Prenatal Diagnosis and Molecular Cytogenetic Characterization of a Small Supernumerary Marker Chromosome Derived From Chromosome 21

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Small supernumerary marker chromosomes (sSMCs) are small supernumerary aberrant chromosomes that are generally equal in size or smaller than a chromosome 20, and cannot be identified or characterized by conventional cytogenetic banding techniques [1–3]. sSMCs can appear in 0.044% of newborn infants and in 0.075% of prenatal cases [1,3,4]. About 70% of sSMCs are caused by a *de novo* event [5], about 70% of sSMCs are originated from acrocentric chromosomes [1,6], and about 70% of *de novo* sSMCs are associated with no phenotypic effects [4]. Prenatal diagnosis of sSMCs gives rise to difficulties in genetic counseling, and identification of the nature of the aberrant chromosome requires molecular cytogenetic technologies [4,7–10]. We present our experience of the prenatal diagnosis and molecular cytogenetic characterization of an sSMC derived from chromosome 21 using fluorescence *in situ* hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA).

A 36-year-old woman, gravida 2, para 1, underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Amniocentesis revealed an sSMC, which was C-band positive and nucleolar organizing region-stain positive. The parental karyotypes were normal. The karyotype was 47,XX,+mar (Figure 1).

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The sSMC hybridized with a centromere 13/21-specific α-satellite DNA probe (D13Z1/D21Z1) (cep13/21) (Cytocell, Adderbury, Oxfordshire, UK) (Figure 2). MLPA was used to determine the origin of the sSMC using a SALSA MLPA P181 centromere kit (MRC-Holland, Amsterdam, the Netherlands) (Figure 3). The results of MLPA indicated a duplication of the 21q11.2 segment containing the *STCH* and *SAMSN1* genes. FISH determination of the duplications of the *STCH* and *SAMSN1* genes was performed using the bacterial artificial chromosome clone probes RP11-138O15 and RP11-392H8 at 21q11.2. FISH determination of 21q21.1 involvement utilized the 21q21.1-specific bacterial artificial chromosome clone probes RP11-89M24 and RP11-109H14. FISH revealed *STCH* and *SAMSN1* gene duplications in the fetus with sSMC(21) (Figure 4). No RP11-89M24- or

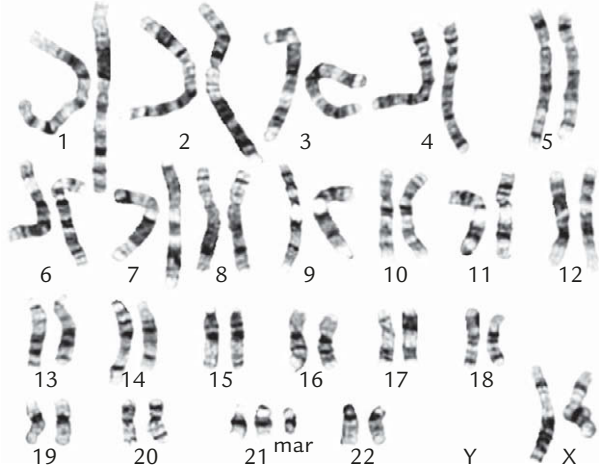

Figure 1. The G-banded karyotype. mar = marker chromosome.

RP11-109H14-positive hybridization signals were detected on the sSMC (Figure 5). The karyotype was 47,XX,+mar .ish der(21)(D13Z1/D21Z1+,RP11-138O15+,RP11-392H8+,RP11-89M24-,RP11-109H14-).mlpa 21q11.2(P181)×3, or 47,XX,+min(21)(pter→q11.2:). Level II ultrasound findings were unremarkable, but the parents elected to terminate the pregnancy.

This case shows the limitations of the cep13/21 (D13Z1/D21Z1) probe and the usefulness of the MLPA centromere kit for the identification of an sSMC derived from chromosome 21. The cep13/21 probe, along with cep14/22 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, cep13/21 recognizes the centromeres of both chromosomes 13 and 21. MLPA was first described by Schouten et al [11]. It is a molecular method suitable for detecting gene dosage abnormalities in a wide range of conditions using relative quantification of multiple DNA target sequences in one polymerase chain reaction. It requires an input of 20 ng or more of DNA, but no living cells or cell cultures. It can be automated and can produce results in as little as 30 hours [12]. MLPA involves the amplification and quantification of the probes added to the samples [13]. An MLPA kit for rapid aneuploidy diagnosis is commercially available. The SALSA MLPA P181 centromere kit (MRC-Holland, Amsterdam, the Netherlands) contains 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 (MRC-Holland). Each probe is close to the centromere of a specific chromosome. The P181 centromere kit contains 46 different probes and is designed to detect deletions/duplications of the designated pericentromeric regions of each chromosome.

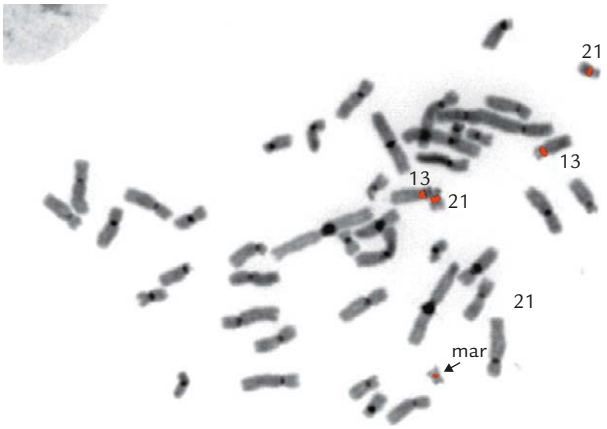

Figure 2. Fluorescence *in situ* hybridization using an α-satellite probe D13Z1/D21Z1 (cep13/21) (spectrum red) showing positive hybridization signals on two chromosomes 13, two chromosomes 21 and the marker chromosome (mar).

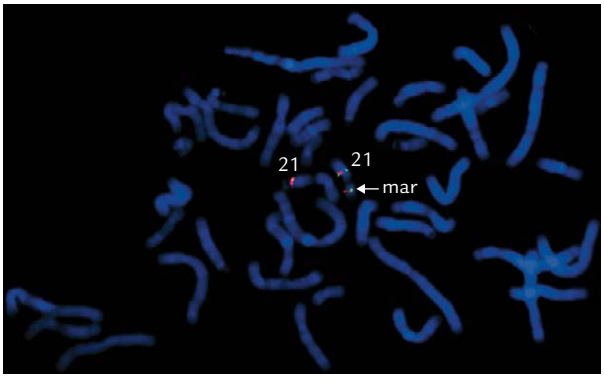

Figure 4. Fluorescence *in situ* hybridization using 21q11.2-specific bacterial artificial chromosome clone probes RP11-138O15 (spectrum green) encompassing the *STCH* gene and RP11-392H8 (spectrum red) encompassing the *SAMSN1* gene showing positive hybridization signals on two chromosomes 21 and the marker chromosome (mar), indicating duplications of the *STCH* and *SAMSN1* genes.

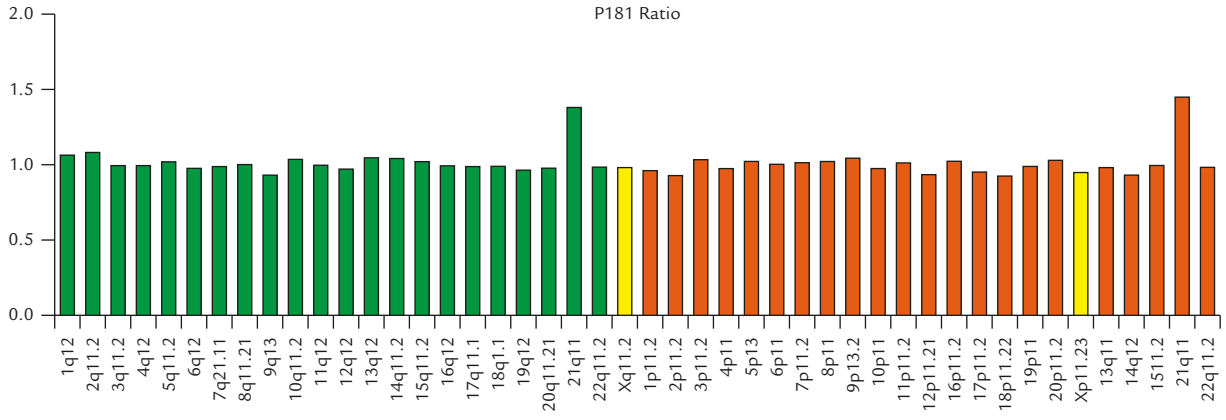

Figure 3. Multiplex ligation-dependent probe amplification using a SALSA MLPA P181 centromere kit showing three copies of two targets on chromosome 21q11.2, consistent with the diagnosis of marker chromosome 21 and a duplication of 21q11.2.

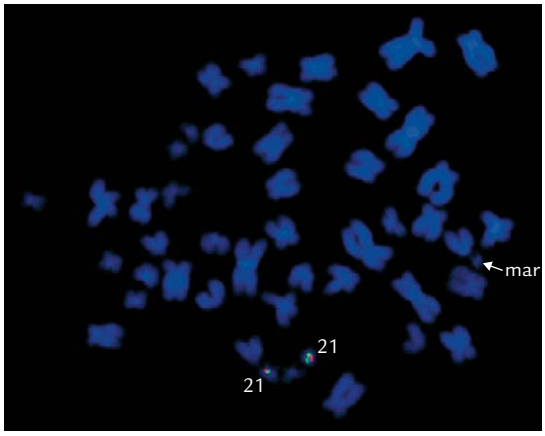

Figure 5. Fluorescence *in situ* hybridization study using 21q21.1-specific bacterial artificial chromosome clone probes RP11-89M24 (spectrum green) and RP11-109H14 (spectrum red) showing positive hybridization signals on two chromosomes 21 and no signals on the marker chromosome (mar).

For chromosome 21, the designated regions are *STCH* and *SAMSN1* at 21q11.2. This MLPA centromere kit is especially useful for rapidly identifying the chromosome origin of an sSMC. In addition to MLPA, array comparative genomic hybridization (aCGH) is able to detect DNA dosage imbalances, including deletions and duplications, in the pericentromeric euchromatic regions and is useful for the characterization of the genomic imbalance in sSMC.

About 13% of SMCs are derived from chromosomes 13 and 21 [14]. In a study of 137 patients with SMCs, Crolla et al [14] found that 59.1% (81/137) were mosaic and 40.9% (56/137) were non-mosaic. One hundred and nine cases had known parental origins, 69.7% (76/109) were *de novo*, 19.3% (21/109) were maternally inherited, and 11.0% (12/109) were paternally inherited. Among 112 SMCs with FISH results, Crolla et al [14] found that 34.8% (39/112) were SMC(15), 12.5% (14/112) were SMC(13/21), 11.6% (13/112) were SMC(14), and 8.9% (10/112) were SMC(22). The current case had a duplication encompassing only the 21q11.2 region, with no involvement of the Down syndrome critical region. The Down syndrome critical region has been designated as 21q22→qter and is hypothesized to harbor the most influential genes, of which extra copies are responsible for most of the features of Down syndrome [15,16].

To date, at least eight patients with an sSMC(21) derived from inv dup(21)(q10)-(q11.2), min(21)(pter-:p11.1→q11.2:-q21.1:), der(21)t(21;22)(q11.1;p11.2) or r(21)(::p11.2→q21.1::) have been documented, with no clinical findings [17]. However, at least 12 patients with an sSMC(21) derived from inv dup(21)(q21.1)-(q21.2), inv dup(21)(p10), or min(21)(pter→q11.2:-q11.2~21.1:-q21.1:-q22.1:-q22.13:) have been documented with clinical findings [17]. Duplication of 21q11.2 and/or 21q21.1 can be associated with phenotypic abnormalities. Baldwin et al [18] reported an SMC(21) with 50% mosaicism for 1.6 Mb pericentromeric constitutional euchromatin encompassing 6–10 genes at 21p→q11.2 in a postnatal case with fine motor skill difficulty, mild dysmorphic features, and a small penis. Liehr [17] reported a 62-year-old man who had 25% mosaicism for min(21)(pter→q11.2~21.1:) with psychomotor retardation, severe developmental delay, no sexual development, short stature, microcephaly, brachycephaly, a large nose and behavioral problems. Liehr [17] also reported a newborn boy with 33% mosaicism for min(21)(pter→q11.2~21.1:) with scoliosis, fine motor skill delay, eating problems, and a depressed nasal bridge, and a 2-year-old boy with an SMC(21) of der(21) (pter→q11.1::q11.1→p11.1 or p11.1→q11.1::p12→q21.1:) and developmental delay. Prenatal diagnosis of sSMC(21) has remained a diagnostic challenge and should alert clinicians to the possibility of Down syndrome with the involvement of gene dosage increases in the Down syndrome critical region.

Acknowledgments

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