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CASE REPORT

Prenatal detection and characterization of a small supernumerary marker chromosome (sSMC) derived from chromosome 22 with apparently normal phenotype†

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Objective To present prenatal findings and molecular cytogenetic characterization of a small supernumerary marker chromosome (sSMC) derived from chromosome 22 with apparently normal phenotype.

Case and Methods An amniocentesis was performed at 15 weeks' gestation and a small marker chromosome in the female fetus of a twin pregnancy was noted. A second amniocentesis was performed at 18 weeks; G-banding analysis on amniotic cells confirmed the small marker chromosome found in the female fetus. Both parents and the male twin fetus had normal karyotypes. Spectral karyotyping (SKY), Fluorescence *in situ* hybridization (FISH) analyses with chromosomal specific whole chromosome painting probe (WCP 22) and alphoid satellite DNA probe (D22Z4) were used to identify the origin of the sSMC. The make-up of the sSMC was characterized by further FISH studies with chromosome region specific probes. The twin babies were delivered normally at 35 weeks' gestation. The female neonate with sSMC did not show any dysmorphic features, except for a type II atrial septum defect (ASD) at birth. She was found to be developing and growing normally at her 2-year follow-up.

Results Conventional G-banding study confirmed the presence of a sSMC with bi-satellites. SKY and FISH with D22Z4 probes showed that the marker originated from chromosome 22. FISH studies using 4 locus-specific DNA probes in the 22q11.2 region (N25 probe to detect the D22S75 locus within the velocardiofacial syndrome/DiGeorge syndrome (VCFS/DGS) critical region, a clone to detect the Bid locus just distal to the cat eye syndrome (CES) critical region and two clones 77H2 and 109L3 to detect the proximal end of the CES critical region, (*CECR*2 and *CECR*7), did not reveal any hybridization signal with the marker chromosome. The karyotype of the fetus was 47,XX,+mar. ish der(22) (SKY+,D22Z42+,*CECR*7−,*CECR*2−, BID−,D22S75−).

Conclusion The supernumerary marker chromosome in this case was a *de novo* inv dup(22)(q11.2) and contained a duplicated proximal long arm region <400 kb from the centromere; it did not appear to affect the phenotype of the child. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: marker chromosome 22; small supernumerary marker chromosome (sSMC); SKY; CES critical region; VCFS/DGS critical region

INTRODUCTION

Small supernumerary marker chromosomes (sSMCs) are structurally abnormal chromosomes equal in size or smaller than chromosome 20 and cannot be identified or characterized unambiguously by conventional cytogenetic banding techniques (Liehr *et al*., 2004). sSMCs are present in about 0.043% of newborn and in 0.076% of prenatal cases (Liehr *et al*., 2004). Until very recently, there was no way of predicting precisely the outcome of the pregnancy when a *de novo* sSMC is ascertained prenatally. About 70% of sSMCs derive from acrocentric chromosomes (Viersbach *et al*., 1998; Liehr *et al*., 2004). In general, the risk for an abnormal phenotype is about 7% when *de novo* sSMC deriving from chromosomes 13, 14, 21 and 22 ascertained prenatally (Crolla, 1998; Warburton, 1991). Patients with small derivatives of chromosome 15 tend to have a normal phenotype and sSMC derived from chromosomes 13, 21 and 14 also appear to have low risk of abnormalities

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(Vierbach *et al*., 1998). The 22q11 region is particularly susceptible to chromosomal rearrangement and sSMC formation because it contains various low copy repeat sequences (Edelmann *et al*., 1999; Shaikh *et al*., 2000; Crolla *et al*., 2005). Three different congenital malformation syndromes are known to occur in this region: cat eye syndrome (CES), derivative 22 syndrome [der(22)] and velocardiofacial syndrome/DiGeorge syndrome (VCFS/DGS) (McDermid and Morrow, 2002). However, sSMC cases with inv dup(22) (q10 → q11.2) do not necessarily result in abnormal phenotypes (Mears *et al*., 1994; Gravholt and Friedrich, 1995; Vierbach *et al*., 1998; Hastings *et al*., 1999; Engelen *et al*., 2000; Lohmann *et al*., 2000; Bartels *et al*., 2003; Starke *et al*., 2003; von Eggeling *et al*., 2003). Bartsch *et al*. (2005) recently identified six diagnostically relevant intervals on chromosome 22q by fluorescence in situ hybridization (FISH) and proposed using DNA probes corresponding to these intervals to characterize sSMC. In this study, we used a combination of spectral karyotyping (SKY) and FISH with chromosome-specific and locus-specific DNA probes in the region 22q11 to delineate the breakpoint of a prenatally detected sSMC originated from chromosome 22.

CASE REPORT AND METHODS

A 34-year-old Taiwanese woman, gravida 1, para 0, abortion 0, who had become pregnant after having undergone controlled ovarian hyperstimulation, *in vitro* fertilization and embryo transfer was referred to us from a local clinic to evaluate an earlier amniocentesis finding of abnormal karyotyping (47,XX,+mar) in one of her twin fetuses (twin-A). Family history was noncontributory except for her long-term infertility. Sonography demonstrated a dichorion–diamnionic twin pregnancy with normal fetuses. A second amniocentesis was performed at 18 weeks' gestation to confirm the aberrant karyotype. Conventional G-banding revealed that both parents and one of the twin fetuses (twin-B, male) had normal karyotypes. However, an extra small bi-satellited marker chromosome was observed in all the 20 cells analyzed (47, XX, +mar) from the other twin (twin-A, female). The SKY analysis identified that the marker chromosome was derived from chromosome 22 (Figure 1(a)). FISH studies with chromosome 22 specific alphoid satellite DNA probe D22Z4 (Rocchi *et al*., 1994) under stringent conditions showed that the marker chromosome was positive for D22Z4 (Figure 1(b)). These findings indicated that the marker originated on chromosome 22 and might retain the chromosome 22 centromeric/pericentromeric region 22p11.1- q11.1. Further FISH experiments were conducted using locus-specific DNA probes for fine mapping of the marker chromosome breakpoint within the VCFS/DGS and CES critical regions. LSI DiGeorge N25 probe (Vysis) was used to detect the D22S75 locus and nearby CTP and CLTD genes (Carlson *et al*., 1997) located within the VCFS/DGS critical region at 22q11.2. BAC clone (RPCI11-91O6) (CHORI BAC/PAC resources) was used to detect BID locus at the distal end of CES critical region (McDermid and Morrow, 2002). Two clones, 77H2 and 109L3 which cover the *CECR2* and *CECR7 loci* in the CES critical region were also used to map the breakpoints about 800 and 400 kb away from the centromere, respectively (Footz *et al*., 2001). No hybridization signals were observed in the marker chromosome by any of the four DNA probes used; however, positive hybridization signals of the 4 locus-specific probes were detected in chromosome 22s (Figure 2(a–d)). Together, G-banding, SKY and FISH studies revealed that the karyotype of fetus-A was 47, XX,+mar.ish der(22) (SKY+, D22Z4+,*CECR*7−,*CECR*2−,BID−,D22S75−). Our molecular cytogenetic studies indicated that the marker chromosome did not contain the N25 locus in the VCFS/DGS critical region and did not contain the BID, CECR2 or CECR7 gene sequences within the CES critical region. Therefore, the breakpoint of the marker should have occurred somewhere <400 kb from the centromere (Footz *et al*., 2001) (Figure 3). The pregnancy was allowed to continue. At 35 weeks' gestation,

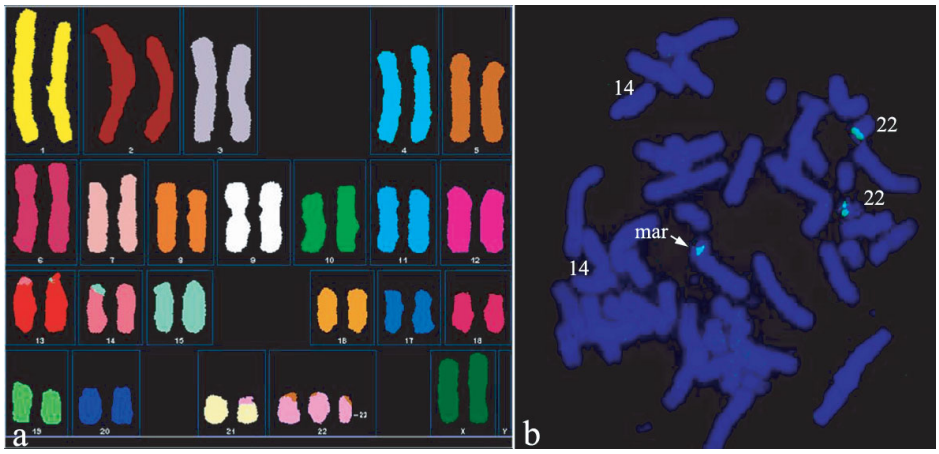

Figure 1—Spectral karyotyping (SKY) and FISH analysis of the marker chromosome. (a) SKY shows that the marker chromosome (indicated) is derived from chromosome 22. (b) FISH with a chromosome-specific alphoid satellite probe (D22Z4) shows positive hybridization signals on chromosome 22s as well as on the marker (indicated by an arrow). Chromosome 14s is also indicated

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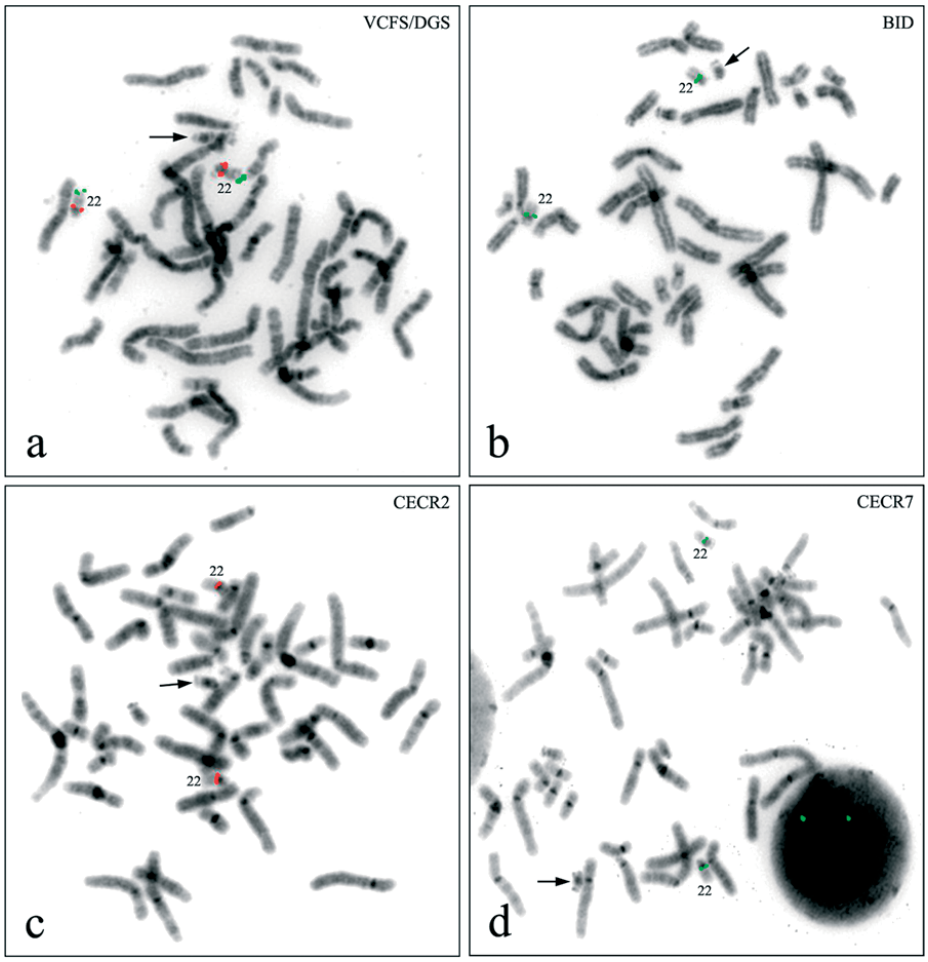

Figure 2—FISH study with locus-specific probes located within the velocardiofacial syndrome/DiGeorge syndrome (VCFS/DGS) critical region and cat eye syndrome (CES) critical region. (a) A metaphase cell hybridized with LSI DiGeorge N25 region probe (Vysis). Both number 22 chromosomes (indicated) show presence of the spectrumorange N25 probe and the specteumgreen LSI ARSA control probe. The G-banding patterns of chromosomes are visible by the inverted DAPI counterstain and show the bi-satellite nature of the marker, which lack a hybridization signal (indicated by an arrow). (b) The same metaphase as in Figure 1(b) hybridized with a BAC clone probe, RPCI11-91O6(CHORI) containing the BID locus in the CES critical region. Both chromosome 22s (indicated) show presence of the probe with green fluorescent signals, whereas the marker chromosome (indicated by an arrow) shows lack of hybridization, indicating that the BID locus is missing. (c) A metaphase cell hybridized with 77H2 clone probe of the CECR2 locus. Both chromosome 22s have positive red hybridization signals (indicated). The marker chromosome has no hybridization signal (indicated by an arrow), which indicates the absence of CECR2 locus in the marker. (d) A metaphase cell and an interphase cells hybridized with 109L3 clone probe containing the CECR7 locus. Both number 22 chromosomes (indicated) show presence of the109L3 probe (green signals). The marker chromosome shows absence of hybridization signal (indicated by an arrow), indicating that the CECR7 locus is not present. Two positive green hybridization signals are seen in the interphase cell

premature rupture of the membrane occurred and two babies were delivered normally. The neonatal course of both babies was uneventful. A serial examination revealed that twin-A did not have any dysmorphic features except for type II atrial septum defect (ASD). She was developing normally [head girth: 50 cm (50th percentile), body length: 92 cm (95th percentile), body weight: 12 kg (50th percentile)] comparable to her normal karyotype twin brother at her 2-year follow-up. Spontaneous closure of type II ASD was also noted.

DISCUSSION

Great achievement has been made in recent years, both in the characterization of sSMC and in exploration of its clinical impact. sSMC have been characterized by molecular cytogenetic study for their chromosomal origin in over 1500 patients (Liehr *et al*., 2004). A number of clinical syndromes associated with sSMC have also been documented including the der(22) syndrome and CES (Fraccaro *et al*., 1980; Schinzel *et al*., 1981), Pallister–Killian syndrome (Peltomaki *et al*., 1987) and i(18p) syndrome (Callen *et al*., 1990). A large portion of sSMCs (30.3%) have been shown to have originated from inverted duplication of chromosome 15, inv dup(15) (Liehr *et al*., 2004). It has been noticed that patients with small inv dup(15) have normal phenotypes but those with large inv dup(15) show phenotypic anomalities (Viersbach *et al*., 1998). It is now known that the clinical severity of sSMC with inv dup(15) is associated with the dosage of the Prader-Willi/Angelman syndrome critical region (Nietzel *et al*., 2003). However, about 62% of sSMC are not correlated with a

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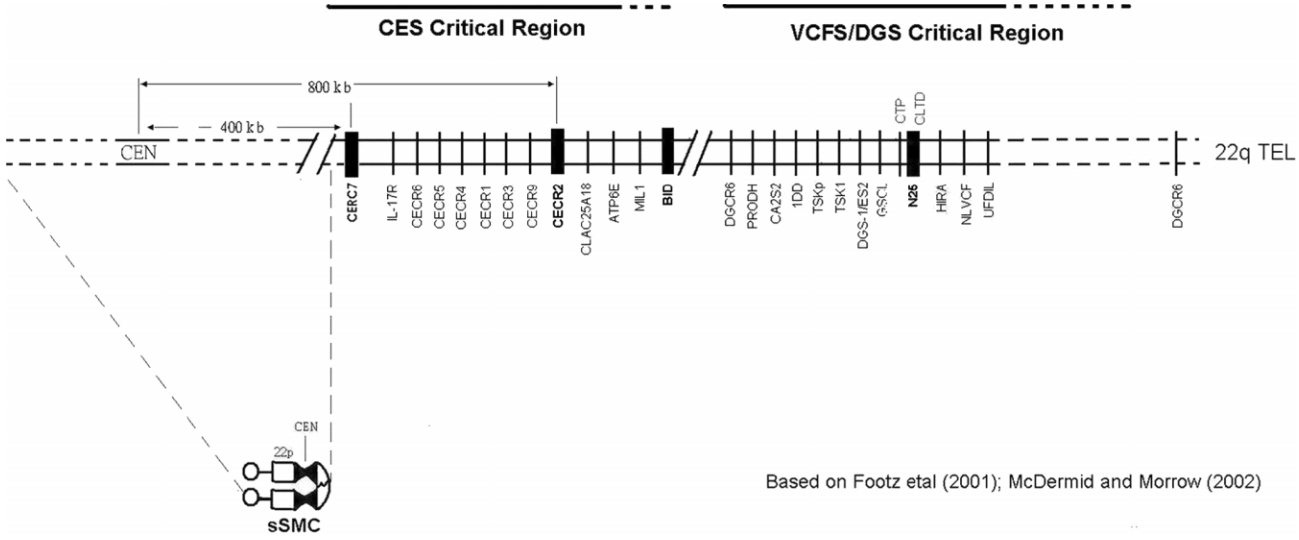

Figure 3—A graphic presentation of the small supernumerary marker chromosome (sSMC) and a map of the CES and VCFS/DGS critical regions in 22q11.1-q11.2. Horizontal lines show relative order of the genes in those regions. Locus-specific probes used in the study are shown by bold horizontal lines. Distances between the CECR2 and the centromere, and CECR7 and the centromere are indicated. Breakpoint of the sSMC could have occurred somewhere in the 22q11.1, <400 kb from the centromere

specific clinical syndrome (Liehr *et al*., 2004). Furthermore, based on the recent studies by Starke *et al*. (2003) and Liehr *et al*. (2004), it is believed that sSMCs without euchromatin and no uniparental disomy (UPD) are harmless, for example, it was reported that a patient with maternal UPD 22 did not have clinical abnormalities (Bartels *et al*., 2003). Duplications in the juxtacentromeric region of 2q, 3p, 4q, 5q, 7p, 8p, 17p, 18p, 19p, 19q and 22q have been shown to be clinically insignificant. At least 22 patients with sSMC(22) derived from chromosome region 22q10-22q11.2 have been documented to be clinically normal (see electronic-database: sSMC derived from chromosome 22 created by Liehr *et al*., 2004). Among those, crossover breakpoints occurred at 22q10-22q11.1 in 17 patients and at 22q11.1-22q11.2 in the remaining 5 patients. sSMCs in most of the patients were due to inverted duplication of a part of chromosome 22, inv dup(22)(q10-q11.2) resulting from crossover errors in meiosis (Schreck *et al*., 1977).

In this study, we were able to map the crossover breakpoint of an sSMC(22) somewhere <400 kb away from the centromere in 22q11.1 by using region specific DNA probes. The breakpoint location is somewhere between interval 1 (centromere heterochromatin) and interval 2 (the CECR) of 22q as defined by the FISH study reported by Bartsch *et al*. (2005). This sSMC has centromeric/pericentromeric heterochromatin but may contain very minimal amounts of euchromatin in the proximal long arm of chromosome 22. The affected neonate's phenotype appeared to be normal with the exception of type II ASD, which spontaneously closed before the age of two; the infant is growing and developing normally. Thus, the sSMC(22) observed in this study will add another case to the growing list of benign sSMC derived from chromosome 22. We were unable to determine the exact amounts of euchromatin present in the sSMC. Therefore, the possibility that the neonate may develop some mild features of CES cannot be completely ruled out. This risk was mentioned to the parents during prenatal counseling. Parental origin studies of the sSMC and microsatellite analysis to exclude a UPD of the sSMC's sister-chromosome were not performed.

In conclusion, we have presented the prenatal diagnosis of a *de novo* sSMC derived from chromosome 22 in a second-trimester fetus. This sSMC did not contain the region covering the CTP-ClTD genes in the VCFS/DGS critical region and also did not harbour the BID, CECR 2 and CECR 7 loci in the CES critical region. Therefore, the breakpoint must have occurred at the proximal region of 22q11.1 <400 kb from the centromere. The sSMC appeared to have little effect on the fetal phenotype and the neonate continues to develop normally. Molecular cytogenetic analyses enable a more detailed and accurate characterization of the marker, which is helpful in prenatal counseling and in preventing unnecessary termination of pregnancy.

ELECTRONIC-DATABASE INFORMATION

sSMC derived from chromosome 22. http://mit-n.mti.uni-jena.de/~huwww/MOL_ZYTO/sSMAC/22.htm

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