

Cognitive Deficit, Learning Difficulties, Severe Behavioral Abnormalities and Healed Cleft Lip in a Patient with a 1.2-Mb Distal Microduplication at 22q11.2

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Key Words

aCGH · Cleft lip · Microduplication 22q11.2 · MLPA · *SMARCB1* · *SNRPD3*

Abstract

The 22q11.2 duplication syndrome has been recently characterized as a new entity with features overlapping the 22q11.2 deletion syndrome. Most 22q11.2 duplications represent reciprocal events of the typical 3-Mb deletions extending between low copy repeat (LCR) 22-A and LCR22-D. It has been suggested that the clinical manifestations observed in patients with 22q11.2 microduplications may range from milder phenotypes to multiple severe defects, and this variability could be responsible for many undetected cases. Here, we report on a patient with a 1.2-Mb microduplication at 22q11.2 spanning LCR22-F and LCR22-H which harbor the *SMARCB1* and *SNRPD3* genes. The patient presented healed cleft lip, mild facial dysmorphism, cognitive deficit, and delayed language development associated with severe behavioral problems including learning difficulties and aggressive behavior.

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The term genomic disorder is typically used to describe a gain (duplication) or loss (deletion) of a specific chromosomal region, associated with a clinical genetic syndrome that may present with congenital anomalies or with impairment in neurological and cognitive function [Emanuel, 2008].

Although both deletion and duplication events should occur in equal proportions, microduplications of the 22q11.2 region are about half as frequent as microdeletions. A recent study suggested that the frequency of 22q11.2 deletions is slightly higher than that of 22q11.2 duplications in sperm cells because intrachromatid events, in contrast to interchromatid events, only result in deletions [Turner et al., 2007; Gu et al., 2008].

Most commonly duplicated is a region of 3 Mb extending from low copy repeat (LCR) 22-A to LCR22-D, which encompasses nearly 40 genes. Less common is a smaller 1.5-Mb duplication extending from LCR22-A to LCR22-B. Duplications with other sizes in this interval have been seldom reported [Edelmann et al., 1999; Shaikh et al., 2001; Coppinger et al., 2009].



Fig. 1. Facial front view (A), side views (B, C) and tooth aspects (D) of the patient.

The phenotypical spectrum of patients with proximal 22q11.2 microduplications is diverse, with symptoms ranging from mild learning disability and mild dysmorphic facial features to severe mental retardation and multiple congenital malformations [Ensenauer et al., 2003; Boudewyns et al., 2012]. More than 50 index cases with 22q11.2 duplications involving the distal LCR22s have been reported so far [Descartes et al., 2008; Ou et al., 2008; Lundin et al., 2010; Shimojima et al., 2010; Tan et al., 2011; Wincent et al., 2011]. Similar to the proximal duplications, there seems to be a high rate of familial transmission [Ensenauer et al., 2003; Hassed et al., 2004; Portnoi et al., 2005; Ou et al., 2008], and the phenotypes vary among family members carrying the duplications [Descartes et al., 2008; Wincent et al., 2011].

In the present study, we report on a patient with 'healed' cleft lip and cognitive deficit carrying an atypical 1.2-Mb distal microduplication at 22q11.2 which was inherited from the mother with a near normal phenotype. Cleft lip is an unusual finding within the clinical spectrum of the velocardiofacial (VCF) syndrome, reinforcing the view that all children with cleft lip and/or palate should routinely be tested for VCF syndrome [Bashir et al., 2008; Nugent et al., 2010] despite of controversies [Ruiter et al., 2003; Barisic et al., 2008].

Material and Methods

Case Report

The proband (fig. 1A–D) was an 8-year-old boy born at 39 weeks of gestation as the second child from a normal 39-year-old mother and her normal non-consanguineous 46-year-old husband. Pregnancy was normal, at term, and delivery was through cesarean section. The proband's birth weight was 3,600 g (75th

percentile) and birth length 52 cm (95th percentile). Neuropsychological development was delayed as well as language development with first words at age 2 years. Clinical examination at age 8 years and 4 months showed a height of 127.5 cm (25th percentile), weight of 23 kg (25th percentile), and OFC of 51 cm (50th percentile). Routine biochemical and hematological exams were normal. Echocardiogram showed no structural heart abnormality. His facial features appeared mildly dysmorphic with low frontal hair line, downslanted palpebral fissures, deep set eyes, high nasal bridge and alar hypoplasia, mild malar hypoplasia, healed cleft lip at left, and large central incisors. He presented as an immature boy with moderate cognitive impairment, and his behavior ranges from apathy to extreme anxiety, with dependence on the mother for any decision.

Ethical approval was obtained for this study from the IRB at Hospital de Reabilitação de Anomalias Craniofaciais, Bauru, Brazil. Blood samples from patient and parents were obtained after informed consent.

Genetic Studies

Routine chromosome analysis was performed on GTG-banded metaphases prepared from cultured peripheral blood according to standard protocols.

MLPA was performed on DNA from peripheral blood lymphocytes, extracted with the Puregene Blood Core Kit C (Qiagen). Kit SALSA P250 for DGS/VCFS (MRC-Holland) was used. The kit tests 26 loci on 22q11, 2 on 22q13, 2 on 4q, 3 on 8p, 2 on 9q, 6 on 10p, and 4 on 17p. Reaction was performed on a MyGenie™ 96 thermocycler (Bioneer). Amplification products were identified using capillary electrophoresis on ABI3500 (Applied Biosystems). Data analysis was made against up to 5 control samples using the GeneMarker v1.95 software.

To perform array-comparative genomic hybridization (aCGH), Agilent oligonucleotide arrays (Human Genome CGH Microarray Kit 4×105K, Agilent Technologies) were used according to the manufacturer's instruction. Results were interpreted with DNA analytics_4.0.76 software (Agilent Technologies). Genomic positions were defined using NCBI36/hg18.

Fig. 2. MLPA results of the patient and his mother. Duplications of the probes are shown as red dots. Normal copy numbers are indicated as green dots, and the control probes are blue dots. Data were analyzed with GeneMarker v1.95.

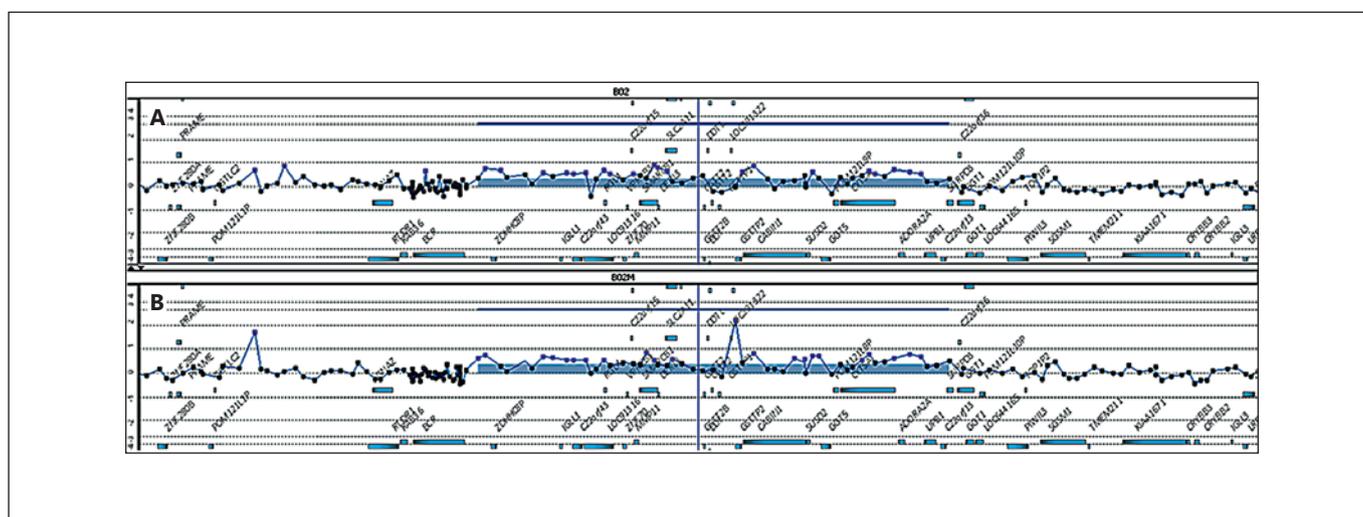
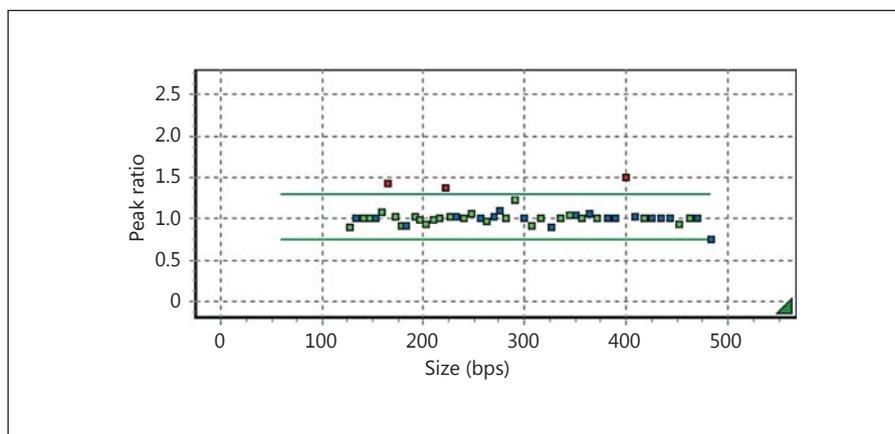


Fig. 3. aCGH profile of the 22q11.23 region showing the duplication in the patient (A) and his mother (B).

Results

The patient had a normal 46,XY karyotype. The MLPA screening with the P250 kit showed a proximal LCR22 F–H duplication (fig. 2), which was inherited from his healthy mother. In order to define the breakpoints more precisely, whole genome aCGH was performed and showed a 1.2-Mb interstitial duplication affecting the 22q11.23 region between the genomic positions 22,026,101–23,290,049 bp (according to NCBI build 36-hg18) in both patient and mother (fig. 3). No other CNVs were found. Because the maternal grandparents were not available for study, we were not able to determine if this duplication was *de novo* in the mother or inherited.

Discussion

The chromosome 22q11.2 region contains 8 different chromosome 22-specific LCRs, designated LCR22-A to LCR22-H, which are known to mediate recurrent microdeletions and microduplications by non-allelic homologous recombination (NAHR). Although both deletions and duplications are expected to occur in equal proportions as a result of reciprocal LCR-mediated events, only 50 cases of 22q11.2 microduplications have been reported so far [Portnoi, 2009]. Probably, microduplications are underdiagnosed by karyotype analysis and FISH.

In the present study, our diagnostic strategy for evaluation of the proband with cognition deficit was to use first

MLPA (MLPA P250), designed to detect microdeletions and microduplications. Further fine mapping was performed with aCGH which showed a 1.2-Mb duplication that maps distally to the typically deleted region (TDR) extending from LCR22-A to LCR22-D.

Due to the wide clinical variability and reduced penetrance, 22q11.2 microduplication syndrome, in some instances, has been considered a non-pathogenic polymorphism; however, clinical manifestations and wide clinical and molecular heterogeneity led most authors to define it as a new genomic duplication syndrome [Ensenauer et al., 2003; Portnoi, 2009]. The phenotypic spectrum observed in patients with 22q11.2 microduplications is diverse, with symptoms ranging from mild learning disability and mild dysmorphic facial features to severe mental retardation and multiple congenital malformations, speech delay, behavioral problems, hearing loss, growth delay, urogenital abnormalities, muscular hypotonia, congenital heart malformation, velopharyngeal insufficiency with or without cleft palate or hypernasal speech, seizures, and bladder exstrophy, among others [Ensenauer et al., 2003; Ou et al., 2008; Wentzel et al., 2008; Portnoi, 2009], but with no apparent phenotype-genotype correlation [Shimajima et al., 2010]. Unlike 22q11.2 deletion syndrome, the majority of the 22q11.2 duplications are inherited from a mildly affected or clinically normal parent. As previously described for microdeletions, patients with microduplications distal to the TDR have also been reported. These cases were characterized by phenotypic variability and lack of a common facial gestalt with apparently contradictory clinical features between the patients [Shaikh et al., 2001; Tan et al., 2011; Pebrel-Richard et al., 2012].

In the patient reported here, the cognitive and behavioral findings were those usually observed both in 22q11.2 deletions and duplications, whereas physical signs were mild and unremarkable, except for the presence of healed cleft lip. Most of the cases with milder forms of cleft lip are sporadic; however, it has been reported as a subtle expression of the bronchio-oculo-facial syndrome [Lin et al., 2009]. Cleft lip/palate has been seldom found both in 22q11.2 microdeletion as well as in microduplication; in the latter, it was reported only in 2 instances [Portnoi et al., 2005; Fernandez et al., 2009].

The patient presented here carried an atypical duplication inherited from his mother, who showed a normal mental development and no dysmorphic features. The distal chromosome 22q11.2 microduplication may act as a susceptibility factor, requiring a modifying factor for a clinical phenotype to emerge. The putative modifiers are likely to be different in each system, thus producing in-

terindividual variability [Tan et al., 2011], while regulation of neuronal function through epigenetic control of gene transcription seems to be an important mechanism related to intellectual deficiency [van Bokhoven and Kramer, 2010].

The 22q11.2 F–H region harbors *SNRPD3* and *SMARCB1* genes, both involved in tumorigenesis. Recently, the *SNRPD3* gene was related to aberrant splicing events, a mechanism established as direct cause of several human disorders [Khan et al., 2012]. In relation to *SMARCB1*, depending on the nature of the mutation that alters its function, diverse forms of cognitive deficits could result due to a mechanism involving epigenetic regulators [Day and Sweatt, 2011; Kleefstra et al., 2012]. It is probable that *SMARCB1* could be one of the main genes involved in cognition deficit in 22q11.2 deletion and/or duplication.

In conclusion, we report on a patient presenting cleft lip, cognitive deficit and mild dysmorphic features, who carries an inherited 22q11.2 duplication. This case adds to the literature supporting the pathogenic involvement of 22q11.2 duplication in intellectual deficiency and the incomplete penetrance and variable expressivity of this altered region which makes the genotype-phenotype correlation very difficult. The diagnoses in these patients are clearly necessary due to the implications toward prognosis, management, and counseling.

Acknowledgements

We thank the patient and his mother for their collaboration. Genetic analysis was supported by grants from FAPESP (2011/07012-9).

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