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Inherited and *de novo* 22q11.2 distal duplications in two patients with autistic features, speech delay and no dysmorphology

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Abstract. In a screen of patients by fluorescence in-situ hybridization and array comparative genomic hybridization in the past two years (July 2007–July 2009), we identified two patients with duplications in the 22q11.22-23, occurring outside the common DiGeorge syndrome/valocardiofacial syndrome region. Fluorescent in-situ hybridization, multiplex ligation-dependent probe amplification and high density bacterial artificial chromosomes and oligo arrays were used to identify the extent of the duplications. In one patient the duplication extended from LCR22-E/5 to LCR22-H/8, which is similar to recently described 22q11.2 distal duplications, while in the second patient, a *de novo* duplication was identified extending between LCR22-E/5 to LCR22-F/6. The second proband also harbored a *de novo* 15q14 duplication, complicating phenotype interpretation. The patients were affected with speech delay and autistic features, but neither reported cardiac concern or dysmorphic features.

Keywords: 22q11.2, duplication, mental retardation, autism, tic behavior, speech, DiGeorge syndrome/valocardiofacial syndrome

1. Introduction

Chromosomal rearrangements on chromosome 22q11.2 are associated with a wide and variable clinical phenotype including DiGeorge/velocardiofacial syndrome (DGS, OMIM #188400; VCFS, OMIM #192430), the supernumerary marker chromosome for cat eye syndrome (CES, OMIM #115470), and autism/autism spectrum disorders [1–4]. The 22q11.2 deletion syndrome, also referred to as DGS/VCFS, is the most common genomic deletion syndrome, with frequencies reaching 1:3000–1:4000 live births [1,5–7]. Patients present with cardiac defects, developmental delay, and characteristic facial features, while a subset present with reduced or absent thymus gland and hypocalcaemia [1,8–10]. Adults with 22q11.2 deletions can also present with psychiatric problems, schizophrenia and bipolar disorder [4,10,11].

The presence of several low copy repeats (LCR) at the 22q11.2 region, numbered A-H (alternatively numbered LCR22-2, -3a, -3b, -4-8), has been shown to contribute to the occurrence of such deletions [12–14]. In DGS/VCFS patients, a deletion of 3 Mb, between LCR-A and LCR-D, or a nested deletion of 1.5 Mb, within LCR-A and LCR-B, account for the

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majority of rearrangements detected and is referred to as the common DGS/VCFS region. The phenotype of the 22q11 deletion syndrome seems independent of deletion size [15], and interestingly, can present differently within carriers within the same family [16,17]. Recently, a new 22q11.2 rearrangement was recognized. The 22q11.2 distal deletion syndrome (OMIM #611867) was described in patients with prenatal and postnatal growth delay, developmental delay, pre-term delivery, and some dysmorphic features. The deletions were either 1.4 or 2.1 Mb in size, occurring between LCR 4 and LCR 6, with different breakpoints [18–21].

Theoretically, duplications on 22q11.2 should be as common as deletions as a result of non-allelic homologous recombination (NAHR) between LCRs [22–25]. However, it has been recently established that not all NAHR phenomena generate both anomalies (deletions and duplications). For instance, intrachromatid NAHR generates only deletions by a so called non-homologous end joining (NHEJ) mechanism [26,27]. Turner et al. [28] further reported that some deletions are generated at a higher rate than their reciprocal duplications in the male germline.

Microduplications in the 22q11.2 region were identified in several patients and a new syndrome was suggested, 22q11.2 duplication syndrome. Patients showed variability of presentations including in most patients some form of cognitive disability, with some displaying velopharyngeal insufficiency, downslanting palpebral fissures, and hypertelorism [29,30]. Some patients were described with a triplication at 22q11.2 [31]. Patients with duplications at the same 3 Mb common DGS/VCFS region showed some overlapping features with DGS/VCFS patients, including cardiac and characteristic facies [22]. The variability in the phenotype of 22q11.2 microduplications seems to be a hallmark of rearrangements in this region [32,33]. Recently, several patients were identified that harbored interstitial duplications distal to the common DGS/VCFS region. Patients generally suffered from developmental delay, language delay, and dysmorphic features [34–36].

We have identified several patients harboring duplications in the 22q11.2 region. Two patients, however, harbored duplications outside the common DGS/VCFS region, and we describe the detection of these 22q11.2 distal duplications and the phenotypes presented in the patients and compare our findings to those described by others.

2. Materials and methods

2.1. Patients

Twenty-six probands, 20 males and six females were analyzed by karyotyping, array comparative genomic hybridization (aCGH) and/or fluorescence in-situ hybridization (FISH), depending on ordering physician, to rule in/rule out in patients with DGS/VCFS or for indications of developmental delay, mental retardation, or autism/autistic like features. Follow-up of a subset of these patients with multiplex ligation-dependent probe amplification (MLPA) analysis was performed to better define the duplication region.

2.2. Bacterial artificial chromosomes (BAC)-microarray and oligo-microarray analysis

Array CGH was performed using Quest Diagnostics' ClariSure™ arrays, which contained approximately 1350 BAC clones, undergoing minor modifications with each build until a more recent upgrade to bring the BAC total to over 3000 FISH and/or end-sequence verified BAC clones. Patients were tested in duplicate in dye swap analysis. Briefly, patient test DNA is labeled using the BioPrime Array CGH Genomic Labeling System (Invitrogen, US) with Cy3 and Cy5 dCTP-conjugated dyes (GE Healthcare, US) as described by the manufacturers. Similarly labeled control pooled sex-mismatched DNA was used as the reference sample. Cot-1 DNA was combined with each labeled DNA pair and column purified (PureLink PCR Purification Kit, Invitrogen), ethanol precipitated, then re-suspended in hybridization solution (Pronto, Universal Hybridization Kit, Corning Life Sciences, US). Prior to hybridization, printed slides were pretreated using the Pronto, Universal Background Reduction treatment, including pre-soak and pre-hybridization as described by the vendor. Hybridization occurred for a minimum of 20 hr at 42 °C. Post-hybridization slides were washed, centrifuged briefly to spin dry, scanned for image acquisition (GenePix 4000B Scanner, Molecular Devices, US) and processed using GenePix Pro (version 6.0). Data was normalized using the autosomal BAC spots by LOWESS "pin-wise" normalization with the InfoQuant CGH software package (InfoQuant, Ltd., version 3.0.5). Spots with intensities less than 2X signal to noise were omitted, as were those flagged by GenePix, replicates greater than 2 standard deviation (SD) from the replicates mean, and single replicates.

Individual arrays and the consensus data were analyzed using thresholds set at 4 times the mean autosomal standard deviation (SD) as similarly outlined by Vermeesch [37].

2.3. FISH

Chromosome analysis was performed using standard protocols. FISH was performed using standard procedures with the BAC clones of concern as probes to confirm the duplications. FISH probes were home-made by labeling with Spectrum Orange (Abbot, Abbot Park, IL) by nick translation. Analysis of the genomic duplication was performed by visualizing two color FISH images showing three hybridization signals for the experimental probe and two signals for the aryl sulfatase A gene (ARSA) control probe (DiGeorge Region Probe Vysis, US) in interphase nuclei and an enhanced signal at the proximal region of one chromosome 22 in metaphases. At least 50 interphase nuclei and 10 metaphase spreads were examined.

2.4. MLPA analysis

Samples were analyzed using the MRC-Holland DGS MLPA mix (Catalog # P250; Amsterdam, Holland) according to manufacturer's instructions. Briefly, 100–200 ng DNA were denatured and mixed with hybridization oligos overnight at 60 °C. Heat-stable ligase was added and an aliquot was used for PCR amplification using PCR mix reagents provided in the kit. Amplified fragments were mixed with Rox-500 size standard and Hi-Di Formamide (both from Applied Biosystems, Foster City, CA) and loaded onto ABI 3100 (Applied Biosystems). Results were analyzed using GeneMapper® software (Applied Biosystems) and dosage calculated using SeqPilot® Software (JSI, Germany). All samples were analyzed in duplicates at least twice.

3. Results

3.1. Screening patients for 22q11.2 deletions and duplications

Twenty-six probands, 20 males and six females, were analyzed by karyotyping, aCGH and/or FISH, depending on the ordering physician, to rule in/rule out DGS/VCFS or for indications of developmental delay, mental retardation, or autism/autistic like features. Table 1 lists the 26 patients and their phenotypes. As a reference laboratory, we are unable to provide further detailed clinical information as we are limited by what the ordering physician provides on the requisition. Parental follow-up studies were ordered and performed in nine families. The inheritance in these nine families: two *de novo*, one paternal and six maternal. The duplication size ranges from 202 Kb to 2,907 Kb with almost half of the cases falling in the 2 Mb range. Variations of duplication size with family members were not observed.

3.2. Detection of 22q11.2 distal duplication

As duplications in the DGS/VCFS are becoming increasingly recognized, we sought to determine the breakpoints of these duplications to better define the region involved in the phenotype of these patients and to better compare duplications identified in our laboratory to those identified elsewhere. When we were able to isolate DNA of sufficient quality, we used MLPA analysis, and were able to confirm duplications in several patients. Most patients harbored duplications in the common DGS/VCFS 3 Mb region. We discuss below two patients that showed evidence of duplication distal to the common DGS/VCFS region.

3.3. Patient #8

The first patient, a 7-year-old male, was born to a 29-year-old mother after a 38-wk pregnancy with no known complications. The patient presented to a child neurology clinic for evaluation of episodes of staring with unresponsiveness for a few seconds. The family also reported concerns about increased tic behavior, including shoulder shrugging and chin rolling with increased blinking. The patient exhibited echolalia and delayed language acquisition, with speech becoming understandable around 3.5 years. There was no history of cardiac concern or frequent infections. The patient was diagnosed with mental retardation and mild autism and pervasive developmental disorder by other specialists. Family history was remarkable for tic disorder in the father and a sibling with autism. After identification of the duplication in the proband, the mother submitted a blood sample for analysis.

Table 1. Characteristics and molecular and cytogenetics results of patients with 22q11.2 duplications

Patient no	Chromosome	CGH/FISH results	Inheritance	Clinical information																				
				Age	Sex	Mental retardation	Developmental delay	Learning disability	Heart defect	Velopharyngeal insufficiency/cleft palate	Hypotonia	Dysmorphic features	Behavioral problems	Growth delay	Hearing loss	Polydactyly	Urogenital abnormalities	Autism spectrum disorder	Seizure disorder	Tethered cord	Positive family history	Rule out DiGeorge syndrome	No symptoms	No information provided
1	46,XY,dup(22)(q11.2)	ish dup(22)(q11.2q11.2)(HIRA++ or HIRA enh)/nuc ish(HIRAx3),(ARSAx2)	Maternal	2 yr	M		+			+														
2	46,XY,dup(22)(q11.2q11.2) de novo		De novo	18 mo	M		+				+													
3	46,XY	ish dup(22)(q11.2q11.2)(TUPLE1enh)/nuc ish (TUPLE1X3),(ARSAX2)	Maternal	20 mo	M		+		+			+										+		
4	46,XX	ish dup(22)(HIRA++,or HIRA enh)/nuc ish(HIRAx3)	Unknown	22 yr	F																+		+	
5	46,XY	arr cgh 22q11.21(CTD-2300P14->CTD-2137N5)x3/ish dup(22)(q11.2q11.2) (HIRA enh)/nuc ish(HIRAx3),(ARSAx2)	Maternal	11 yr	M								+	+							+			
6		ish dup(22)(q11.2q11.2)(TUPLE1++ or enh)/nuc ish(TUPLE1x3)(ARSAx2)	Unknown	5 yr	F																	+		
7	46,XY	arr cgh 22q11.21(CTD-2300P14->CTD-2137N5)x3	Maternal	10 yr	M		+	+				+			+						+			
7(S)	n/p	arr cgh 22q11.21(CTD-2300P14->CTD-2137N5)x3/ish dup(22)(q11.2)(q11.2)(TUPLE++ or enh), nuc ish (TUPLE1x3)	Paternal	3 yr	M																+		+	
8	n/p	arr cgh 22q11.23(CTD-3006M2->RP11-549H7)x3	Maternal	7 yr	M	+	+											+						
9	46,XY	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Maternal	4 yr	M		+	+				+	+			+								
10	n/p	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Unknown	24 yr	M		+	+				+												
11	46,XY	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	De novo	1 day	M				+	+		+			+									
12	n/p	arr cgh 22q11.21(RP11-466H9->CTD-2536F14)x3	Maternal	5 yr	M		+					+	+				+							
13	n/p	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Maternal	6 yr	F		+																	
14	46,XX,dup(15)(q11.2q13)	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Unknown	24 yr	F																			+
15	n/p	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Unknown	9 yr	M																			+
16	46,XY	ish dup(22)(q11.2q11.2)(HIRA enh)	Unknown	52 day	M																			+
17	46,XY	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Maternal	3 yr	M		+					+						+					
18	n/p	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Unknown	8 yr	M		+	+				+												
19	46,XY	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Unknown	4 yr	M		+		+								+		+	+				
20	46,XY	arr cgh 22q11.21(CTD-2300P14->RP11-505B16)x3	Unknown	1 day	M				+															
21	n/p	arr cgh 22q11.21(CTD-2008M3->CTD-2280M24)x3	Unknown	2 yr	M																			+
22	n/p	arr cgh 22q11.21q11.22(CTD-2506I16->CTD-2363M19)x3	Not paternal	8 yr	M		+	+	+															
23	n/p	arr cgh 15q14(CTD-2266H12, RP11-347I18)x3, 22q11.22q11.23(CTD-2517E7->CTD-3006M2)x3	De novo	4 yr	F		+											+						
24	n/p	arr cgh 22q11.21(CTD-2636K18->CTD-2280M24)x3	Unknown	1 yr	M		+				+	+		+										
25	n/p	arr cgh 22q11.21(CTD-3060F3)x3	Unknown	8 yr	F	+	+	+				+												
26	n/p	arr cgh 22q11.21(RP11-505B16,CTD2280M24)x3	Unknown	3 day	F							+		+										

S = Sibling; x3 = Three copies of region probed detected; M = Male; F = Female.

The father of the proband was lost to follow-up. No other information on the sibling brother was available.

In this patient, aCGH analysis showed a duplication on 22q11 region (Figure 1A). BAC-microarray results for this patient showed that the BAC clones duplicated at 22q11.23, CTD3006M2 and RP11-549H7, which were outside the common DGS/VCFS FISH, coordinates chr22: 22,9 Mb-24.3 Mb (hg19). Analysis using clone CTD-3006M2, located between LCR E/5 and LCR F/6 (Fig. 3), showed presence of three fluorescent signals in interphase cells and an enhanced signal on metaphase spreads (Fig. 1B). To better pinpoint the breaking points of the duplications, analysis using MLPA showed duplication had occurred outside the common 3 Mb region (Fig. 1B) between LCR22-E/5 and LCR22-H8. We further utilized NimbleGen oligo-microarray analysis to better define the end of the duplication. Due to the low amount of residual material, DNA was whole-genome amplified and analyzed using NimbleGen 385K oligo array according to manufacturer's instructions. The results showed the duplication spanning ~1.85 Mb (Fig. 1D), extending from LCR22-E/5 to LCR22-H/8 with no overlap with the DGS/VCFS common deletion region. The duplication was identified also in the mother's DNA. No unusual findings were reported for the mother.

3.4. Patient #23

The second patient was a 4-year-old female who was born to a 31-year-old mother and a 33-year-old. father. There was no report of complications during pregnancy. The patient presented with autism-related

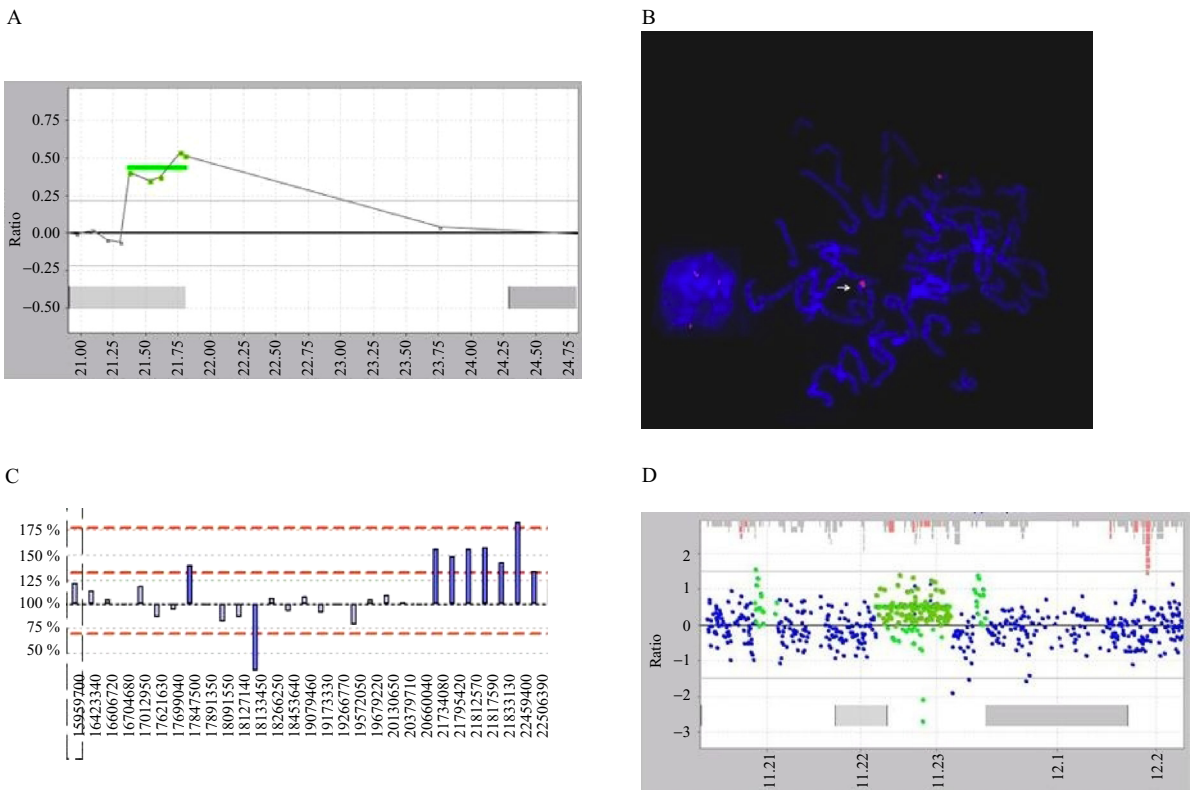

Fig. 1. Identification of 22q11.2 distal duplication in proband 1. (A) Detection of 22q11.2 distal duplication in proband 1 by array comparative genomic hybridization using 1-Mb high density comparative genomic hybridization array (our second generation ClariSure arrays) localized the duplication outside the DiGeorge/velocardiofacial syndrome region located between base pairs 22,949,883->24,265,623. (B) The duplication was confirmed by fluorescence in-situ hybridization using bacterial artificial chromosomes clone CTD-3006M2 which hybridizes to 22q11.23. Fluorescence in-situ hybridization showed 3 hybridization signals in an interphase nucleus and an enhanced signal on the dup(22q) in a metaphase spread (arrow). (C) Multiplex ligation-dependent probe amplification probe analysis showed the duplication encompassed at minimum a region between coordinates Chr22: 21795420-22506390. (D) Whole genome-amplified DNA tested on NimbleGen 375K array showed the duplication localized between Chr22: 21.54 Mb – 23.39 Mb, spanning ~1.85 Mb.

repetitive behavior, occasional toe walking, and speech delay, but without notable dysmorphic features. The patient showed normal magnetic resonance imaging, and normal levels of lactate and organic acids, which are usually elevated in autistic patients. The patient had no siblings but history was remarkable for a maternal cousin with speech delay. The parents submitted blood samples for analysis after abnormal aCGH findings were identified in their daughter.

Array CGH showed a duplication of 15q14 clones CTD-2266H12 and RP11-347I18 (Fig. 2A), coordinate chr15: 34.2 Mb-34.4 MB (hg19), and a duplication of clones CTD-2517E7 and CTD-3006M2 on 22q11.22-23 (Fig. 2C), coordinates: 22.9 Mb-23.7 Mb (hg19). To our knowledge the 15q14 duplication described here, is novel as only large duplications of 15q14 have been described (see discussion). FISH analyses confirmed the duplications on 15q14 (Fig. 2B) and 22q11.2 (Fig. 2D). The 22q11.22-23 duplication was further characterized using MLPA, and only probes located between LCR22-E/5 and LCR22-F/6 were duplicated (Fig. 2E), coordinates 21734080-21833130, making this 22q11.2 distal duplication identified in this proband the smallest distal duplications identified to date (Fig. 3). It is possible that the phenotype in the patient can be a result of both duplication events.

Following the identification of the abnormalities in the proband's DNA, parental blood samples were submitted for analysis. It was remarkable that neither

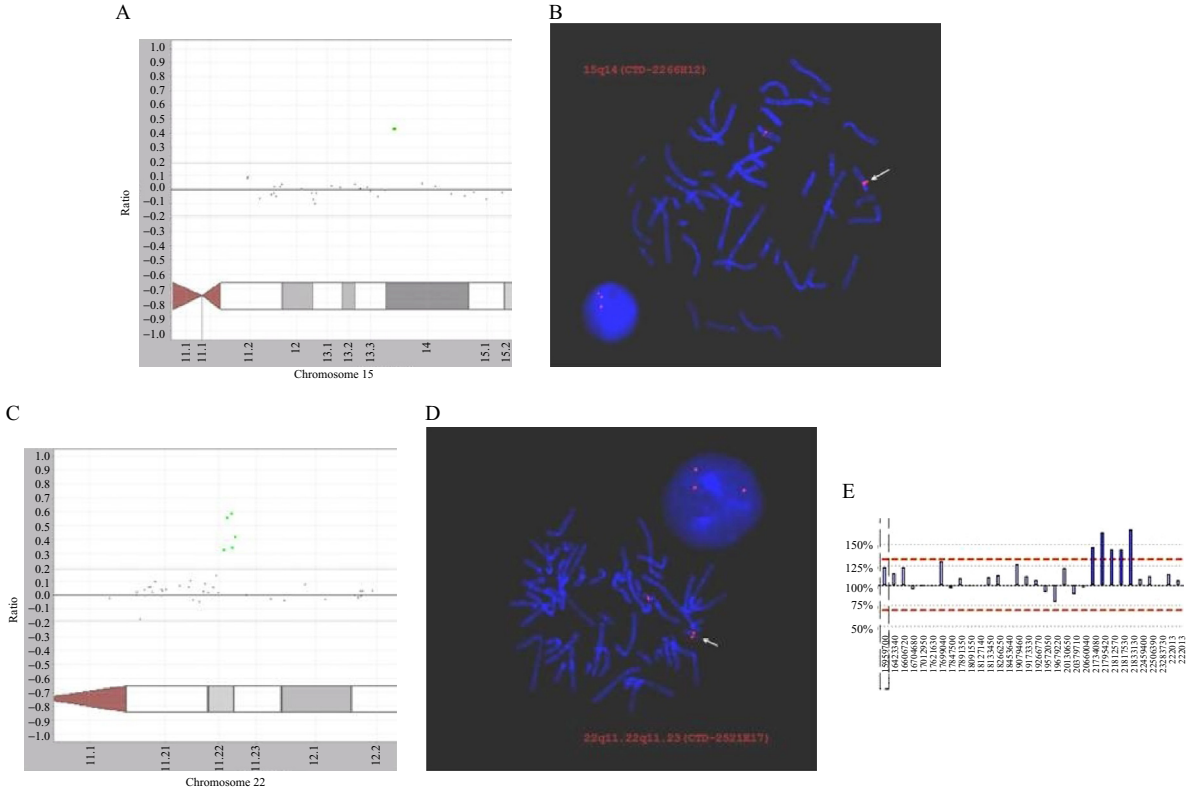

Fig. 2. Detection of 15q14 duplication and 22q11.2 distal duplication in proband 2. (A) Array comparative genomic hybridization results showing the 15q14 duplication located between base pairs 34,182,853->34,411,701. (B) Fluorescence in-situ hybridization (FISH) confirmation of the duplications on 15q14 using bacterial artificial chromosomes clone CTD-2266H12. Three signals are apparent in an interphase nucleus and an enhanced signal of the 15q14 on a metaphase spread (arrow). (C) Array comparative genomic hybridization results showing the 22q11.2 distal duplication located between base pairs 22,949,883->23,691,664 in the proband. (D) FISH confirmation of the duplications on 22q11.2 using BAC clone CTD-2521E17. Three signals are apparent in an interphase nucleus and two signal on 22q11.2 on a metaphase spread (arrow). FISH analyses using HIR histone cell cycle regulation defective homolog A (HIRA/TUPLE1) (located on 22q11.21) and aryl sulfatase A gene (ARSA) (located on 22q13.3) probes showed no duplication (data not shown). (E) Multiplex ligation-dependent probe amplification analysis to fine map the duplication showed duplication of probes on Chr22 located between base pairs 21795420-21833130, located between LCR22-E/5 and LCR22-F/6.

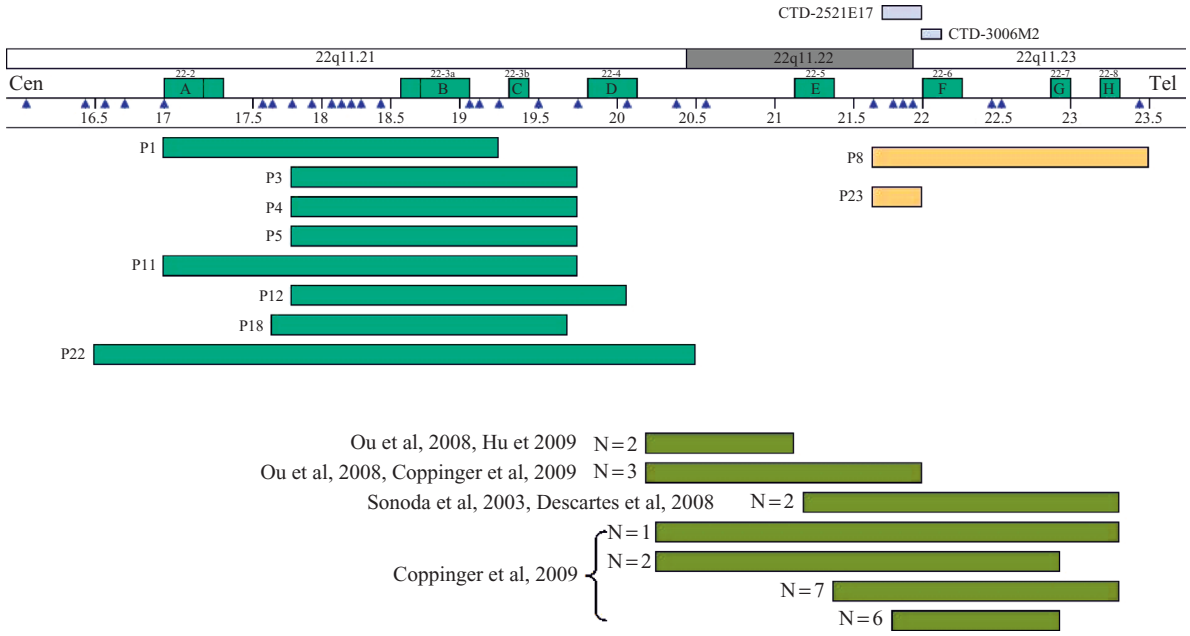

Fig. 3. Schematic representation of the location of 22q11.2 duplications from probands (orange) and other patients (green) using multiplex ligation-dependent probe amplification analysis and interpretation of informative probes (blue arrowheads). Extent of duplications is shown based on multiplex ligation-dependent probe amplification results. Boundaries of duplications are likely flanked by LCR22 repeats. Identifiers to the left of each green or orange bars indicate patient number (Table 1). The blue bars show location of bacterial artificial chromosomes clones. Distal duplications thus far reported in the literature are shown in olive-green colored-bars.

showed a duplication of either region suggesting both duplication events detected in the proband were *de novo* occurrences.

4. Discussion

The total number of patients with true distal duplications on 22q11.2 region, i.e. duplications between LCR22-E/5 and LCR22-H/8, has now reached 17: one described by Sonoda et al. [34], one described by Descartes et al. [35], thirteen described by Coppinger et al. [36], and two described in this report. Ou et al. [24], Coppinger et al. [36] and Hu et al. [37] also described patients with other 22q11.2 distal duplications; however, these patients had their proximal duplication breakpoint at LCR22-D/4. As we attempt to define the phenotype associated with 22q11.2 distal duplications, it might be useful to focus on patients with duplications within LCR22-E/5 to LCR22-H/8.

The patient from Sonoda et al. [34] appeared to harbor a 2 Mb duplication extending from LCR22-E/5 to LCR22-H8 and suffered from poor growth, high arched palate, dysmorphic features, partial syndactyly, atrial septal defect, and speech delay. The duplication was not inherited from the mother, but paternal DNA was not available. The patient described by Descartes et al. [35] failed to thrive, showed developmental delay and had relative macrocephaly. The duplication was 2.1 Mb is size, located between LCR22-E/5 and LCR22-H/8 and paternally inherited. The father, paternal uncle, and cousins all reported learning difficulties. The proband's sister, who also inherited the distal duplication, is, however, phenotypically- and developmentally-normal. The proband described by Descartes et al. [35] shared some features with patients 2, 3 and 4 of Coppinger et al. [36] in the size of the duplication, cephalic effects, some dysmorphic features and developmental delay. The first proband we describe here, who also had a duplication that encompassed LCR22-E/5 to LCR22-H/8, shared the developmental delay phenotype, but also suffered from mild autism, speech delay and pervasive developmental delay. It is notable that family history included a father with tic behavior and a brother with autistic features. After identification of the duplication in our proband, the mother submitted a blood sample for analysis. Using aCGH, we confirmed the presence

of the duplication in the mother, who is reportedly normal. The duplication seemed to harbor the same breakpoints as in the proband. The sibling's DNA was not available for analysis.

In total, seventeen patients thus far with duplications encompassing LCR22-E/5 to LCR22-H/8 have been described. Based on information from six of the patients with available clinical information (one from Descartes et al. [35], three from Coppinger et al. [36] and one from this study) a presentation of developmental delay and speech delay is exhibited, and duplication is generally inherited (three maternal and one paternal, while two are still of unknown origin).

The second proband we describe is a 4-year-old female who harbored *de novo* duplications on 15q14 and 22q11.2. Two clones were duplicated on the aCGH, CTD-2266H12 and RP11-347I18 from 15q14, while several clones were duplicated on the 22q11.2 region. The 15q14 duplication we describe here appears novel, as all other duplications involving 15q14 published to date are large [38,39]. We saw another 15q14 duplication in our series of aCGH analyses (>9000 consecutive cases) prior to the current one, and that duplication was large, involving 1.5 Mb (R.O. unpublished observations). Considering the rarity of the event, and even though the CNV database refers to the region as CNV (http://projects.tcag.ca; accessed July 6, 2009), it is unlikely that this duplication is benign; however, full interpretation of the effects on the probands phenotype are complicated by the presence of the 22q11.2 LCR22-E/5 to LCR22E/6 duplication in the same patient. The LCR22-E/5 to LCR22E/6 duplication is novel as it has not been described before and is the smallest of the 22q11.2 distal duplications described thus far. One case of a deletion on 22q11.2 involving the same region was described before [40] in a patient with heart abnormality but very minor dysmorphic features and no cognitive or psychological issues. That patient inherited the deletion from her normal father. Some patients with 22q11.2 distal duplication present with phenotypes similar to 22q11.2 distal deletions [18,36], but in our proband, the phenotype is distinct from that of the Rauch et al. [40] deletion patient. However, it is premature to conclude phenotype association with deletion type, especially with the complicated interpretation due to 15q14 duplication present in the second proband we describe.

The dosage of the critical 22q11.2 region seems important, and it is thought that genes contribute to cognitive and psychiatric well being, as well as heart and facial development of the fetus [41]. The variable presentation of clinical phenotypes associated with 22q11.2 rearrangement syndromes, even where cases are familial [25,31,33,36], regardless of the size of the rearrangement [15] might be due to 1) decreased or increased dosage of certain genes on 22q11.2 harboring polymorphisms that influence their expression or their protein product levels, or 2) to dosage-dependent interaction between genes deleted/duplicated on 22q11.2 and genes on other chromosomes. Therefore, it might be worthwhile to massively sequence patients with 22q11.2 rearrangements to determine SNPs and small insertions/deletions in this critical region and to determine whether there is a reason for variable presentation. Similarly, changes in the dosage of genes in this critical region and polymorphisms or dosage changes in other genes on other chromosomes that interact with genes on 22q11.2 region could lead to the variable presentation. Therefore, comprehensive studies are still needed to explain the variable presentation.

In cases of inherited rearrangements, and where parents show no phenotype, extensive family history of psychological or cognitive behavior, that would require asking grandparents of probands, may not be feasible, and therefore, some information from childhood of parents that involves cognitive-psychological manifestations may be lacking. On the other hand, delayed presentation of symptoms has been reported [11,42], and follow-up in parents and relatives of patients with inherited rearrangements might prove useful.

In summary, we present two cases of 22q11.2 distal duplications, with one harboring an additional alteration at 15q14. The phenotype of the duplication of LCR22-E/5 to LCR22-H/8 in the first proband showed mild autism and developmental delay. In the second proband the phenotype could be the result of the two novel duplications described.

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