

Case Report

Molecular characterization of microduplication 22q11.2 in a girl with hypernasal speech

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ABSTRACT. We present a 12-year-old girl with karyotype 46,XX. A comparative genomic hybridization array revealed a 3.172-Mb microduplication on 22q11.2. This chromosome 22q11.2 region microduplication has been described in patients with variable phenotypes; a large majority of them have identical 3-Mb duplications. The girl presented mild mental motor retardation, facial dysmorphism consisting of a long narrow face, widely spaced eyes, downslanting palpebral fissures, broad nasal base, short philtrum, thin upper lip, micro/retrognathia, low set and retroverted ears, microcephaly, high-arched palate, hypoplastic teeth, and hypernasal speech. She had delayed psychomotor development and behavioral problems. Molecular characterization of patients differs greatly among reports and detailed molecular characterization and documentation are needed to better understand the effects of these duplications. This description of the phenotype of a patient with microduplication on 22q11.2 will contribute

to the growing knowledge regarding deletions and duplications of the 22q11.2 region; this is important to conclude whether 22q11.2 duplication is a microduplication syndrome or not.

Key words: Array-CGH; Hypernasal speech; Temporal hair loss; Microduplication 22q11.2; Mental retardation

INTRODUCTION

The chromosome 22q11.2 region is susceptible to well-known genomic disorders (Wentzel et al., 2008). DiGeorge/velocardiofacial syndrome (DGS/VCFS) (22q11.2 deletion syndrome, 22q11.2DS) is the most frequently identified genomic disorder of the region with a prevalence of 4/10,000 births, with only 6 to 25% of deletions having been inherited and the rest arising *de novo* (Wentzel et al., 2008; Grigorenko et al., 2010). Cat eye syndrome (arising from the supernumerary bisatellited marker chromosome) and der(22)t(11;22) syndrome are associated with either decreased or increased gene dosage (Edelmann et al., 1999a). 22q11.2DS is characterized by specific craniofacial features (cleft palate and velopharyngeal insufficiency, 69-100%), thymic and parathyroid defects (17-60%), mild to moderate renal anomalies (36-37%), congenital cardiovascular malformations (49-83%), and a range of cognitive and behavioral impairments (Grigorenko et al., 2010). Low average to borderline range of general cognitive functioning is one of the most consistent findings in the phenotypic presentation of 22q11.2DS (Antshel et al., 2008). Patients with duplications of the same region at 22q11.2 have been reported (de La Rochebrochard et al., 2006; Alberti et al., 2007; Yu et al., 2008). Although some of the features of the 22q11.2 duplication syndrome overlap with the deletion syndrome, most of the symptoms are distinct and seem to vary compared to those of the 22q11.2 deletion syndrome (Wentzel et al., 2008).

Moreover, the whole phenotypic spectrum of 22q11.2 microduplication is not yet fully known, owing to a highly variable and mild phenotype, such that many individuals with microduplications may not undergo testing (Lo-Castro et al., 2009). The common molecular defect is a ~3-Mb deletion in 87% of the cases known as the "typically deleted region", followed by a proximally nested ~1.5-Mb deletion in 7%, and other atypical deletions at chromosome 22q11.2 (Fernandez et al., 2009). Chromosome 22q11.2 is a well-characterized genomic region that is gene-rich and contains multiple region-specific low-copy repeats (LCRs) (McDermid and Morrow, 2002). Eight different LCRs are located in the proximal 22q region, which are presumed to predispose to mediate non-allelic homologous recombinations that result in interstitial deletions, duplications and inversions, as well as unbalanced translocations in this region (McDermid and Morrow, 2002). In theory, although both deletion and duplication events should occur in equal proportions, microduplications of the 22q11.2 region are about half as frequent as the deletions (Yu et al., 2008). Here we present and discuss the clinical and molecular findings of a 12-year-old girl with 46,XX karyotype and a 3.172-Mb duplication on chromosome 22q11.2.

CLINICAL REPORT

The proband is a 12-year-old girl referred to the medical genetics department be-

cause of mild mental-motor retardation and facial dysmorphism. She is the second child of a healthy 26-year-old mother and 32-year-old unrelated father. The first child of the family is healthy, and further family history was unremarkable. She was born by vaginal delivery at term; her birth weight was 3250 g (90th percentile), but length and head circumference were not recorded at that time. At 7 months old, stereotypic head movements were observed by her family. At 10 years old, her weight, length and occipitofrontal circumference were 25 kg (5-10th percentile), 127 cm (3rd-5th percentile), and 48 cm, respectively, and at 12 years old, these were 37 kg (25-50th percentile), 145 cm (10-25th percentile), and 47 cm, respectively. In her child history, eating difficulty, delayed walking without support at 5 years old, and speaking at 6 years old were pointed out. Although she was able to follow normal school without help, she was unsuccessful, and attention deficit disorder was noticed by her teachers. At present, she has hypernasal speech, delayed psychomotor development, and behavioral problems. Physical examination showed a mild facial dysmorphism consisting of a long narrow face, widely spaced eyes, downslanting palpebral fissures, broad nasal base, short philtrum, thin upper lip, micro/retrognathia, low set and retroverted ears, microcephaly, flat occiput, high-arched palate, hypoplastic teeth, pectus excavatum, and bilateral hair loss on temporal regions of head (Figure 1). Assessment of her main organ systems, including cardiac, renal, urogenital, and endocrinological systems, was normal. Audiometric investigation of the patient was normal. Magnetic resonance images (MRI) of the brain showed vermian agenesis and ependymal cyst and mucosal thickness on maxillary sinus and ethmoid cellules.



Figure 1. Image of the patient. Facial dysmorphism consisting of long narrow face, widely spaced eyes, downslanting palpebral fissures, broad nasal base, short philtrum, thin upper lip, and micro/retrognathia. Profile with low set and retroverted ears and hair loss on temporal region of her head is apparent.

GENETIC ANALYSIS

Metaphase slides of the patient and both parents were prepared from lymphocyte cultures of peripheral blood, and chromosome analysis was performed according to standard protocols using GTG banding at an approximately 550-band resolution. Standard chromosome preparations of the patient and her phenotypically healthy parents were analyzed, and the karyotypes were diagnosed as normal for all individuals. Since the karyotype analysis of

the patient was normal, further investigation was performed by array-comparative genomic hybridization (array-CGH) to delineate any chromosomal anomaly. Parents' specimens were unavailable for array-CGH testing at the time of submission. A 3.172-Mb duplication at genomic position 17.041.699 → 20.213.930 bp in the 22q11.2 region was detected by array-CGH, which was performed using a genome wide 180 k oligo array platform (OGT CytoSure, OGT Oxford, UK) (Figure 2).

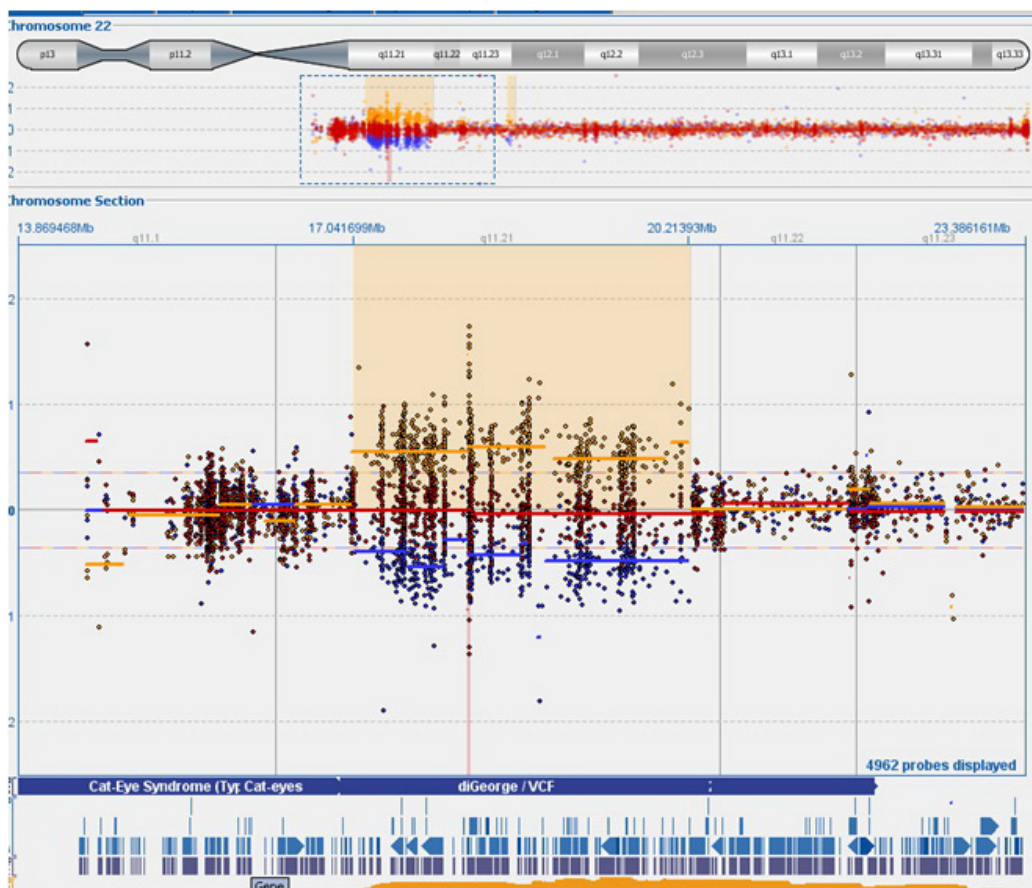


Figure 2. Oligoarray comparative genomic hybridization (CGH) study. Array-CGH showing a 3.172-Mb duplication in the 22q11.2 chromosomal region. VCF = velocardiofacial syndrome.

DISCUSSION

The 22q11.2 deletion syndrome's molecular mechanism has been well studied on the cytogenetic level; the deleted material can range from 3-Mb deletion (~90%) to 1.5-Mb deletion (7%), with the rest having a smaller deletion. The 3-Mb deletion region and the 1.5-Mb region contain more than 45 and 24 genes, respectively (Grigorenko et al., 2010). The peri-

centromeric region 22q11, is rich in regions of segmental duplication. In the 22q11 deletion syndrome, the typical 3-Mb deletion has both of its breakpoints in prominent segmental duplication regions (Grigorenko et al., 2010). The genes *PRODH* (the gene coding for proline dehydrogenase), *TBX1* (member of the Tbox family of transcription factors), *GNBIL* (a gene that encodes a protein of unknown function), *COMT* (the gene encoding catechol-O-methyl transferase), *ARVCF* (the armadillo repeat gene deleted in 22q11.2DS, which may have a role in cell-to-cell communication and intracellular transduction during embryonic development), *DGCR8*, *RANBP1*, *ZDHHC8* (the gene coding for zinc finger- and DHHC domain-containing protein 8), and *PIK4CA* are located in or close to regions of segmental duplication. There is a hypothesis that the syndrome is caused by an alteration of the genetic dosage of a particular gene or a number of genes in the region or disruption of a major regulatory gene, for example, the transcription factor *TBX1* gene (Grigorenko et al., 2010; Scambler, 2010).

The phenotypes previously associated with microduplications of 22q11.2 are extremely variable with features overlapping those of the 22q11.2 deletion syndrome, including heart defects, velopharyngeal insufficiency with or without cleft palate, hypernasal speech, hearing loss, urogenital abnormalities, delayed growth and development, learning disabilities, behavioral problems, and various dysmorphic features (Portnoi, 2009). The mild facial dysmorphic features reported are hypertelorism, superior placement of eyebrows, epicanthal folds, broad nasal bridge, high forehead, downslanting palpebral fissures, smooth philtrum, midface hypoplasia, mild micro/retrognathia, dysplastic large ears, microcephaly, long and narrow facial shape, which were different from dysmorphic features of patients with DGS/VCFC. In addition, a significant number of 22q11.2 microduplications may also be associated with a normal or near normal phenotype (Ensenauer et al., 2003). The reason for the wide phenotypic variation is unknown, but it has been speculated that epigenetic factors as well as non-penetrance may be involved (Yobb et al., 2005). In the recent years, several candidate genes reproducing the phenotype in animal models have been identified in 22q11.2 (Fernandez et al., 2009).

Until more information is available, perhaps we should consider the 22q11.2 syndrome as comprising only those individuals with common 3- or 1.5-Mb proximally nested microduplications (Ou et al., 2008). The typical duplicated region of 3-Mb from LCR-A (LCR22-2) to LCR-D (LCR22-4) contains more than 40 genes, whereas the 1.5-Mb duplicated region extending from LCR-A (LCR22-2) to LCR-B (LCR22-3a) contains the *TBX1* gene, which is a member of the T-box family of transcription factors; it has been shown to be the major disease gene in this region responsible for the DGS/VCFS when over- and under-expressed, explaining why there are many similar features between the 22q11.2 deletion and microduplication syndromes (Portnoi, 2009).

At least 63 individuals from 35 families have been described with duplication 22q11.2 (Courtens et al., 2008). In contrast to the reciprocal microdeletions (22q11.2 microdeletion syndrome), in which 90% occur *de novo*, familial transmission was reported in 70% by Wentzel et al. (2008). The first patient carrying a 3-Mb microduplication on 22q11.2 was a 4-year-old girl who presented moderate developmental delay, velopharyngeal insufficiency and a significant delay in language skills. Her facial features common to those of our patient were narrow face, downslanting palpebral fissures and velopharyngeal insufficiency (Edelmann et al., 1999b). Ensenauer et al. (2003) identified 10 unrelated patients with 22q11.2 microduplications, defining a new 22q11.2 microduplication syndrome distinct from the 22q11.2 deletion syndrome in

a series of 653 patients. Ou et al. (2008) reported 19 individuals from 10 families with various sizes of genomic gains within the 22q11.2 region detected by array-CGH among 7000 samples referred for a variety of indications to undergo clinical testing over the course of several years. Among them, 10 individuals were found to carry 3-Mb microduplications of the common DGS/VCFS region. The common clinical findings of these individuals, similar to those of our patient, were isolated speech delay, hypernasal speech and behavioral abnormality.

Common dysmorphic phenotypes similar to those of our patient were microcephaly, brachycephaly, downslanting eyes, micrognathia, and unusual hair patterns. In patients for whom parental samples were available, they observed a high frequency of familial transmission. Coppinger et al. (2009) screened 22,096 consecutive individuals with idiopathic mental retardation and/or congenital anomalies and reported 18 individuals with duplications of 22q11.21-q11.23. Thirteen of them were the first reported ones with 22q11.2 duplications that do not involve LCR22s A-D. Clinical records for nine subjects revealed shared characteristics, but some clinical features such as macrocephaly and upslanting palpebral fissures were contradicting clinical features (Coppinger et al., 2009). Lo-Castro et al. (2009) reported a 5.3-year-old girl with severe psychomotor retardation, facial dysmorphism, and autism, carrying a 22q11.2 duplication inherited from her mother who had a cleft palate and normal intelligence. She had severe mental retardation, severe autistic disorder and speech delay. Our patient has speech delay, learning disability, cognitive and behavioral disorders such as stereotypic movements of her head, anxiety, aggressivity, and attention deficiency, which are some of the major features of the 22q11.2 duplication syndrome. Courtens et al. (2008) reported on two families with 22q11.2 microduplication and, differently, they had thyroid problems, where both were treated with thyroid hormone. Our patient's thyroid hormone level was normal, but her mother had a thyroid operation and was treated with thyroid hormone. The occurrence of thyroid problem in the mother could be a coincidence, but the possibility of relationship with microduplication is not excluded. Prenatal testing is technically feasible but it is not possible to reliably predict the phenotype from a laboratory finding of 22q11.2 duplication (Wentzel et al., 2008).

The dysmorphic features of the patient presented here are likely the effect of 22q11.2 microduplication. Microduplication 22q11.2 may be largely undetected as a result of a nonspecific/mild phenotype and could be present more frequently in persons without clinical symptoms having a normal to even high intelligence (Courtens et al., 2008). Because it is undetectable by routine G-banded karyotyping, most individuals with 22q11.2 duplication are identified either by array-CGH testing, interphase FISH or multiplex ligation-dependent probe amplification testing for the 22q11.2 deletion syndrome (Stachon et al., 2007). Resolution of just a few hundred base pairs and even down to the single base pair level, as contrasted to conventional cytogenetic analyses with a resolution not better than several tens of thousands of base pairs, now allows high-resolution analyses of cryptic rearrangements (Grigorenko et al., 2010).

The molecular characterization of patients differs greatly between reports and detailed molecular characterization and documentation in the literature, making accurate genotype-phenotype correlations difficult. Future reports of other families are needed to find out whether the delineation of this microduplication 22q11.2 is a true "syndrome" with a very large clinical variability and reduced penetrance or a non-pathogenic polymorphism (Courtens et al., 2008). In conclusion, our patient report will contribute to the growing knowledge regarding constantly new deletions and duplications of 22q11.2 region.

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