Prenatal Diagnosis and Molecular Cytogenetic Characterization of a Small Supernumerary Marker Chromosome Derived From Chromosome 22

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Small supernumerary marker chromosomes (sSMCs) are defined as structurally abnormal chromosomes that cannot be identified or characterized by conventional banding cytogenetics, and are generally equal in size or smaller than a chromosome 20 [1–3]. sSMCs are present in 0.044% of newborn infants and in 0.075% of prenatal cases [1,3–5]. About 70% of sSMCs arise *de novo* [4], about 70% of sSMCs are derived from acrocentric chromosomes [1,6], and about 70% cases of *de novo* sSMCs have no phenotypic effects [5]. Prenatal diagnosis of sSMCs gives rise to difficulties in genetic counseling, and identification of the nature of the aberrant chromosome requires molecular cytogenetic technologies [5,7–10]. We present our experience of the prenatal diagnosis and molecular cytogenetic characterization of an sSMC derived from chromosome 22 using fluorescence *in situ* hybridization (FISH) and array comparative genomic hybridization (aCGH).

A 42-year-old woman, gravida 2, para 0, underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. The woman was phenotypically normal. She had previously experienced one spontaneous abortion. Amniocentesis revealed an sSMC. The sSMC was C-band positive and nucleolar organizing region-stain positive. Cytogenetic analysis of the parents revealed that the mother carried the same sSMC. The karyotype was 47,XX, +mar mat (Figure 1). FISH using a centromere 14/22-specific α-satellite DNA probe (D14Z1/D22Z1) (cep14/22) (Cytocell, Adderbury, Oxfordshire, UK) and a centromere 22-specific α-satellite DNA probe (p190.22; D22Z4 probe reported by Rocchi et al) [11] revealed that the sSMC was positive for D14Z1/D22Z1 (Figure 2) and positive for two D22Z4 signals (Figure 3). The parents decided to continue the pregnancy. A normal female baby weighing 2,828 g was delivered uneventfully at 39 weeks of gestation. She was developing normally at her 4-year follow-up. aCGH analysis using Oligo HD Scan (CMDX, Irvine, CA, USA) showed no genomic imbalance on the pericentromeric euchromatic region of chromosome 22 (Figure 4). The sSMC was inv dup(22)(q10). The karyotype was 47,XX, +mar .ish der(22) (D14Z1/D22Z1+, D22Z4++) or 47,XX, +inv dup(22)(q10).

This case shows the limitations of the cep14/22 (D14Z1/D22Z1) probe and the usefulness of the D22Z4

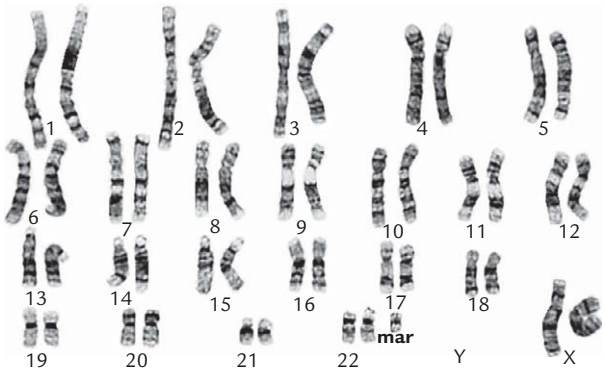

Figure 1. The G-banded karyotype. mar = marker chromosome.

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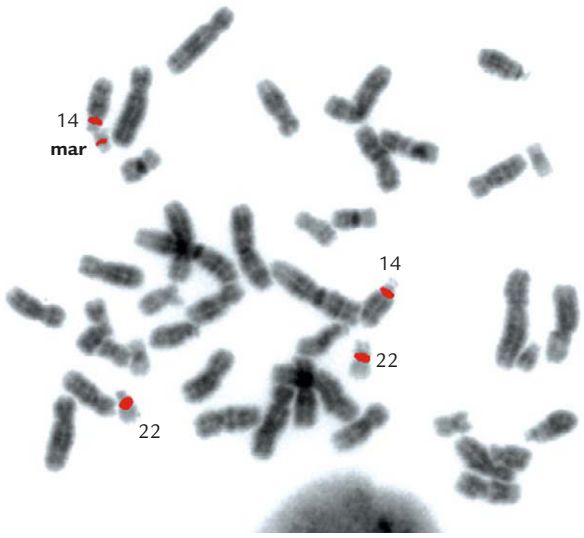

Figure 2. Fluorescence *in situ* hybridization using an α-satellite probe D14Z1/D22Z1, cep14/22 (spectrum red) showing positive hybridization signals on two chromosomes 14, two chromosomes 22 and the marker chromosome (mar).

Figure 3. Fluorescence *in situ* hybridization using an α-satellite probe D22Z4 showing positive hybridization signals on two chromosomes 22 and the marker chromosome (mar). The marker chromosome contains two positive signals for D22Z4.

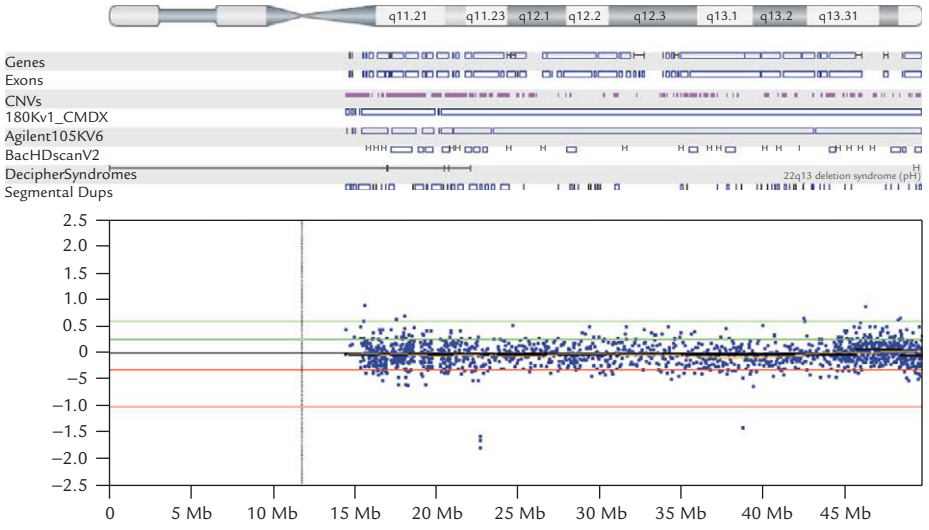

Figure 4. Oligonucleotide-based array comparative genomic hybridization using Oligo HD Scan showing no genomic imbalance in the pericentromeric euchromatic region of chromosome 22.

(p190.22) probe and aCGH in the identification of an sSMC derived from chromosome 22. The cep14/22 probe, along with cep13/21 and cep15, can be used for the rapid identification of an acrocentric chromosome-derived sSMC with positive C-banding and nucleolar organizing region-staining. However, cep14/22 recognizes the centromeres of both chromosomes 14 and 22. In contrast, the alphoid p190.22 (D22Z4) probe specifically recognizes the centromere of chromosome 22 under high stringency hybridization conditions [11], and is therefore useful for differentiating between chromosomes 22 and 14 when the sSMC is hybridized with cep14/22 (D14Z1/D22Z1). Other chromosome 22 centromere-specific probes include p22/1:2.1 (D22Z2) [12] and D22Z3 [13,14]. aCGH has the ability to detect DNA dosage imbalances, including deletions

and duplications, in the pericentromeric euchromatic regions and is useful for characterizing the genomic imbalance in the sSMC. Multiplex ligation-dependent probe amplification (MLPA) is commercially available for rapid aneuploidy diagnosis. The SALSA MLPA P181 and P182 centromere kits contain one probe for the short arm and one probe for the long arm of chromosomes other than acrocentric chromosomes, and two probes for the long arm of chromosomes 13, 14, 15, 21 and 22. Each probe is located close to the centromere of a specific chromosome. The designated regions of chromosome 22 are *CECR5* and *CECR1* at 22q11.1 for the P181 centromere kit, and the designated regions of chromosome 22 are *CECR1* at 22q11.1 and *SLC25A18* at 22q11.21 for the P182 centromere kit [15]. A high-definition MLPA (MLPA-HD) 22q11 kit has recently been developed to detect copy-number changes at 37 loci encompassing a 3-Mb region on 22q11, including the critical region for DiGeorge syndrome/velocardiofacial syndrome (DGS/VCFS), cat eye syndrome (CES) and commonly deleted distal regions [16].

About 9% of SMCs are derived from chromosome 22 [17]. The 22q11 region is susceptible to chromosomal rearrangements leading to DGS/VCFS, CES and t(11;22)der(22) syndrome, all three of which have breakpoint regions harboring a similar low-copy repeat (LCR) known as LCR22 [18,19]. Homologous recombination events between LCR22s during meiosis have been implicated in DGS/VCFS and CES, and the sites of chromosome breakage on 11q23 and 22q11 in der(22) syndrome occur in the unstable AT-rich palindromic sequences leading to nonhomologous recombination mechanisms [19–22]. At least 61 patients with an sSMC(22) derived from inv dup(22)(q10)-(q11.21) or min(22)(pter-:p11.2 → q11.1:-q11.21:) have been documented to date, with no clinical findings [23]. However, at least 106 patients with an sSMC(22) derived from inv dup(22) or inv dup(22)(q11.21)-(q11.23) have been documented with CES or clinical abnormalities [23]. CES (OMIM 115470) is usually associated with an sSMC(22) presenting as inv dup(22)(q11) and has a highly variable phenotype, including coloboma of the iris, anal atresia with fistula, down-slanting palpebral fissures, preauricular tags and/or pits, mild hypertelorism, cardiac defects, renal malformation, normal or near-normal mental development in 44% of patients, mild or moderate mental retardation in 48% of patients and severe mental retardation in 7% of patients [24–26]. Prenatal diagnosis of sSMC(22) has remained a diagnostic challenge and should alert clinicians to the possibility of CES with the involvement of trisomy or tetrasomy of the CES chromosome region candidate genes, such as *CECR1-CECR9* at 22q11 [19,26–30].

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