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Supernumerary Marker Chromosomes Derived From Chromosome 6: Cytogenetic, Molecular Cytogenetic and Array CGH Characterization

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Abstract

Supernumerary marker chromosomes (SMC) are relatively common in prenatal diagnosis. As the clinical outcomes vary greatly, a better understanding of the karyotype-phenotype correlation for different SMCs will be important for genetic counseling. We present two cases of prenatally detected *de novo*, small SMCs. The markers were present in 80% of amniocyte colonies in Case 1 and 38% of the colonies in Case 2. The SMCs were determined to be derived from chromosome 6 during postnatal confirmation studies. Although the sizes and the chromosomal origin of the SMCs in these two cases appeared to be similar, the clinical outcomes varied. The clinical manifestations observed in Case 1 included small for gestational age, feeding difficulty at birth, hydronephrosis, deviated septum and dysmorphic features, while the phenotype is apparently normal in Case 2. Array comparative genomic hybridization (CGH) was performed and showed increase in dosage for approximately 26 Mb of genetic material from the proximal short and long arms of chromosome 6 in Case 1. Results of array CGH were uninformative in Case 2, either due to mosaicism or lack of detectable euchromatin. The difference in the clinical presentation in these two patients may have resulted from the difference in the actual gene contents of the marker chromosomes and/or the differential distribution of the mosaicism.

Keywords

marker chromosome; array CGH; chromosome 6; FISH

INTRODUCTION

Supernumerary marker chromosomes (SMC) are frequently encountered during prenatal diagnosis, occurring in 0.8–1.5 per thousand pregnancies [Bartsch et al., 2005; Benn and Hsu, 1984; Blennow et al., 1994; Brondum-Nielsen and Mikkelsen, 1995; Hook and Cross, 1987; Sachs et al., 1987]. Fluorescence in situ hybridization (FISH) based identification of

SMC shows approximately 50% to be derived from chromosome 15, for which there are well established karyotype-phenotype correlations [Blennow et al, 1994; Crolla et al., 1995]. The remaining SMC generally have poor karyotype-phenotype correlation, with phenotypes ranging from normal to dysmorphic features and/or developmental delay [Crolla, 1998]. This variability is a function of the euchromatin content of the SMC, the level of mosaicism and tissue distribution of the SMC. More recently, the application of pericentromeric FISH probe and array CGH have resulted in improved characterization of SMC and emerging genotype-phenotype correlations [Ballif et al., 2007; Liehr et al., 2006a].

The identification of a SMC at prenatal diagnosis is challenging for genetic counseling, as little or no accurate clinical or prognostic information can typically be provided for many SMC. Therefore, investigation of a large number of SMC cases and their genetic content is important to gain a better understanding of karyotype-phenotype correlations. In this study, we present cytogenetic, fluorescence *in situ* hybridization, array CGH analysis and clinical findings of two cases of SMC derived from chromosome 6.

CLINICAL REPORTS AND CYTOGENETIC ANALYSIS

Case 1—Amniocentesis was performed on a 26-year-old G3P2 woman due to a positive maternal serum screen in the second trimester indicating an increased risk for neural tube defects. Cytogenetic analysis of amniocyte and peripheral blood lymphocyte cultures, and GTG banding were performed following standard methods. Chromosome analysis of amniocytes showed a small SMC (Fig 1a) in 12/15 colonies examined: 47,XY,+mar[12]/46,XY[3]. The amniotic fluid AFP level was normal. Chromosome analysis on both phenotypically normal parents showed normal karyotypes in 50 cells examined, although low level mosaicism in the parents cannot be completely ruled out. After genetic counseling the parents elected to continue the pregnancy. The patient experienced preterm labor at 29 weeks gestation and was on bed rest for the remaining term of the pregnancy. The baby was born at 40 weeks of gestation based on ultrasound dating. The baby appears full term. However, his birth weight was 1.93 kg (<5th centile) and birth height was 48.26 cm (<5th centile). At 19 days of age, the infant was found to be symmetrically small for his age (<<5% for height, weight and head circumference). Chromosome analysis from peripheral blood confirmed the presence of the SMC: 47,XY,+mar[15]/46,XY[5]. He had hypotonia and feeding difficulties during the first 2 years of age. At 4 years of age, he had developmental delay, speech delay, epilepsy, mild dysmorphic features, hydronephrosis and vesico-urethral junction obstruction that required multiple surgeries.

Case 2—A 35-year-old G1P0 woman was referred for genetic counseling and amniocentesis due to advanced maternal age and a positive maternal serum screen in the second trimester indicating an increased risk for Down syndrome. Chromosome analysis of amniocytes showed a small SMC (Fig 2a) in 6/16 colonies examined: 47,XX,+mar[6]/46,XX[10]. The amniotic fluid AFP level was normal and both phenotypically normal parents had normal karyotypes in 50 cells examined, although low level mosaicism cannot be completely ruled out. The pregnancy was continued and the baby was delivered at full term without complications. At 2 years of age, the child was reported to be phenotypically and developmentally normal. Chromosome analysis from peripheral blood confirmed the presence of the SMC: 47,XX,+mar[8]/46,XX[12].

Fluorescence *In Situ* Hybridization Studies

FISH was performed postnatally with combinations of SpectrumAqua[™], SpectrumGreen[™] and SpectrumOrange[™]-labeled centromere probes (Vysis), according to the manufacturer's instructions. Both SMC showed signal with the D6Z1 probe (specific for the centromere

region of chromosome 6), indicating a chromosome 6 origin (Figs 1b, 2b). However, the D6Z1 probe did not hybridize to the entire SMC in both cases, indicating the presence of euchromatic material on the SMCs. Additional FISH analysis with a whole chromosome paint probe for chromosome 6 (Cambio, Cambridge, UK) was performed according to the manufacturer's instructions. In both cases, hybridization signals were observed over the entire SMC, confirming that the SMCs were derived from chromosome 6 (Figs 1c, 2c). Hybridization with the whole chromosome painting probe suggests that the SMCs contain euchromatic material.

Array CGH Analysis

Array CGH analysis was performed using DNA from the postnatal blood specimens using a microarray consisting of 2464 BAC, PAC and P1 clones printed in triplicate (HumArray 2.0) as previously described [Rauen et al., 2002; Snijders et al., 2001].

Array CGH analysis of Case 1 showed a gain of 20 contiguous clones CTD-2118F18 (6p21.2) to RP11-164C22 (6q11.2) [genomic coordinates: 36,702,000–62,577,198; Human March 2006 assembly (hg18, NCBI build 36)], approximately 26 Mb, and also a gain of clone RP11-43B19 at 6q26 (Fig. 3). Clone RP11-43B19 has not been reported to be a polymorphic clone (Database of Genomic Variants; <http://projects.tcag.ca/variation/>). However, it does substantially overlap with clone CTD-2310B5, which does show copy number variation in normal individuals [Wong et al., 2007]. Thus, the gain shown in clone RP11-43B19 is most likely a polymorphic copy number variant. No parental samples were available for array CGH analysis.

Array CGH showed no copy number changes of chromosome 6 in Case 2 (data not shown), either due to a lower level of mosaicism of the SMC(6) [i.e. below the level of detection], or that the SMC(6) contained no detectable euchromatin. The clones on the array flanking the centromere were RP3-445N2 (6p12.1) and RP11-164C22 (6q11.1). Thus a small SMC within this region (a maximum predicted size of 5.9 MB) would not be detected by the array.

DISCUSSION

We describe two cases of prenatally ascertained supernumerary marker chromosomes derived from chromosome 6 [SMC(6)]. Prenatally ascertained SMCs derived from chromosome 6 are rare with only nine cases reported. Seven of these cases are summarized in Table I. We have excluded from Table I the prenatal case reported by Chen et al. [2006] in which the proband had multiple SMCs including an SMC(6), since the phenotype is likely confused by the presence of other abnormalities. Of interest, Liehr et al. [2006b] noted that SMC derived from chromosome 6 are over-represented in cases with multiple SMCs (33%), although the significance of this observation is unknown. The prenatal SMC(6) case of Sala et al. [2005] was also excluded as it was a neocentromere-containing SMC derived from distal 6q, 6q26→qter, whereas the majority of SMC(6) contain variable amounts of pericentromeric chromosome 6 material (Table I). We have included the case reported by Crolla et al. [1998] (case 6 in Table I) as it reports prenatal findings despite a postnatal ascertainment.

Comparison of the prenatally diagnosed cases of SMC(6) to date shows some karyotype-phenotype correlation (Table I). Patients fall into three broad categories; no detectable euchromatin with a normal phenotype, detectable euchromatin with a normal phenotype and detectable euchromatin with an abnormal phenotype. Where the SMC(6) were characterized for euchromatic content, the genotype-phenotype comparisons summarized in Table I are consistent with observations from a larger series of postnatal SMC(6) cases reported by

Liehr et al. [2006a]; the absence of euchromatin or only small amounts of euchromatin from the centromere to distal 6p12.1 or proximal 6q12 is associated with a normal phenotype, whereas abnormal phenotypes and/or development delay are associated with euchromatin content extending beyond these regions Liehr et al. <http://www.med.uni-jena.de/fish/sSMC/06.htm#Start06>. Three cases in Table I (cases 3–5) fall into the normal phenotype categories with no or minimum euchromatin within the region of distal 6p12.1 to proximal 6q12. Two of these reports [Bartsch et al., 2005; Liehr et al., 2006a] described small SMC(6) with no detectable euchromatic content with FISH. Both pregnancies went to term and resulted in normal infants. Phenotype and development were normal in both patients at the time of follow up. These cases are consistent with the observation that minute SMCs with no detectable euchromatin have a low risk of phenotypic abnormality [Cotter et al., 2005]. The 3rd patient reported by Leite et al., [2006] with the SMC(6) containing 6cen→6p12 had a normal phenotype and is also consistent with the genotype-phenotype predictions mentioned above [Liehr *et al*, 2006a].

Concomitantly, the presence of a large amount of euchromatin extending beyond distal 6p12.1 and proximal 6q12 is associated with an abnormal phenotype. One of the previously reported cases (Table I, case 6) and case 1 of the current study fall into this category. The SMCs in Case 1 of the current study and the case reported by Crolla [1998] contained the proximal short arm region of chromosome 6 and had abnormal phenotypes. Therefore, trisomy for the short arm of chromosome 6 for at least 25 Mb (6cen to CTD-2118F18) appears to be associated with an abnormal phenotype. One of these cases [Crolla, 1998] had some of the features associated with uniparental disomy of the normal chromosome 6 homologs. This SMC(6) was maternal in origin and UPD studies showed the patient to also have paternal UPD6. The patient did have transient neonatal diabetes which is associated with patUPD6. However, the presence of extra euchromatin in the marker chromosome most likely contributes to the other abnormal manifestations.

The remaining two cases of SMC(6) with uncharacterized euchromatic content presented with normal phenotypes (Table I, cases 2 and 7). Case 2, presented here, had a normal phenotype, possibly due to the lower level of mosaicism of the SMC(6), or perhaps less euchromatin present that was not detected by the array. Array CGH has been shown to identify mosaicism down to 20% in some cases [Ballif et al., 2006]. In this case, cultured lymphocytes showed 40% of metaphases with the SMC. However, whether this percentage is representative of all nucleated cells from which DNA was extracted is unknown. The case described by Hastings et al. [1999] was clinically and developmentally normal at 5 months. The SMC(6) in this fetus was also present in the phenotypically and developmentally normal mother.

Secondary to euchromatin characterization of SMC(6), which is an important predictor of phenotype, there are other variables that might affect the severity of the phenotype in SMC(6) patients. UPD testing may be warranted as a SMC(6) may be the result of a trisomy rescue event secondary to a chromosome 6 non-disjunction. PatUPD6 is associated with transient neonatal diabetes [James et al., 1995], as in the patient reported by Crolla et al., [1998]. Postnatally, UPD6 testing is likely only warranted when transient neonatal diabetes is present. However, UPD6 analysis should be considered in all prenatal SMC(6) cases. Parental karyotype analysis should also be performed to determine whether a SMC is *de novo* or familial, as the presence of the same SMC in a phenotypically and developmentally normal parent makes a normal phenotype in the fetus more likely. However, care should be taken with this approach, as the tissue distribution and percentage of the SMC in the fetus may be different from that in the parent. The tissue distribution of the SMC can also affect the severity of the phenotype. A low or high percentage of cells with a SMC in one tissue may not necessarily correlate with a low or high risk, respectively, of an abnormal

phenotype. It appears that the most accurate predictor of phenotype is the euchromatic content of a SMC(6). Currently, chromosome microarray analysis is the most effective approach to characterize the euchromatic content of a SMC. However, if the percentage of cells with the SMCs is below the threshold of detection of array analysis, as may be the situation for Case 2 presented here, it may be uninformative. Higher density arrays have enriched coverage over the pericentromeric regions, and may have identified euchromatin on the SMC of Case 2. Some commentators have raised concerns over the prenatal use of high-density arrays citing the higher incidence of variants of unknown significance (VOUS) which raise uncertainty of phenotypic outcome [Lichtenbelt et al., 2011]. The use of more targeted arrays with lower incidence of VOUS are recommended in prenatal diagnosis [ACOG Committee on Genetics, 2009]. Alternative strategies, such as the acro-FISH approach and the use of pericentromeric FISH probes, may be more effective for characterization of SMCs with low level mosaicism [Liehr *et al*, 2006a; Starke *et al.*, 2003]. Additionally, enrichment of SMC DNA by microdissection for array CGH has also been reported [Backx et al., 2007].

In conclusion, array CGH is a powerful tool for the characterization of SMC. Together with the use of targeted FISH approaches in cases of low level mosaicism for SMC, it has led to a more accurate genotype-phenotype correlation. For future cases, these tools should be used in all cases of SMC, particularly prenatally ascertained SMC. The presence of mosaicism in a large number of these cases further adds to the complexity in predicting the outcomes. Therefore, the continued investigation of a large number of SMC cases and a better understanding of the genetic content of the SMC is important for improved delineation of karyotype-phenotype correlation.

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Figure 1.

Figure 2.

Figure 3.

Table 1

Reported cases of prenatally diagnosed supernumerary marker chromosomes derived from chromosome 6.

Cases	Reference	% Abnormal Cells	Inheritance	UPD6	Euchromatin Content	Phenotype
1	Present report, Case 1 Huang et al., [2006] Case 56	AF-80% PB-75 %	<i>de novo</i>	NR	6p21.2→6q11.2	small for gestational age, developmental delay, epilepsy, mild dysmorphic face
2	Present report, Case 2 Huang et al., [2006] Case 57	AF-38% PB-40%	<i>de novo</i>	NR	Present, not characterized	Normal at 2 years
3	Bartsch et al., [2005] Case 38	83%	<i>de novo</i>	Biparental	None detected r(6)(p11q11)	No congenital anomalies, normal development at 7 months.
4	Liehr et al., [2006a] Case 6-2	84%	<i>de novo</i>	Biparental	None detected 6p11.1→q11.1	Normal child born
5	Leite et al., [2006] Case 2	AF-74% CB-87%	<i>de novo</i>	NR	6p12→q11.1	Normal at 4 years
6	Crolla et al., [1998] Case 5	74%	<i>de novo</i>	patUPD6	6p21.2→6cen	Intrauterine growth retardation, mild developmental delay, epicanthal folds, thick upper lip and prominent cheeks; transient neonatal diabetes
7	Hastings et al., [1999] Case 11	mosaic, % NR	maternal	NR	Present, not characterized	Clinically and developmentally normal at 5 months. No transient neonatal diabetes. Mother normal.

NR – not reported/not determined; AF – amniocytes; CB – cord blood; PB – peripheral blood.