Prenatal Diagnosis and Molecular Cytogenetic Characterization of a Small Supernumerary Marker Chromosome Derived From Chromosome 8

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SUMMARY

Objective: To present prenatal diagnosis and molecular cytogenetic characterization of a small supernumerary marker chromosome (sSMC) derived from chromosome 8 by multiplex ligation-dependent probe amplification (MLPA), fluorescence *in situ* hybridization (FISH), spectral karyotyping (SKY) and array comparative genomic hybridization (aCGH).

Case Report: A 42-year-old woman, gravida 6, para 3, underwent amniocentesis at 19 gestational weeks because of advanced maternal age. Amniocentesis revealed a *de novo* ring-shaped sSMC in all 13 colonies of the amniocytes. The karyotype was 47,XY,+mar. The MLPA showed duplications of 8p11.21-specific probes. At 24 gestational weeks, level II ultrasound revealed a left multicystic kidney in the fetus. Other internal organs were unremarkable. Repeat amniocentesis revealed a karyotype of 47,XY,+mar[25]/46,XY[2]. The sSMC was characterized by SKY and FISH, which showed a chromosome 8 origin of the sSMC. Oligonucleotide-based aCGH demonstrated a 4.4-Mb duplication of 8p11.21q11.1 [arr cgh 8p11.21q11.1 (42,637,263-47,062,180)×3]. The karyotype was 47,*XY*,+*r*(8)(p11.21q11.1)[25]/46,*XY*[2]. Polymorphic DNA marker analysis revealed no uniparental disomy for chromosome 8. The woman elected to continue the pregnancy and at 34 gestational weeks, a 1,820 g male baby without craniofacial dysmorphism was delivered. At the age of 1 month, the infant was apparently normal except for left multicystic kidney disease and mild ventriculomegaly.

Conclusion: MLPA, SKY and aCGH are helpful in genetic counseling of prenatally detected sSMCs by providing the immediate information on the origin and the genetic contents of the sSMC. [*Taiwan J Obstet Gynecol* 2010; 49(4):500–505]

Key Words: aCGH, chromosome 8, MLPA, prenatal diagnosis, SKY, small supernumerary marker chromosome

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Introduction

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]. The sSMCs are present in 0.044% of newborn infants and in 0.075% of prenatal cases [1,3,4]. Around 70% of sSMCs arise *de novo* [5], and about 70% of sSMCs are derived from acrocentric chromosomes [1,6], and 70% of *de novo* sSMCs have no phenotypic effects [4]. Prenatal diagnosis of sSMCs gives rise to difficulties in genetic counseling, and requires molecular cytogenetic technologies to identify the nature of the aberrant chromosome [4,7–11]. Here we present our experience of prenatal diagnosis and molecular cytogenetic characterization of an sSMC derived from chromosome 8 by multiplex ligation-dependent probe amplification (MLPA), fluorescence *in situ* hybridization (FISH), spectral karyotyping (SKY) and array comparative genomic hybridization (aCGH).

Case Report

A 42-year-old woman, gravida 6, para 3, underwent amniocentesis at 19 gestational weeks because of advanced maternal age. Amniocentesis revealed a ring-shaped sSMC in all 13 colonies of the amniocytes. The karyotype was 47,XY,+mar (Figure 1). The parental karyotypes were normal. MLPA was applied to determine the origin of the sSMC using the SALSA MLPA Kit P181-A2/P182-B1 Centromere (MRC-Holland bv, Amsterdam, the Netherlands; Figure 2). The result of MLPA indicated a duplication of the 8p11.21-specific probe 05722-L05161 containing the *FNTA* gene and the 8p11.21-specific probe 06244-L06278 containing the *HOOK3* gene. No duplication was found in the 8q11.21-specific probe 06239-L05745 (*KIAA0146* gene) and the 8q11.21-specific probe 05757-L05195 (*PRKDC* gene). At 24 gestational weeks, level II ultrasound revealed a left multicystic kidney in the fetus. Other internal organs were unremarkable. Repeat amniocentesis revealed a karyotype of 47,*XY*,+mar[25]/46,*XY*[2]. The sSMC was characterized by SKY using 24-color SKY probes (Applied Spectral Imaging, Carlsbad, CA, USA) and FISH using a WCP8-specific gene probe (Cytocell, Adderbury, Oxfordshire, UK; Figures 3 and 4). SKY and FISH showed a chromosome 8 origin of the sSMC. Oligonucleotide-based aCGH demonstrated a 4.4-Mb duplication of 8p11.21q11.1 [arr cgh 8p11.21q11.1 (42,637,263-47,062,180)×3] (Figure 5). The aCGH results indicated a duplication of 8p from the 8p11.21-specific probe A_16_P18291023 (42,637,263 bp) (NCBI Build 36) to the 8p11.1-specific probe A_16_P18292593 (43,647,122 bp) and a duplication of 8q at the 8q11.1-specific probe A_14_P133856 (47,062,180 bp) [Cyto Scan gene chip (Agilent customer array, Changhua Christian Hospital, Changhua, Taiwan)]. The karyotype was 47,XY,+r(8)(p11.21q11.1)[25]/46,XY[2]. Polymorphic DNA marker analysis of the cultured amniocytes revealed no uniparental disomy for chromosome 8. The woman elected to continue the pregnancy and at 34 gestational weeks, a 1,820-g male baby without craniofacial dysmorphism was delivered by cesarean section because of maternal preeclampsia. At the age of 1 month, the infant had a weight of 2,090 g (<3rd centile) and a height of 43 cm (<3rd centile). He was apparently normal except for left multicystic kidney disease and mild ventriculomegaly.

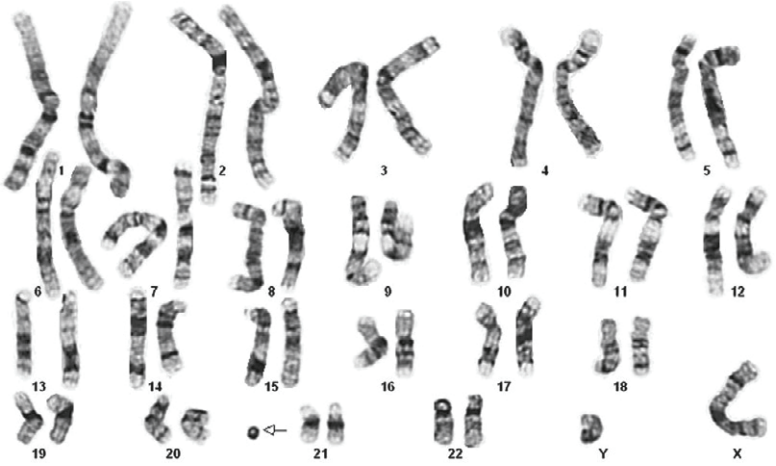

Figure 1. The G-banded karyotype of 47,XY,+r(8)(p11.21q11.1). The arrow indicates a supernumerary marker chromosome (SMC) derived from chromosome 8.

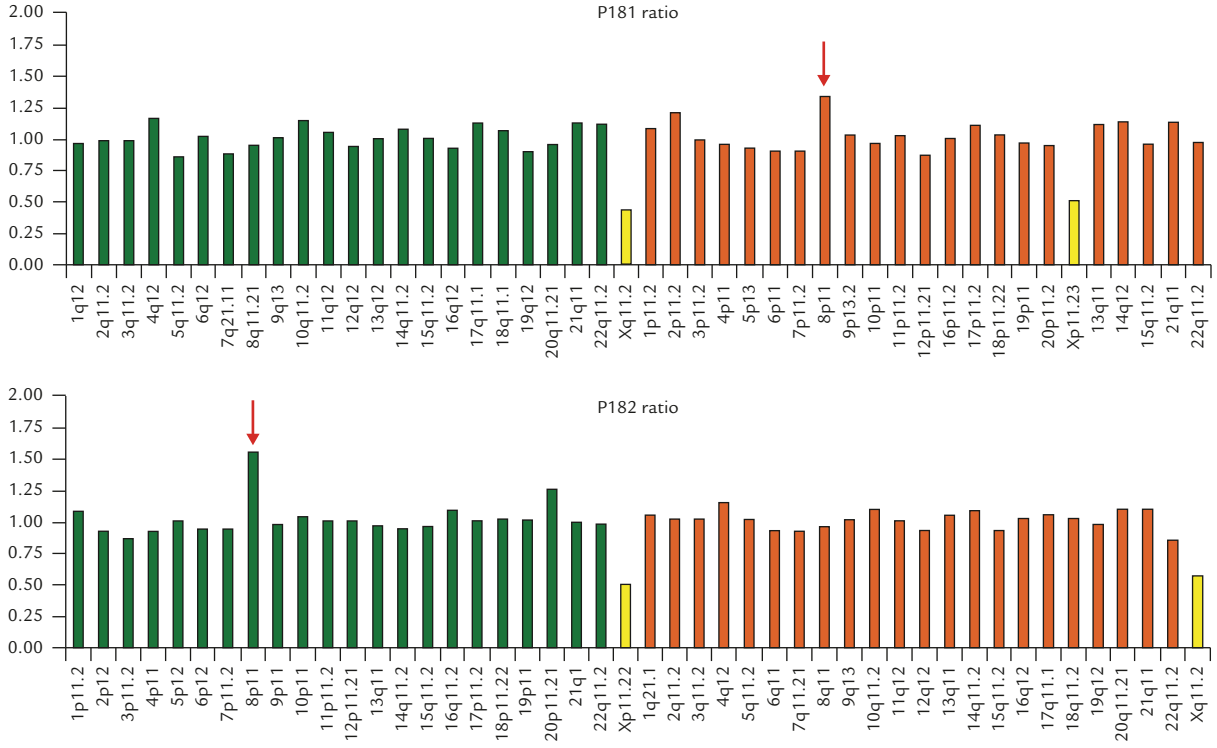

Figure 2. Multiplex ligation-dependent probe amplification shows three copies of two targets on chromosome 8p11 consistent with the diagnosis of marker chromosome 8 and a duplication of 8p11. Arrows indicate the chromosome 8p11 targets.

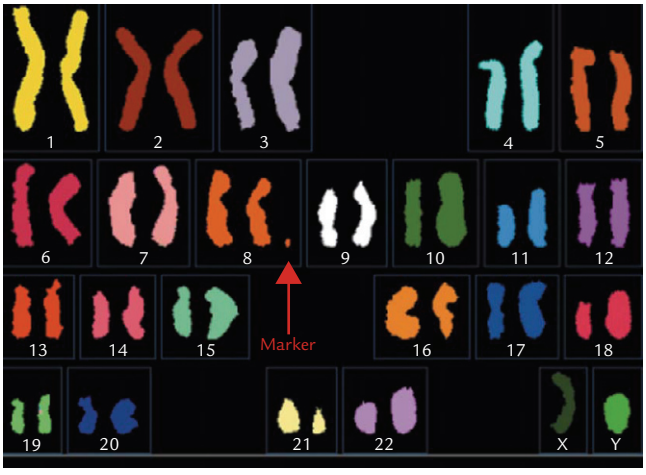

Figure 3. Spectral karyotyping using 24-color sky probe shows an sSMC derived from chromosome 8.

Discussion

Our presentation shows the usefulness of the MLPA Centromere kit, SKY and aCGH in the identification of an sSMC derived from chromosome 8. MLPA, first described by Schouten et al [12], is a molecular method to detect gene dosage abnormalities in a wide range of conditions by relative quantification of multiple DNA target sequences in one polymerase chain reaction with the input of 20 ng or more DNA but without the requirement of living cells or cell cultures. It can be easily automated and results can be obtained within 30 hours [13]. In MLPA, the probes added to the samples are amplified and quantified [14]. The MLPA kit for rapid aneuploidy diagnosis is commercially available. Both the SALSA MLPA P181-A2 and P182-B1 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 SALSA MLPA Kit P181-A2/P182-B1 Centromere is designed to detect deletions/duplications of the designated pericentromeric regions of each chromosome. For chromosome 8, the designated regions are *FNTA* and *HOOK3* at 8p11.21, and *KIAA0146* and *PRKDC* at 8q11.21. This MLPA Centromere kit is especially useful for rapid identification of the chromosome origin of an sSMC. In addition to MLPA and SKY, aCGH has the ability to detect DNA dosage imbalance including deletions and duplications in the pericentromeric euchromatic regions and is useful for the characterization of the genomic imbalance in the sSMC.

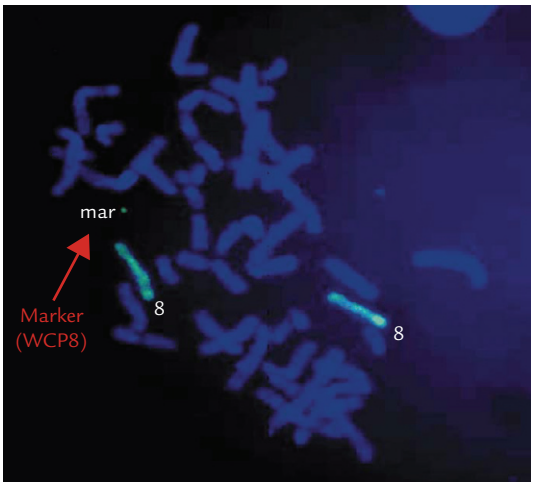

Figure 4. Fluorescence *in situ* hybridization using chromosome 8 whole chromosome painting probe shows a chromosome 8 origin of the small supernumerary marker chromosome. mar=marker chromosome; WCP=whole chromosome pointing probe.

To date, at least 11 patients with an sSMC(8) derived from r(8)(::p23.1→q11::), min(8)(:p11.22~11.21→q11.1:), r(8)(::p11.21→q11.1::), min(8)(:p11.21→q11.1:), r(8)(::p11.21→q11.21::), min(8) (:p11→q11:), min(8)(:p11.1→q11.21:), r(8)(::p11.1→q12.1::), min(8)(:p11→q11.2:) or r(8)(::p10→q12::) have been documented without clinical findings [2,15–22]. However, at least 10 patients with an sSMC(8) derived from r(8)(::p11.22~11.21→q11.1::), min(8)(:p11.21→q11.1:),min(8)(:p11.22~11.21→q11.1:),min(8)(:p11.21~11.22→q10:), dic(8;8)(::p11.21→q11.1::p11.21→q11.1::), r(8)(::q10→ p11.2::p11.2→ q10::) or r(8)(::p11→q11::) have been documented with clinical findings [2,22–27].

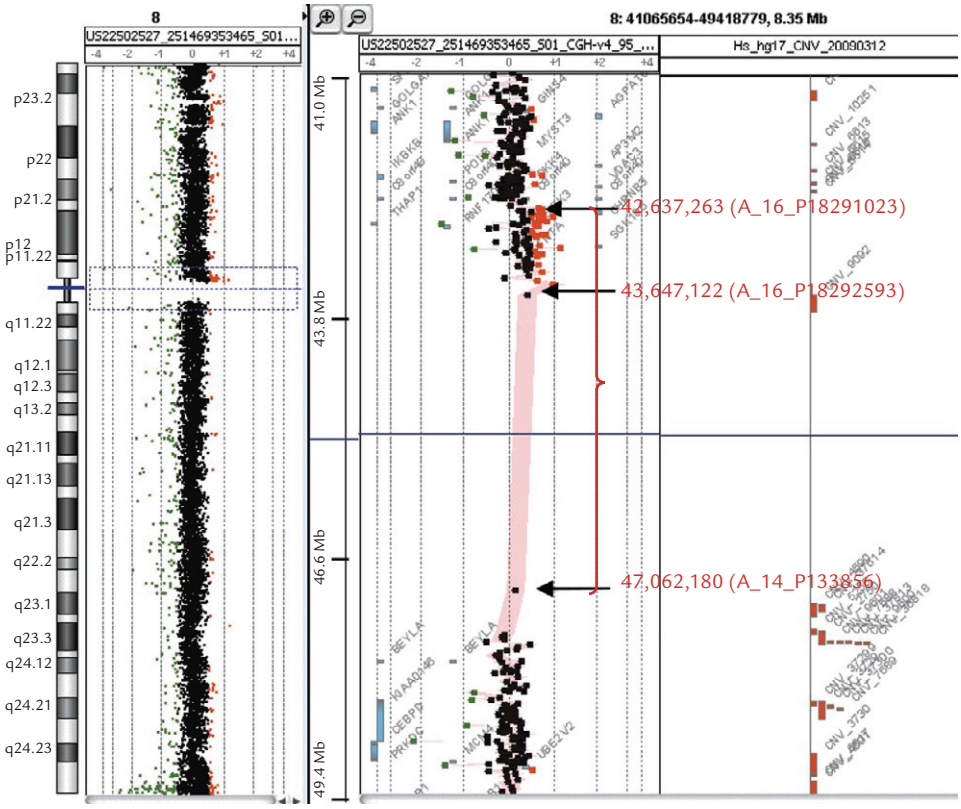

Figure 5. Oligonucleotide-based array comparative genomic hybridization (aCGH) demonstrates a 4.4 Mb duplication in 8p11.21→q11.1 [arr cgh 8p11.21q11.1 (42,637,263-47,062,180)×3]. The aCGH shows a duplication of 8p from the 8p11.21-specific probe A_16_P18291023 (42,637,263 bp) (NCBI Build 36) to the 8p11.1-specific probe A_16_P18292593 (43,647,122 bp) and a duplication of 8q at 8q11.1-specific probe A_14_P133856 (47,062,180 bp).

An SMC(8) with a duplication of p11.21→q11.1 can be associated with clinical findings. Batanian et al [23] reported a 5-year-old boy with 72% mosaicism for min(8)(:p11.21→q11.1:), attention deficit disorder, seizures and developmental delay. Batanian et al [23] also reported an 11-year-old girl with an SMC(8) or min(8)(:p11.22~p11.21→q11.1:), anomalous pulmonary venous return, idiopathic thrombocytopenia, precocious puberty and obesity. Anderlid et al [24] reported a 3-year-old boy with 27% mosaicism for r(8)(p10) *de novo* or r(8)(::p11→q11::), mental retardation and behavior problems. Herry et al [25] reported a 29-year-old man with 76% mosaicism for r(8)(::p11→q11::) and mild intellectual delay but no dysmorphic features. Brecevic et al [26] reported a female infant with 60% mosaicism for r(8)(::p11.21→q11.1::) and global developmental delay. Liehr et al [2] reported a 43-year-old man with 60% mosaicism for dic(8;8)(::p11.21→q11.1::p11.21→q11.1::), developmental delay, severe mental retardation and autistic behavior. Ballif et al [27] reported a patient with an SMC(8) or min(8)(:p11.22~p11.21→q10:) and developmental delay. Ballif et al [27] also reported a patient with 47% mosaicism for r(8)(::q10→p11.2::p11.1→q10:) and developmental delay. Liehr [22] reported a 15-year-old boy with SMC(8) or r(8)(::p11.22~11.21→q11.1::) and atypical autism. Liehr [22] also reported a 3-year-old girl with an SMC(8) or min(8)(:p11.21→q11.1:), developmental and mental retardation, and psychomotor deficiencies.

The present case had a 1-Mb 8p duplication encompassing 8p11.21-p11.1. The duplicated segment contained eight functional genes: *CHRNB3*, *CHRNA6*, *THAP1*, *RNF170*, *HOOK3*, *FNTA*, *HGSNAT* and *A26A1*, and seven hypothetical genes: *FLJ23356*, *LOC100131789*, *LOC643654*, *LOC347028*, *LOC100128173*, *LOC100130474* and *LOC100130767*. The genes *CHRNB3* and *CHRNA6* are associated with nicotinic acetylcholine receptors. Mutations in the *THAP1* gene are related to autosomal dominant torsion dystonia-6. RNF170 is a putative LAG1-interacting ring finger protein. The Golgi-associated HOOK3 protein is a member of the microtubule-binding proteins. The gene *FNTA* encodes the α subunit of CAAX farnesyltransferase and *HGSNAT* encodes heparan-α-glucosaminide N-acetyltransferase, with mutations of *HGSNAT* associated with an autosomal recessive disorder of mucopolysaccharidosis type IIIC. A26A1 or POTE8 is a prostate-, ovary-, testis-expressed protein on chromosome 8. The present case clinically manifested as multicystic kidney disease and ventriculomegaly but no dysmorphic features. An increase of the genes within the duplicated segment in this case has not previously been reported to be associated with multicystic kidney disease or ventriculomegaly.

With the advent of MLPA, SKY and aCGH, *de novo* non-acrocentric sSMCs can be well characterized by molecular cytogenetic technologies. We conclude that MLPA, SKY and aCGH are helpful in genetic counseling of prenatally detected sSMCs by providing immediate information on the origin and genetic contents of the sSMC.

Acknowledgments

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