Original Article

Cytogenetic and Genome Research

Cytogenet Genome Res
DOI: 10.1159/000341570

Accepted: Mai 21, 2012
by M. Schmid
Published online: August 10,2012

Wide Clinical Variability in Cat Eye Syndrome Patients: Four Non-Related Patients and Three Patients from the Same Family

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Key Words
Cat eye syndrome · Chromosome 22 · Marker chromosome · Small supernumerary marker chromosome

Abstract
A small supernumerary marker chromosome (sSMC) derived from chromosome 22 is a relatively common cytogenetic finding. This sSMC typically results in tetrasomy for a chromosomal region that spans the chromosome 22p arm and the proximal 2 Mb of 22q11.21. Using classical cytogenetics, fluorescence in situ hybridization, multiplex ligation-dependent probe amplification, and array techniques, 7 patients with sSMCs derived from chromosome 22 were studied: 4 non-related and 3 from the same family (mother, daughter, and son). The sSMCs in all patients were dicentric and bisatellited chromosomes with breakpoints in the chromosome 22 low-copy repeat A region, resulting in cat eye syndrome (CES) due to chromosome 22 partial tetrasomy 22pter→q11.2 including the cat eye chromosome region. Although all subjects presented the same chromosomal abnormality, they showed a wide range of phenotypic differences, even in the 3 patients from the same family. There are no previous reports of CES occurring within 3 patients in the same family. Thus, the clinical and follow-up data presented here contribute to a better delineation of the phenotypes and outcomes of CES patients and will be useful for genetic counseling.

Small supernumerary marker chromosomes (sSMCs) are a frequent finding in cytogenetic diagnosis [Bartels et al., 2003]. They are reported in 0.043% of newborn infants, 0.077% of prenatal cases, 0.433% of mentally retarded patients, and 0.171% of subfertile individuals [Liehr and Weise, 2007]. The chromosomal origin of the marker is usually determined using fluorescence in situ hybridization (FISH). Among sSMCs, approximately 20% are derived from chromosome 22 [Liehr, 2012]. Different sSMCs containing the 22q11.2 region have been described, including the typical bisatellited cat eye syndrome (CES, OMIM #115470) chromosomes and the sSMCs 22 derived from the recurrent balanced t(11;22)(q23;q11) translocation that results in Emanuel syndrome [Carter et al., 2009]. These rearrangements result in 4 or 3 copies of the proximal 22q region, all producing overlapping phenotypes among the syndromes [Bartsch et al., 2005; Allotey et al., 2008]. CES is a rare syndrome char-

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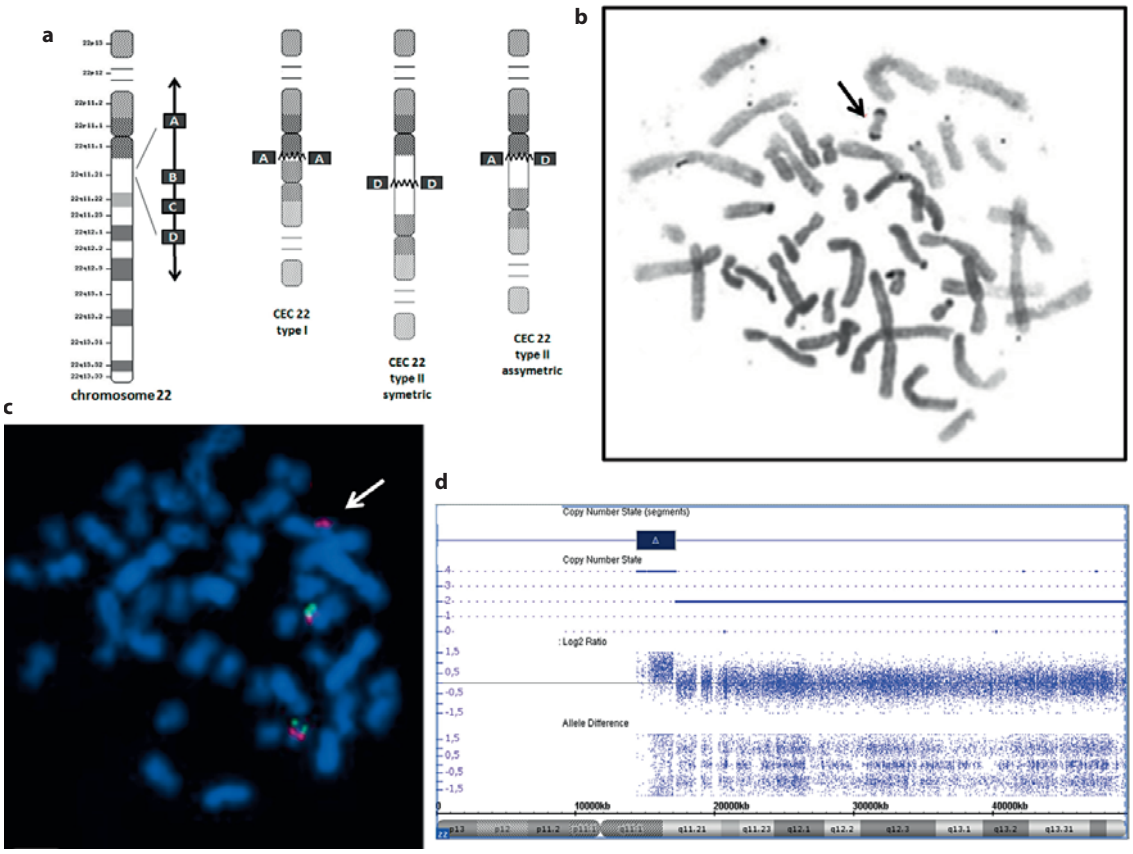

Fig. 1. a Classic and molecular cytogenetic characterization of the CEC. Ideogram of normal chromosome 22, CEC type I, symmetric CEC type II, and asymmetric CEC type II, with the respective breakpoints indicated by black rectangles. **b** NOR-staining showing the bisatellited marker (arrow). **c** FISH with RP11-81B3 (red signal) and with RP11-479G10 (green signal) probes in the CEC type I (arrow). **d** Array from Patient 4 showing 4 copies of the proximal region of chromosome 22.

acterized by the tetrasomy of the region that spans the short arm of chromosome 22 and part of 22q11, usually in the form of an inv dup(22) [Mears et al., 1994]. The cat eye chromosome (CEC) originates from breakpoints in the low-copy repeat (LCR) regions LCR-A and LCR-D located on 22q11.2 [Mears et al., 1994]. Therefore, the CEC is classified based on the location of its breakpoints [McTaggart et al., 1998]. The small CEC (type I) is symmetrical, with both breakpoints located within LCR-A, and the larger CEC (type II) may be either symmetrical, with both breakpoints in LCR-D, or asymmetrical, with breakpoints located in each of the 2 LCRs (fig. 1a). The phenotype of CES is highly variable and includes ocular coloboma, pre-auricular anomalies, cleft palate, congenital heart malformations (particularly total anomalous pulmonary venous return and tetralogy of Fallot) and renal and anorectal anomalies [Berends et al., 2001]. A website displaying sSMC findings, created by Thomas Liehr (http://www.med.uni-jena.de/fish/sSMC/00START.htm, accessed March 2012), shows that the majority of cases are sporadic, although some familial cases have been reported. We found only 1 report of a marker chromosome present in 3 subjects from a 3-generation family, but this was a heterochromatic harmless sSMC [Nelle et al., 2010]. Here, we report 7 patients, 4 non-related and 3 from the same family, with sSMCs derived from chromosome 22, resulting in partial tetrasomy of chromosome 22 (CES).

Patients

This research was approved by the institutional ethics review board of the Universidade Federal de São Paulo, and informed consent was obtained from all of participants and/or their repre-

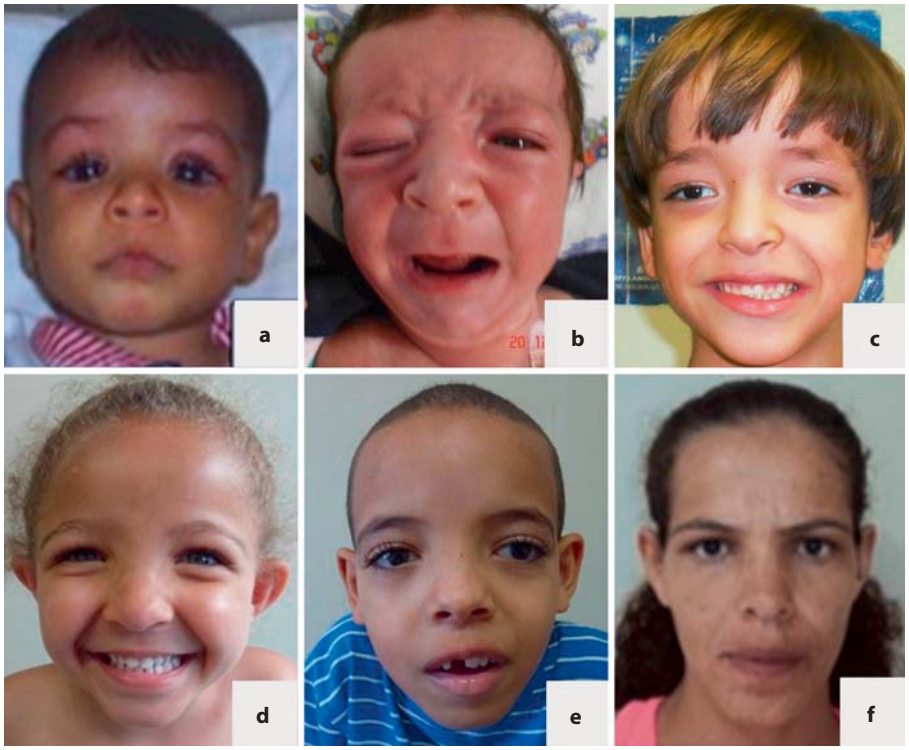

Fig. 2. The faces of Patients 1 (**a**), 3 (**b**), 4 (**c**), 5 (**d**), 6 (**e**), and 7 (**f**) at 4 and 2 months, 4, 4, 6, and 26 years, respectively.

sentatives. Seven Brazilian CES patients were studied (table 1; fig. 2). All patients were examined by clinical geneticists who requested a karyogram. They were clinically evaluated with complete clinical and anthropometric exam and detailed phenotypic description. All patients were examined by 2-dimensional echocardiography, abdominal ultrasound, and complete ophthalmic and audiological assessment, except for Patient 1 who died prematurely due to cardiac complications.

Patient 1

This patient presented as a 4-month-old female (fig. 2a), the first child of young and non-consanguineous parents, born at 39 weeks of gestation with normal measurements. The patient was reported to look tired after breast-feeding. Physical examination showed that her body weight was 4,410 g (<3rd percentile), length was 61.5 cm (50th percentile), and head circumference (OFC) 41 cm (50th percentile). The patient had a prominent forehead, downslanting palpebral fissures, ocular hypertelorism, epicanthic folds, cup-shaped ears with bilateral preauricular skin tags, a flattened nasal bridge, downturned corners of the mouth, and mild malar hypoplasia. Echocardiography showed type B interruption of the aortic arch. The patient had cardiac complications and died at the age of 5 months [Belangero et al., 2009].

Patient 2

This patient presented as a 1-month-old female, the second child of young and non-consanguineous parents, born at term at 3,090 g (50th percentile), 49 cm (50th percentile), and a head circumference of 34 cm (50th percentile). The patient presented with ocular hypertelorism, low set ears with bilateral preauricular tags, a long philtrum, and thin upper lip. The patient's hands presented with a bilateral transverse crease and clinodactyly of the fifth fingers. The patient had an umbilical hernia and anal atresia with a rectovaginal fistula at birth which was corrected by surgery. Perimembranous interventricular communication was diagnosed by echocardiography. Ophthalmologic evaluation detected a bilateral coloboma of iris, retina, and optic nerve. During follow-up, the patient presented with recurrent pulmonary infections due to heart disease. The patient's cognitive and motor development were normal at 3 years when she was clinically re-evaluated.

Patient 3

This patient presented as a 2-month-old male (fig. 2b), the third child of young and non-consanguineous parents, born at term at 3,125 g (50th percentile), 51 cm (50th percentile), and with a head circumference of 34 cm (50th percentile). The patient presented with a large anterior fontanel, prominent forehead, downslanting palpebral fissures, ocular hypertelorism, epicanthal folds, low set ears with bilateral preauricular tag, retrognathia, and a posterior cleft palate. Echocardiography showed total anomalous pulmonary venous return. Ophthalmologic evaluation revealed iris coloboma on the right eye. At the age of 6 years, the patient has fecal incontinence.

Patient 4

This patient presented as a 4-year-and-7-month-old male (fig. 2c), the first son of a young, non-consanguineous couple. His

Table 1. Clinical features of the evaluated patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (last follow-up)	4 months	3 years	2 months	4 years 7 months	4 years 9 months	6 years 3months	26 years
Developmental delay	nd	+	+	+	–	+	–
Ocular coloboma	NA	+	+	+	–	–	–
Preauricular tags/pits	+	+	+	+	+	+	+
Cleft palate	–	–	+	–	–	–	–
Skeletal defects	–	–	–	–	+	–	–
Heart defect	AAI type B	PIC	TAPVR	TAPVR	–	–	–
Urogenital defect	–	+	–	–	–	–	–
Anal defect	–	+	–	–	+	–	–
Hearing loss	–	–	–	+	–	–	+
Other characteristics	–	clinodactyly of fifth fingers, umbilical hernia	–	accessory spleen	strabismus	strabismus, shortening of the fifth finger	–

AAI = Aortic arch interruption; PIC = perimembranous interventricular communication; TAPVR = total anomalous pulmonary venous return; + = present; – = absent; nd = not determined.

mother had 2 previous pregnancies in another relationship, a set of twins and one early spontaneous abortion. The patient was born at 31 weeks gestation due to a maternal pulmonary embolism at 1,750 g (75th percentile) and 42 cm (50th percentile), and remained in the hospital for 5 months. Upon physical examination, the patient's weight was 11 kg (<3rd percentile), height was 85 cm (3rd percentile), and head circumference was 50 cm (50th percentile). He presented with a prominent forehead, mild facial asymmetry, downslanting palpebral fissures, ocular hypertelorism, right iris coloboma, epicanthal folds, a high and wide nasal bridge, and a pre-auricular tag on the right ear. Abdominal ultrasound revealed the presence of an accessory spleen to the left. Cardiac anomaly with total anomalous pulmonary venous return was diagnosed at birth, and 2 surgeries were performed for correction. The patient evolved with mild motor developmental delay.

Patient 5 (Sister of Patient 6 and Daughter of Patient 7)

This patient presented as a 1-year-and-9-month-old female (fig. 2d), first twin, born from the 5th gestation of a non-consanguineous young couple who had previously had 2 first trimester spontaneous abortions and 2 living children. The second twin (of unknown zygosity) died at the age of 7 months from complications of a congenital heart defect. Upon physical examination, the patient's weight was 8,820 g (<3rd percentile), height was 78 cm (<3rd percentile), and head circumference was 46.4 cm (10th percentile). The patient presented with a prominent forehead, downslanting palpebral fissures, ocular hypertelorism, convergent strabismus, low set ears with a bilateral preauricular pit and tag, low hairline, high and wide nasal bridge, long philtrum, and prominent lips. Other anomalies included imperforate anus and a radial defect with a hypoplastic left thumb. Ophthalmologic evaluation was normal, and no cardiopathy was detected. The patient's motor development was delayed during the first 2 years of life. Since that time, the patient has presented with normal cognitive and motor development.

Patient 6 (Brother of Patient 5 and Son of Patient 7)

This patient presented as a 5-year-and-3-month-old male (fig. 2e), the second child of Patient 7, born at term at 2,595 g (<10th percentile), 47 cm (3rd percentile), and a head circumference of 34 cm (50th percentile). He presented with fetal distress and generalized infection in the immediate postnatal period. On physical examination, the patient's body weight was 15.2 kg (3rd percentile), height 104 cm (3rd percentile), and head circumference 50 cm (25th percentile). He presented with downward slanted palpebral fissures, ocular hypertelorism, convergent strabismus and bilateral pre-auricular pits without tags, and a bilateral short fifth finger. Echocardiography, ophthalmologic exam, and audiometry were normal. The patient presented with motor delay in the first 2 years of life and current difficulty in school.

Patient 7 (Mother of Patients 5 and 6)

The 24-year-old mother of Patients 5 and 6 (fig. 2f) was born from the third gestation of non-consanguineous parents. Physical examination showed normal height, weight, and head circumference. The patient has a prominent forehead, left pre-auricular tag, high nasal bridge, and normal interpupillary distance. At age 22, she presented signs of hypertension and began clinical follow-up with a cardiologist. Echocardiography showed mitral and tricuspid regurgitation and mild left ventricular systolic dysfunction due to diffuse hypokinesia. Ophthalmologic examination was normal, and audiometry showed a mild conductive dysfunction in the right ear. Her motor and cognitive development were normal.

Methods

Cytogenetic Analyses

Phytohaemagglutinin-stimulated lymphocyte cultures were performed using blood samples from the patients and their parents. Fifty metaphase cells with G-banding, 10 with nucleolar organizer region (NOR) staining, and 10 with C-banding were analyzed from each patient. All patients showed a 47,XX or XY,+mar karyotype in all cells analyzed. C-banding and NOR-staining showed that the marker chromosomes were dicentric and bisatellited (fig. 1b). With the exception of Patients 5 and 6, whose mother (Patient 7) presented with the same marker chromosome, all patients' parents showed normal karyotypes.

In Patient 1, FISH was performed using DiGeorge/VCFS-Tuple1 (Cytocell, Cambridge, UK) with the centromeric 14/22 (D14Z1/D22Z1) probe (Vysis, Des Plaines, Ill., USA) and cosmid probes as previously reported [Belangero et al., 2009]. In all other patients, FISH was performed using a BAC probe for the cat eye critical region (RP11-81B3) proximal to LCR-A, a DiGeorge critical region probe (RP11-479G10), which maps between LCR-A and LCR-B, and a subtelomeric control probe (RP13-202L15), which maps to distal 22q, as previously reported [Kulikowski et al., 2010]. All marker chromosomes hybridized with the RP11-81B3 probe but not with the RP11-479G10 and RP13-202L15 probes, confirming the marker chromosomes as CEC type I (fig. 1c).

Molecular Analysis

Genomic DNA was extracted from the peripheral blood leukocytes of the patients and their parents according to standard procedures. Multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA P250 DiGeorge Syndrome Kit (MRC-Holland, Amsterdam, Netherlands) revealed increased amplification of *IL17RA, SLC25A18, BID, MICAL3,* and *USP18* probes corresponding to the cat eye region in all patients. In Patient 4, high-resolution breakpoint mapping performed with the Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 array (Affymetrix, Inc., Santa Clara, Calif., USA) showed 4 copies of the proximal region of chromosome 22, described as arr 22q11.1q11.21(14,435,171–17,190,083)×4, according to NCBI 36/hg 18, indicating a breakpoint at LCR-A (fig. 1d). No other relevant genomic imbalance was found. Thus, all patients presented as 47,XY or XX,+idic(22)(pter→q11.21::q11.21→pter). ish idic(22)(RP11-81B3+,RP11-479G10-).mlpa(P250)×4.

Results and Discussion

We report a cytogenetic and molecular study of 7 patients with CES who presented sSMC 22 with breakpoints located at LCR-A, characterizing them as CEC type I, the most common type of marker chromosome 22 [Emanuel, 2008]. These small marker chromosomes are believed to originate through a U-strand exchange mechanism due to the presence of LCRs in 22q11.2 that predispose these chromosomes to deletions, duplications, and inversions through non-allelic homologous recombination [McTaggart et al., 1998]. The clinical phenotype of CES patients is quite variable, ranging from mild disability to severe intellectual disability and/or malformations leading to early death [Berends et al., 2001; Rosias et al., 2001; Rosa et al., 2010]. According to Schinzel [2001], this chromosome aberration is especially unique in its high variability in clinical expression; some parents with no apparent phenotypes or only minor features have children with the full range of CES syndrome phenotypes. In fact, some patients may present only subtle abnormalities and could be underdiagnosed, as observed for Patient 7 who was diagnosed only because of her children. As shown in table 1, Patient 5 presents a more severe phenotype than her brother, Patient 6, and her mother, Patient 7. Congenital heart defect is a recurrent characteristic in CES, although different types of cardiac defects have been described in this syndrome [Berends et al., 2001; Rosias et al., 2001]. Four of the 7 CES patients studied here had congenital heart defects. Patient 2 exhibited defective development of the cardiac outflow tract, i.e. a conotruncal defect (perimembranous interventricular communication), while Patients 1, 3, and 4 had defects due to the anomalous development of the derivatives of the caudal pharynx, including the caudal pharyngeal arches (aortic arch interruption and total anomalous pulmonary venous return). Heart defect is an important cause of morbidity and mortality in CES children, and therefore an early diagnosis of the syndrome can improve follow-up, preventing further health complications. Although Patient 5 did not present with a heart defect, her twin died due to cardiac complications. Interestingly, all patients showed preauricular tags or pits. In fact, preauricular tags/pits are the most consistent feature in CES [Rosias et al., 2001], and only 41% of CES patients have the classical combination of iris coloboma, anal anomalies, and preauricular anomalies [Berends et al., 2001]. Clinically, multiple variations of sSMC 22 have been described with no real correlation to the classical phenotype [Allotey et al., 2008]. In 2 of the patients in the present study the developmental delay may be attributed to complications in the perinatal period. Some genes within and adjacent to the cat eye chromosome region (CECR) were proposed to be involved in the CES phenotype. The *CECR1* gene has been tentatively correlated with the heart and facial defects in CES [Riazi et al., 2000], the *CECR2* gene may be involved in chromatid remodeling [Jeanmougin et al., 1997; Collingwood et al., 1999; Winston and Allis, 1999], and the *MIL1* and *BID* genes may affect the physical and mental development of CES patients [Footz et al., 2001]. Different siz-

es of duplications of the distal CECR have been reported, but no clear genotype-phenotype correlation has been shown [Berends et al., 2001; Coppinger et al., 2009], suggesting that other factors such as epigenetic effects or epistasis with other currently unidentified genes may influence the clinical manifestation of the disease. Our findings emphasize the great clinical variability in CES even in familial cases with the same marker chromosome. These findings also highlight the importance of performing chromosome examination even in asymptomatic parents to determine the risk of recurrence and provide more conclusive genetic counseling for these families.

Acknowledgments

We thank Dr. Beverly S. Emanuel and April M. Hacker for the molecular study of Patient 1 and Dr. Rosane S. Canonaco for the examination of Patient 4.

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