A de novo sSMC(22) Characterized by High-Resolution Arrays in a Girl with Cat-Eye Syndrome without Coloboma

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Key Words

Cat-eye syndrome • Microarrays • Phenotypic variability • 22q supernumerary marker

Abstract

Cat-eye syndrome (CES) results from trisomy or tetrasomy of proximal 22q originated by a small supernumerary marker chromosome (sSMC). Two critical regions for the major clinical features of CES (CESCRs) have been suggested; however, CES clinical presentation often does not correlate with the sSMC genetic content. We report here a CES girl without coloboma and carrier of a de novo type I sSMC(22) as determined by G- and C-banding, NOR staining and microarrays. This sSMC included 6 distal genes outside the original CESCR and led to a tetrasomy for 22q11.1–22q11.21. The patient's final karyotype was 47,XX,+psu dic(22)(q11.21).arr 22q11.1q11.21(15,250,000–17,035,860)×4 dn. The amplified region outside of CESCR included some genes that may be related to neurologic, heart and renal abnormalities. Conversely, even though the amplification included the *CECR2* gene, a major candidate for eye features, there was no coloboma in

the patient. The genetic delineation of the present sSMC further strengthens that the CES clinical presentation does not fit completely with the duplicated genetic content and that CES is actually a genomic disorder. Furthermore, since we observed no mosaicism, we believe that other mechanisms might be behind the variability of CES phenotypes as well, mainly those related with functional interactions among amplified genes.

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The Schmid-Fraccaro or cat-eye syndrome (CES) is characterized by unilateral or bilateral ocular coloboma, downslanted palpebral fissures, pre-auricular pits or tags, heart and renal malformations, anal fistula and/or atresia, and neuropsychomotor disability [Rosias et al., 2001; Romagna et al., 2010; OMIM 115470]. The fact that the phenotype is highly variable is illustrated by the coloboma being present in only ~50% of patients [Jezela-Stanek et al., 2009]. The CES results from a partial trisomy or tetrasomy of proximal 22q due to a small supernumerary marker chromosome (sSMC) [Mears et al., 1994; Mark et

Fig. 1. **A** Craniofacial features of the patient. **B** Left pre-auricular tag. **C** Dysplastic right ear.

al., 2005; Bélien et al., 2008; Jezela-Stanek et al., 2009], and less commonly from an interstitial duplication of the CES critical region (CESCR) [Meins et al., 2003]. The original CESCR spans around 2 Mb, from the centromere to the D22S57 marker [Mears et al., 1994], and includes the *CECR1* and *CECR2* genes as major candidates for heart/facial and neurologic/eye features, respectively [Rhazi et al., 2000; Banting et al., 2005]. In the CES, 3 sSMC types (I, IIa-b and III) related to low copy repeats in 22q11.2 (LCR22s) have been described [Bartsch et al., 2005a; Bélien et al., 2008]. We report here a CES girl without coloboma but carrying a de novo type I sSMC(22) in order to provide further insights about CES phenotypic variability.

Clinical Report and Methods

Patient Data

The girl is the 3rd child of a G4P3A1 mother and an unrelated father. Prenatal ultrasonography disclosed right microtia, anal atresia, small dysplastic right kidney, and oligohydramnios. At birth, she was diagnosed with patent ductus arteriosus, which closed spontaneously. Her craniofacial features (fig. 1) included broad and prominent forehead, sparse hair, downslanting palpebral fissures, bilateral ptosis of the eyelids, telecanthus, strabismus, facial asymmetry, right microtia, left pre-auricular tag, long philtrum, thin upper lip, and micro-/retrognathia; breast hypoplasia was detected, too. Noticeably, a detailed ophthalmologic inspection did not show any eye coloboma. She underwent surgical treatment for the anal atresia and rectovaginal fistula. On ex-

amination at 2 years, her weight (8,000 g) and height (77 cm) were below the 3rd centile. She also presented delayed psychomotor development.

Cytogenetic Analyses

The initial cytogenetic analyses of the patient, her parents and a sister with cleft lip and palate were made on G-banded chromosomes obtained from 72-hour lymphocyte cultures. To confirm the presence of 2 centromeres and satellites in the sSMC, C-banding and NOR staining were realized.

Array Comparative Genomic Hybridization

To ascertain the chromosomal origin and segmental composition of the sSMC, a high-resolution genomic scan using Affymetrix GenomeWide SNP 6.0 platform was performed in the patient and her parents. Analysis of the arrays was performed using Genotyping Console v4.0 and ChAS v1.2 software. All samples were taken after an informed consent had been signed.

Results

The patient's G-banded karyotype was 47,XX,+mar [30]; karyotypes of the patient's relatives were normal. C-banding and NOR staining confirmed that the stable sSMC was bisatellited and pseudodicentric (fig. 2). In addition to identifying the origin of the marker to be chromosome 22, the microarray assay disclosed an amplified 2.6-Mb region (genomic position 15,250,000–17,035,860) embracing 1,177 markers (NCBI36/hg18) and entailing a partial tetrasomy for 22q11.1–22q11.21 (fig. 2). There were 4 copies of *XKR3*, *GAB4*, *CECR7*, *IL17RA*, *CECR6*, *CECR5*, *CECR1* (at 22q11.1), *CECR2*, *SLC25A18*, *ATP6V1E1*, *MIL1* (also named *BCL2L13*), *BID*, *MICAL3*, *PEX26*, *TUBA8* and *USP18* (at 22q11.21) genes. The amplification did not overlap with the VCF/DiGeorge locus. Thus, the sSMC was classified as type I with breakpoints within LCR22-3a and flanked by D22S427 and D22S36 [McTaggart et al., 1998; Bartsch et al., 2005a]. Microarray results from the parents were normal. The final karyotype based on ISCN [2009] was 47,XX,+psu dic(22)(q11.21).arr 22q11.1q11.21(15,250,000–17,035,860)×4 dn. The present approach, however, was not sufficient to define the parental chromosomal origin of the sSMC.

Discussion

Here, we thoroughly characterized a constitutional de novo type 1 sSMC(22) with both breakpoints within the LCR22-3a interval but including 6 genes outside the original CESCR (fig. 2).

Fig. 2. **A** Gain profile analysis indicated by log₂ ratio (circled plotting) and copy number state (circled bars) visualization. Blue bar represents gains. **B** Ideograms from normal chromosome 22 and sSMC(22). **C** and **D** Partial metaphases showing the sSMC; **C** NOR staining shows a bisatellited marker (arrow), and **D** C-banding

denoting a pseudodicentric marker (arrow). **E** Diagram (not to scale) denoting CESC and beyond as well as the amplified interval reported here. Black lines along with references indicate the reported breakpoint for each amplified interval in that case.

Breakpoints observed place *USP18* as the more distal gene (within D22S427), and suggest that formation of the present sSMC(22) could have resulted from a double rupture within LCR22-3a and inter- or intrachromosomal 'U-type' reunion during meiosis I, in accordance to other sSMCs [Van Dyke et al., 1977; Wandstrat and Schwartz, 2000; Murmann et al., 2009].

The amplified gene content in the present case agrees with most of CES cardinal clinical features, except for coloboma that was absent in spite of *CECR2* overdosage. This fact suggests that other unknown factors beside *CECR2* amplification are required to generate a coloboma. In line, a 600-kb intrachromosomal triplication spanning only *CECR2*, *SLC25A18* and *ATP6V1E1* genes was described in patients with 3 CES traits but neither coloboma nor mental disability [Knijnenburg et al., 2012].

Currently, 2 CESCrs that altogether extend beyond the D22S57 marker and include *BID* as most distal gene, have been suggested to account for most CES clinical features including mental disorders [Mears et al., 1994; Footz et al., 2001]. In addition, other CES cases with larger amplified regions (even encompassing VCF/DiGeorge locus or further) have been described (fig. 2) [Crolla et al., 1997; Bartsch et al., 2005a; Mark et al., 2005; Bélien et al., 2008; Jezela-Stanek et al., 2009]. Despite these observations, there is no precise correlation between the genetic content of the sSMC(22)s and clinical features [Crolla et al., 1997; Rosias et al., 2001; Bartsch et al., 2005a; Jezela-Stanek et al., 2009]. This fact may be due, among other factors, to mosaicism for sSMC(22)s [Bartsch et al., 2005a, b]. Furthermore, a refined delineation of the sSMC(22)s is often lacking; e.g. just ~11/200 CES cases have been delineated by more sensible techniques as microarrays [Liehr, 2012].

The description of at least 2 CESCrs and multiple amplified intervals within 22q coupled to CES phenotypic variants further strengthens that CES is a genomic disorder or contiguous gene syndrome [McDermid and Morrow, 2002]. Thereby, CES clinical spectrum could partially be due to overdosage of genes outside one or both

CESCrs. Accordingly, the gene content of the present sSMC(22) included, in addition to both CESCrs, 3 distal genes whose overdosage (along with that of *MIL1* and *BID*, suggested by Footz et al. [2001]) could mainly be related to the neurologic impairment in this and other CES patients: *MICAL3* and *TUBA8* are implicated in neuronal migration [Bron et al., 2007; Abdollahi et al., 2009] while *PEX26* is highly expressed in kidneys, brain and heart (UCSC genome browser).

However, since this assertion does not perfectly match, we speculate that positive or negative interactions among amplified genes could represent an alternative mechanism to account for the variability of CES phenotypes. This notion is supported by the observation that *MIL1* and *BID* have opposite functions within the same pathway (inhibition and activation, respectively) and that a probable antisense regulation among them was suggested [Kanehisa and Goto, 2000; Footz et al., 2001]. In addition, an analysis of the protein-protein interaction network for proximally or distally amplified genes in CES exhibited, among many others, functional interactions of the *MIL1* and *BID* genes within the same cluster (see online suppl. figs. 1 and 2, www.karger.com/doi/10.1159/000341632).

Finally, we consider it helpful to finely characterize those cases with only cardinal clinical traits of CES in order to complete the list of genes related to the major features of the disease as well as to perform a functional analysis of the amplified gene content. These approaches will ultimately result in a better disease understanding and classification.

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