP11-70E21	NAHR	-
7	45,947,671–51,263,703	~5.3	Paternal, grandmaternal	Affymetrix SNP 6.0	RP11-541M12	NAHR	arr 12q23.1q23.2(chr12: 95,227,874–100,061,773)×3mat
8	46,400,346–51,237,832	~4.8	Unknown	135K (SignatureChipOS v2.0)	RP11-635L5	NAHR	-
9	46,400,346–51,237,832	~4.8	Maternal	135K (SignatureChipOS v2.0)	RP11-635L5	NAHR	arr 6p25.3p22.1(115,226–126,640,914)×3 der(7)t(6;7)(p22.1;p22.3)
10	46,400,346–51,237,832	~4.8	Paternal	135K (SignatureChipOS v2.0)	RP11-100M24	NAHR	-
11	46,400,346–51,237,832	~4.8	Maternal	135K (SignatureChipOS v2.0)	RP11-100M24	NAHR	-
12	46,400,346–51,237,832	~4.8	Unknown	135K (SignatureChipOS v2.0)	RP11-140C5	NAHR	arr 14q31.3q32.11(88,853,671–88,965,421)×1
13	49,062,854–52,062,367	~3.0	Unknown	135K (SignatureChipOS v2.0)	RP11-1005F22	?	-
14	49,062,854–52,062,367	~3.0	Paternal	135K (SignatureChipOS v2.0)	RP11-1005F22	?	-
15	45,927,753–51,581,847	~5.7	de novo	Affymetrix SNP 6.0	None	NAHR	?
16	48,871,525–50,765,047	~1.9	de novo	244K Agilent	None	NAHR	Confined placental mosaicism: arr cgh 2(×3)
17	46,400,346–51,237,832	~4.8	Unknown	135K (SignatureChipOS v2.0)	RP11-140C5	NAHR	-
18	46,400,346–51,237,832	~4.8	Unknown	135K (SignatureChipOS v2.0)	RP11-140C5	NAHR	-
19	46,384,979–52,085,077	~5.7	Unknown	BCM V8.0 OLIGO	RP11-563N6	?	arr 15q21.2q21.3(49,846,193–51,236,840)×3
20	46,384,979–51,265,056	~4.9	Maternal	BCM V8.0 OLIGO	RP11-70E21	NAHR	arr 9q34.3(139,662,925–140,138,901)×3mat

Table 2

Summary of the Results of Microarray Studies and Phenotypic Features of Additional Unrelated Individuals with Microdeletions of 10q11.21q11.23

Patient	Age	Gender	Indication	Coordinates (hg18)	Size (Mb)	FISH verification	Parental origin	Microarray platform	Additional copy number changes
21	4 years	F	Mild DD/ID, DF	49,121,974–50,641,724	~1.5	-	Unknown	BCM V7.1 OLIGO	-
22	1 year	F	N/A	48,102,606–50,641,752	~2.5	RP11-563N6	Unknown	BCM V7.4 OLIGO	arr 9q34.2(135,752,638–135,912,152)×3
23	8 years	M	DD	46,384,979–51,672,034	~5.3	-	Unknown	BCM V8.1 OLIGO	-
24	11 years	F	Cleft palate	46,400,346–51,237,832	~4.8	RP11-635L5	Paternal	135k (SignatureChipOS v2.0)	-

DD, developmental delay; DF, dysmorphic features; ID, intellectual disability; N/A, not available.

Table 3

Summary of the Results of Microarray Studies and Phenotypic Features of 17 Unrelated Individuals with Microduplications of 10q11.21q11.23

Patient	Age	Gender	Indication	Coordinates (hg18)	Size (Mb)	FISH verification	Parental origin	Microarray platform	Additional copy number changes
25	4 years	M	Autism, speech delay	49,062,900–52,085,077	~3.0	RP11-70E21	Unknown	BCM V8.1 OLIGO	arr 12p13.2(11,897,593–12,310,534)×3
26	4 years	M	Moderate DD, ASDs	46,384,979–51,249,165	~4.9	RP11-70E21	Maternal	BCM V8.1 OLIGO	-
27	3 months	F	Hypotonia, abnormal brain MRI	50,265,332–50,721,613	~0.5	RP11-165A4	Maternal	BCM V8.1 OLIGO	-
28	12 years	M	Moderate DD, ASDs, seizures, DF, MCA	46,384,979–51,265,056	~4.9	RP11-70E21	Unknown	BCM V8.2 OLIGO	-
29	14 years	F	T-cell primary immunodeficiency	47,928,933–50,581,445	~2.7	RP11-635L5	Paternal	SignatureChipWG v1 .0.1	arr Xq25(RP11-229G9)×1 pat
30	24 years	M	DD, DF	49,151,859–52,085,217	~2.9	RP11-1005F22	Maternal	135K (SignatureChipOS v1.1)	arr Xp22.33/Yp11.32(463,937–543,186)×3 pat
31	15 years	M	DD, Mild ID, autistic features	50,239,677–50,581,445	~0.3	RP11-1005F22	Maternal	SignatureChipWG v1.0.1	-
32	8 years	M	DD	47,928,933–50,581,445	~2.7	RP11-10N24	Unknown	SignatureChipWG v1.0.1	-
33	14 years	F	Encephalopathy	46,400,346–51,237,832	~4.8	RP11-100M24	Unknown	135K (SignatureChipOS v2.0)	-
34	6 years	M	DD, DF	43,288,081–55,324,785	~12	RP11-219K22	Unknown	135K (SignatureChipOS v2.0)	arr 10q21.1(56,542,636–56,802,473)×1
35	35 years	M	MCA	47,921,367–51,237,832	~4.7	RP11-635L5	Paternal	135K (SignatureChipOS v2.0)	-
36	14 years	M	DF	49,062,854–52,062,367	~3.0	RP11-100M24	Maternal	135K (SignatureChipOS v2.0)	-
37	3 years	F	DD, DF, seizure disorder	43,288,081–55,324,785	~12.0	RP11-168P8	Unknown	135K (SignatureChipOS v2.0)	arr 15q13.3(29,816,893–30,226,405)×1
38	7 years	F	DD, seizure disorder, skeletal anomalies	49,062,854–52,062,367	~3.0	RP11-1005F22	Unknown	135K (SignatureChipOS v2.0)	-
39	4 years	M	DD	46,497,745–51,472,379	~5.0	N/A	Unknown	Affy SNP 6.0	-
40	22 months	F	Global DD	45,947,671–51,510,978	~5.6	RP11-541M12	Maternal	Affy SNP 6.0	-
41	4 months	M	DD	45,947,671–51,492,393	~5.5	RP11-541M12	Paternal	Affy SNP 6.0	-

ASDs, autistic spectrum disorders; DD, developmental delay; DF, dysmorphic features; ID, intellectual disability; MCA, multiple congenital anomalies; N/A, not available.

Table 4

Summary of Clinical Features of Individuals with Microdeletions at 10q 11.21 q21.1

Patient	1[a]	2	3	4	5	6[b]	7	8	9	10
Age	18 years	7.5 years	11 years	11 months	26 months	11 years	12 years	2 years 5 months	6 years	9 years
Sex	M	M	M	M	M	F	M	F	F	M
Height (centile)	NS	75th–90th	5th	NS	90th–97th	<3rd	84th	5th	5th	90th
Weight (centile)	NS	50th–75th	10th	FTT	50th	<3rd	94th	4th	<3rd	75th–90th
OFC (centile)	NS	50th	50th	NS	80th	49 cm microcephalic	55th	19th	Microcephalic	>97th
Psychomotor and behavioral development	Severe ID (IQ<35), speech delay, poor eye contact	Severe DD, ASDs, anxious, severe speech delay, repetitive behaviors	ASDs, ADHD	DD	Global DD, self-injurious behaviors	DD (IQ = 58)	ADHD, Asperger syndrome, LD (IQ~75)	Global DD, at about 6 month level	DD	Normal
Neurology	Poor motor coordination	NS	Mild leg hyperreflexia, decline in walking skills, normal brain MRI	Seizures, hypomyelination above the tentorium	Agenesis of corpus callosum, diffuse periventricular leukomalacia, hypotonia	Asymptomatic until ~5 years of age. Cerebellar ataxia, significant intentional tremor, spastic paraparesis, increased deep tendon reflexes, central tone not significantly elevated except at ankles, abnormal MRI. CT noted small calcifications within basal ganglia, Hx of tonic/clonic seizures, fine motor impairment	Normal brain MRI	Intractable epilepsy, normal brain MRI, MRS: elevated choline: creatine, hypotonia	Dysgenesis of the corpus callosum, bilateral choroid plexus cysts	Normal
Dysmorphic features	Thin face, small cranium, large ears	High arched palate, thin vermilliion border, thin philtrum, narrow facies, protruding auricles	Large, overfolded ears	NS	Mild telecanthus	NS	Prominent ears, tubular nose with high nasal bridge and a bulbous tip, high arched palate	None	Round face, high forehead, low-set and posteriorly rotated ears, ptosis, short palpebral fissures, straight eyebrows, beaked nose, anteverted nostrils, mild maxillary hypoplasia, thin lips, large uvula, hypoplastic philtrum, hypodontia, pointed chin, low posterior hairline with double hair whorl, thin and webbed neck, submucous cleft palate	Epicanthal folds, mild micrognathia, mild facial asymmetry
Musculoskeletal	Scoliosis, hemivertebra, muscle strength 3/5	NS	None	NS	Scoliosis	Stiffness reported	3–4 toe cutaneous syndactyly bilaterally	NS	Leg length discrepancy, shortened halluces,	Hypoplastic fifth digit phalanges

Patient	1[a]	2	3	4	5	6[b]	7	8	9	10
Other	Anal atresia, multiple impacted teeth, jaw maloclusion, chronic constipation, dysphagia	T&A for sleep apnea, chronic constipation	Fifth finger clinodactyly	Abnormal rectosigmoid ratio, apnea/ hypopnea, chronic constipation	Chronic constipation, possible obstructive sleep apnea	Jaundice at 3 days, elevated liver enzymes, Hx of aniteric sclera, PE tubes, Hx of headache, apneic episode with cynaosis at 4 months. Multiple UTIs 3–5 years of age. Cystourethrogram and renal scan Nl. GERD		Pigmentary retinopathy, nystagmus, swallowing dysfunction, GERD, chronic constipation, renal reflux	arachnodactyly of the left fifth finger Severe valvular pulmonary stenosis, severe tricuspid regurgitation, and hypertrophic right ventricle, PFO versus small atrial secundum defect, vitamin D deficiency, hepatomegaly, lacrimal duct obstruction, obstructive sleep apnea, microphthalmia, blepharophimosis, astigmatism, hypodontia, eczema, sacral dimple, renal hypoplasia and insufficiency, vesicoureteral reflux, urethrovaginal fistula, urethral fistula	Unilateral progressive NSHL, GERD, hypoplastic fifth nails

Patient	11[c]	12	13	14	15	16	17	18	19	20
Age	1 month	11 years 8 months	10 years 10 months	26 months	5 years 8 months	30 week fetus	7 years 8 months	6 years 4 months	20 months	7 months
Sex	M	F	F	M	M	M	M	M	F	F
Height (centile)	90th	10th	<3rd	25th–50th	<5th	25th	57th	25th	73rd	44th
Weight (centile)	97th	25th	<3rd	25th–50th	<5th; FTT	3rd–10th	58th	25th–50th	69th	39th
OFC (centile)	90th	85th	NS	50th–75th	<<3rd: -4 to -5 SD	75th	82nd	0 to +1 SD	47th	90th
Psychomotor and behavioral development	Normal	DD, ADHD, dysgraphia	Global DD, at about 6 years level	DD	Global DD. Dysarthria, difficulties staying on task	NA	Global DD, regression	PDD-NOS, DD	Normal	Normal
Neurology	Mild intermittent ankle clonus	Normal	Myoclonic/ generalized epilepsy (3–4 seizures/week since age 3 years); possible Lennox-Gastaut	Hypotonia	Abnormal brain MRI: significant hypomyelination, hypoplasia of inferior cerebellar vermis, persistent cavum septum vergae, mild ventricular prominence, ataxia, spasticity, mild dysmetria, increased DTRs (3+/4) with clonus, truncal	NS	Seizures, multifocal epileptiform discharges, ESES, hypotonia, clenched muscles, toe walking, normal brain MRI	Normal brain MRI	Normal neonatal head U/S	Hypotonia

Patient	11[c]	12	13	14	15	16	17	18	19	20
					hypotonia with some spasticity					
Dysmorphic features	None	None	Down-slanting eyes	Frontal bossing, rounded cheeks, rounded nasal tip, mildly deficient alae nasi, mild micrognathia	Short palpebral fissures, deep-set eyes, flat midface, prominent maxilla, high palate, mild micrognathia, sparse and thin hair	None	Mild ptosis, short columella	None	Round face, prominent eyes, epicanthal folds, depressed nasal bridge, arched palate	NS
Musculoskeletal	NS	NS	Ulnar deviation of hands, elbow contractures	NS	Delayed bone age—12–15 months at age 22 months brachydactyly	NS	Short first toes	NS	NS	Right hand anomaly (same as in father)
Other	Small umbilical hernia	Recurrent UTIs, severe chronic constipation, myopia, T&A	Fifth finger clinodactyly, renal reflux, resolved, seizures in paternal aunt	Micropenis, severe eczema	Congenital nystagmus, congenital cataracts, mild microphthalmia, hypermetropia, poor suck and swallow, GERD, dysphagia	Hypospadias, severe IUGR	Mild optic atrophy, aspiration, GERD, chronic constipation, diarrhea, suspected mitochondrial disorder, immune-suppressed, chronic sinusitis and otitis media	Eczema, hypopigmentation	TAPVR, tachycardia	GERD

ADHD, attention deficit hyperactivity disorder; ASDs, autism spectrum disorders; DD, developmental delay; DTRs, deep tandom reflexes; ESES, electrical status epilepticus during sleep; FTT, failure to thrive; GERD, gastroesophageal reflux disease; ID, intellectual disability; IUGR, intrauterine growth retardation; LD, learning disabilities; MRS, magnetic resonance spectroscopy; NA, not applicable; NS, not specified; NSHL, neurosensory hearing loss; PDD-NOS, pervasive developmental delay-not otherwise specified; PE, pressure equalization; PFO, patent foramen ovale; SD, standard deviation; T&A, tonsillectomy and adenoidectomy; TAPVR, total anomalous pulmonary venous return; UTI, urinary tract infection.

[a] Patient 1's brother with the deletion has ADHD, maloclusion, and mild constipation. Patient 1's father also has ADHD.

[b] Patient 6's mother had stroke at 25 years attributed to foramen ovale.

[c] Patient 11's mother and maternal half brother also carry the 10q11.2 deletion; the brother has speech delay and LD; the mother has a history of speech delay and LD.