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Identification of Autosomal Supernumerary Chromosome Markers (SMCs) by Fluorescent *In Situ* Hybridization (FISH)

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Abstract. *Supernumerary marker chromosomes (SMCs) are rare chromosomal abnormalities resulting in partial trisomy of specific genomic regions with characteristic phenotypic effects. Twenty six cases with autosomal SMCs are reported. Four were identified prenatally and 22 postnatally in children, aged from 8 days to 15 years, who were referred for genetic evaluation because of various congenital anomalies and developmental delay. In 22 of the 26 cases, the SMCs were de novo, in two they were familial and in another two a 11;22 reciprocal translocation was revealed in the mothers. In only one patient was the SMC present in a mosaic form. Sequential fluorescent in situ hybridization studies (FISH) using Whole Chromosome Paint (WCP) probes were performed in order to determine the chromosomal origin of the SMCs. Sixteen of them originated from chromosome 15, five were shown to be an isochromosome 18p and one was derived from chromosome 22, but did not contain the DiGeorge/ VCFS critical region. In two instances, the SMCs were derivatives of chromosome 13 and in two the SMCs resulted from a 11;22 maternal translocation and contained material from both chromosomes 11 and 22. Molecular investigation of two of the patients with an SMC[15] revealed three copies of the SNRPN gene, but the diagnosis of PW/AS due to possible imprinting was excluded in both patients by a methylation-specific PCR. FISH and molecular studies have greatly facilitated the characterization of marker chromosomes. As more SMCs are classified, better genetic counseling and risk evaluation can be achieved.*

In human cytogenetics, supernumerary marker chromosomes (SMCs) are often identified, although they sometimes replace a chromosome in an euploid karyotype. Their occurrence is estimated at 0.3 per 1,000 live births (1). They are of diverse origin and may prove to be isochromosomes, inverted duplications, composite chromosomes resulting from reciprocal translocations, derivative chromosomes with neocentromeres, stabilized deleted chromosomes and rings derived from a single centromere with euchromatin from one or both arms of the original chromosome (1, 2).

Individuals carrying SMCs are usually ascertained during routine karyotyping because of abnormal features and/or developmental delay.The degree of mosaicism is a critical element in the development of the phenotype. If they are identified during prenatal diagnosis, they present an additional problem, since the phenotype or the prediction of intellectual disability can not be determined (3).

Determination of the chromosomal origin of the SMC is of great importance for the genetic burden of the proband and, because of the varying clinical outcome, the genetic counseling of the families. Approximately half of the SMCs are derivatives of chromosome 15 and, among the remaining cases, great variation in chromosomal origin is observed. According to Crolla, 70% of the SMCs are derived from non-acrocentric chromosomes and are not associated with clinical symptoms (3). Also, the phenotypic effect depends on whether the SMC is *de novo* or inherited from one of the parents.

Identification of the SMC by conventional cytogenetic analysis only is almost impossible. For this reason fluorescent *in situ* hybridization (FISH) is most valuable and has been successfully applied for the determination of the chromosomal origin of supernumerary marker or derivative chromosomes.

In the present study, 26 patients with SMCs, identified by the use of FISH, are reported and their phenotypes described.

Materials and Methods

Twenty six unselected patients (15 girls / 11 boys) with autosomal SMCs were included in the study. Four out of 26 were identified prenatally and 22 postnatally in children aged from 8 days to 15 years, referred for genetic evaluation because of various congenital anomalies, developmental delay or learning difficulties.

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Key Words: FISH, supernumerary marker chromosomes (SMCs).

Table I. *Identification of the 26 SMCs by conventional cytogenetic and FISH analysis.*

	Cytogenetic analysis	Fluorescent *in situ* hybridization (FISH)
1	46,XX[55]/47,XX+mar[45] familial*	ish der(15)(WCP15+)
2	47,XX+mar[100] *de novo*	ish der(15)(WCP15+)
3	47,XY+mar[100] *de novo*	ish der(15)(WCP15+)
4	47,XY+mar[100] *de novo*	ish der(15)(WCP15+)
5	47,XY+mar[100] *de novo*	ish der(15)(WCP15+)
6	47,XX+mar[100] *de novo*	ish der(15)(WCP15+)
7	47,XX+mar[100] *de novo*	ish der(15)(WCP15+)
8	47,XY+mar[100] *de novo*	ish der(15)(WCP15+)
9	47,XX+mar[100] *de novo*	ish der(15)(WCP15+)
10	47,XX+mar[100] *de novo*	ish der(15)(WCP15+),ish(15q11.2q13)(SNRPNx3,15qtelx2)
11	47,XX+mar[100] *de novo*	ish der(15)(wcp15+),ish(15q11.2q13)(SNRPNx2,15qtelx2)
12	47,XX+mar[100] *de novo*	ish der(15)(WCP15+), ish(15q11.2q13)(SNRPNx2,15qtelx2)
13	47,XX+mar[100] *de novo*	ish der(15)(WCP15+), ish(15q11.2q13)(SNRPNx2,15qtelx2)
14	47,XX+mar[100] *de novo*	ish der(15)(WCP15+), ish(15q11.2q13)(SNRPNx3,15qtelx2)
15	47,XY+mar[100] *de novo*	ish der(15)(WCP15+),ish(15q11.2q13)(SNRPNx2,15qtelx2)
16	47,XX+mar[100] familial**	ish der(15)(WCP15+),ish(15q11.2q13)(SNRPNx2,15qtel x 2)
17	47,XY+mar[100] *de novo*	ish der(18)(WCP15+, PCP18p+)
18	47,XY+mar[100] *de novo*	ish der(18)(WCP15+, PCP18p+)
19	47,XY+mar[100] *de novo*	ish der(18)(WCP15+, PCP18p+)
20	47,XY+mar[100] *de novo*	ish der(18)(WCP15+,PCP18p+)
21	47,XX+mar[100] *de novo*	ish der(18)(WCP15+, PCP18p+)
22	47,XY+mar[100] *de novo*	ish der(22)(WCP22+), ish (22q11.2)(TUPLEx2,22qtelx2)
23	47,XX+mar[100] *de novo*	ish der(13)(WCP13+)
24	47,XY+mar[100] *de novo*	ish der(13)(WCP13+)
25	47,XY+mar[100]	ish der(11;22)(WCP11+, WCP22+)
26	47,XX+mar[100]	ish der(11;22)(WCP11+, WCP22+)

*Karyotype of the father: 46,XY[94]/ 47,XY+marker[6].
**Karyotype of the mother: 47,XX+marker[100].

Methods. Cytogenetic and molecular cytogenetic studies were performed on chromosomes derived from peripheral blood lymphocytes or amniotic fluid cells. The chromosome preparations were obtained using standard techniques and analyzed by G-banding. In all cases, the parental karyotypes were also analyzed.

In order to identify the origin of the SMC, sequential FISH studies using Whole Chromosome Paint (WCP) probes were performed according to the manufacturer's instructions (Cambio,UK). The chromosome probes were sequentially tested according to their reported frequency of occurrence, starting with chromosome 15. Seven cases, in which an SMC derived from chromosome 15 was identified, were further analyzed with SNRPN/15qtel gene probes (Q-BIOgene, France) (Table I). Five cases (No. 17-21), whose SMC originated from chromosome 18, were further investigated using a Partial Chromosome Paint probe (PCP) for the short arm of chromosome 18 (kindly provided by Dr M. Rocchi, Barri, Italy). Case No. 22 was tested with the TUPLE/22qtel (Q-BIOgene) region - specific probe.

For the molecular analysis of chromosome 15, in cases 10 and 14 genomic DNA of the patient and the parents was extracted with the Qiamp Blood Mini Kit (Qiagen, Germany). Dinucleotide repeat polymorphism (DNRP) analysis was performed, as previously described (4), with six polymorphic markers within the PWS/AS critical region (D15S543, D15S11, D15S10, D15S113, GABRB3, D15S97) and two polymorphic markers (CYP19, D15S87) near the telomere of chromosome 15, to identify possible uniparental disomy (UPD) in the specific loci. The results were visualized on the Visible Genetics Open Gene™ System (Visible Genetics Corp., Canada).

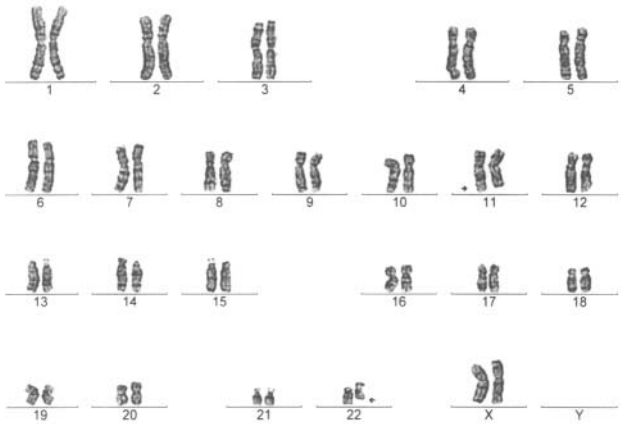

Figure 1. *Karyotype 46,XX, t(11;22) identified in the mother of patient No. 25.*

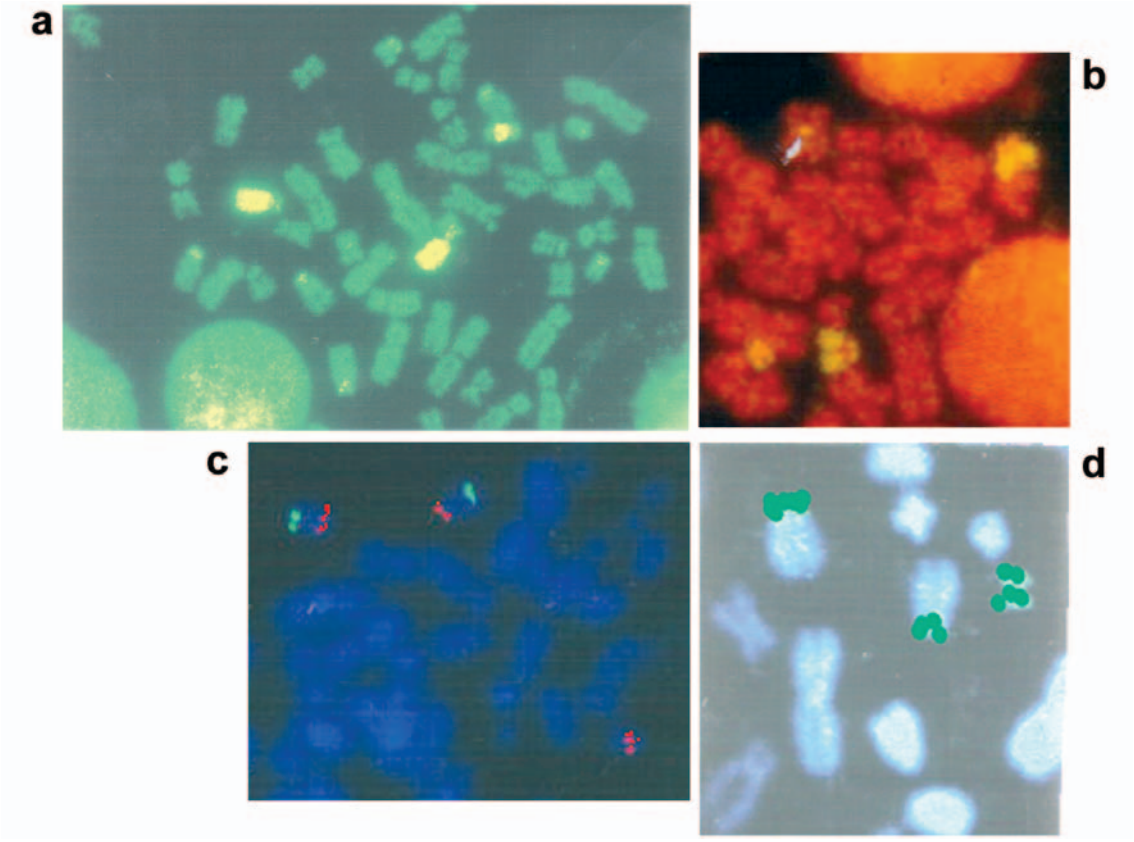

Figure 2. *Identification of the chromosomal origin of the SMC by FISH. (a): SMC[15] identified with WCP 15. (b): SMC[18] identified with WCP 18. (c): FISH analysis in a case with SMC[15] using SNRPN/15qtel probe showing three copies of the SNRPN gene (red signal) and two from the telomere of chromosome 15 (green signal) (case No. 10) (d). Isochromosome 18p identified using PCP for 18p. The whole length of the SMC and the short arms of both chromosomes 18 are painted green.*

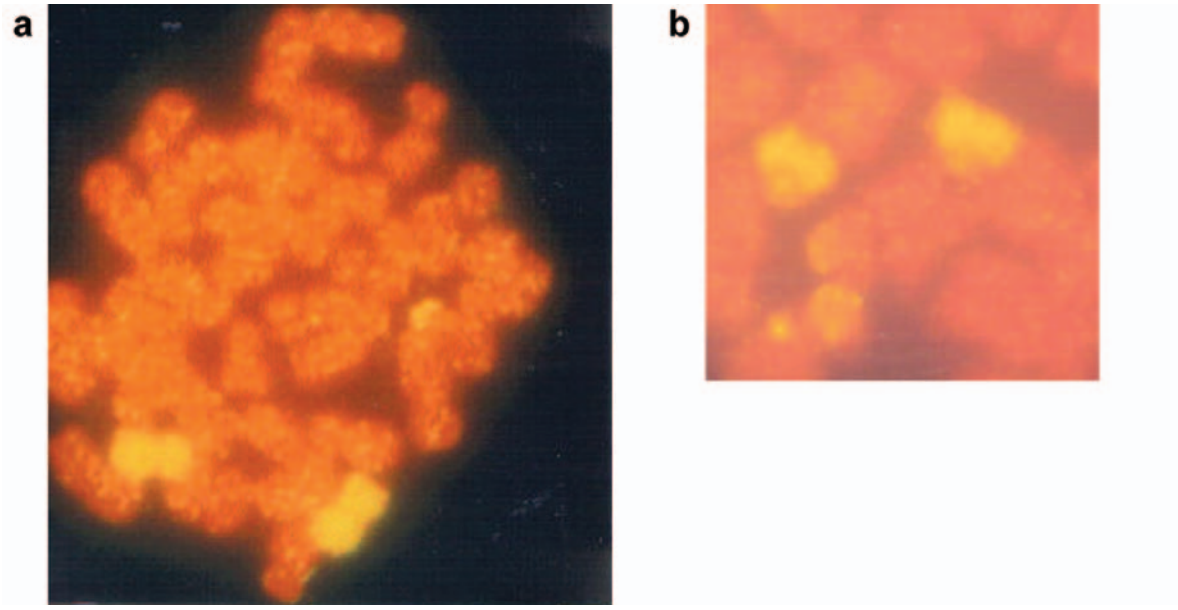

Figure 3. *Identification of SMC der(11;22) by FISH using WCP 11 (Figure a) and WCP 22 (Figure b).*

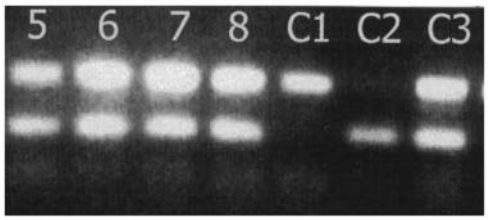

Figure 4. *MSPCR analysis for patients No. 10 (lanes 5 and 6) and 14 (lanes 7 and 8). C1, PWS pattern; C2, AS pattern; and C3, normal pattern.*

Methylation-specific PCR using DNA treated with sodium bisulfate (CpG Genome™ Modification Kit, Q-BIOgene, France) was applied to determine the methylation status of the SNRPN gene in the PWS critical region (5).

Results

Cytogenetic analysis. The cytogenetic and molecular cytogenetic findings of all the cases tested are listed in Table I. The SMC in patient No. 1 was in a mosaic form, while in the remaining 25 patients the SMC was present in all the metaphases analyzed.

In two cases (No. 1 and 16) the SMC was inherited, since it was also identified in the father and the mother of the probands in 6% and 100% of the parental metaphases, respectively. In another two cases (No. 25 and 26), cytogenetic analysis of the parents revealed that, in both cases, the mother was a carrier of a 11;22 reciprocal translocation (Figure 1). In the remaining cases the parental karyotypes were normal.

Molecular cytogenetic analysis. The chromosomal origin of the SMC was identified in all cases by application of sequential FISH hybridizations with WCP (Table I) (Figure 2a and b). In 16 cases, (No. 1-16) (61.5%) the SMCs were derived from chromosome 15. Cases No. 10-16 were further investigated with the SNRPN/15qtel probe to determine if the SMCs contained the SNRPN gene within the PWS/AS critical region. Three copies of the SNRPN gene were identified in cases No. 10 and 14, implying that the SMCs in these cases contained the PWS/AS critical region. None of the cases tested contained material from the telomere of chromosome 15 (Figure 2c).

In five cases (No. 17-21) (19.2%) the SMCs were isochromosomes 18p (i(18p)), since the whole length of the SMC was painted using both WCP 18 and PCP 18p (Figure 2b and d). In case No. 22, the SMC was derived from chromosome 22 and did not contain the DiGeorge/ VCFS critical region, as only two hybridization signals for each probe were detected when TUPLE/22qtel was applied. In cases No. 23 and 24, the SMCs were derivatives of chromosome 13 (7.7%) and in two cases (No. 25, 26) the SMCs contained material from both chromosomes 11 and 22 (7.7%) (Figure 3a and b).

Molecular analysis. Microsatellite analysis of the 15q11-q13 critical region in patients No. 10 and 14 confirmed the presence of three alleles for the PW/AS critical region and demonstrated that the marker chromosome was of maternal origin. In both cases, the diagnosis of PW/AS due to possible imprinting was excluded by a methylation-specific PCR (Figure 4).

Discussion

The identification of 26 autosomal SMCs by conventional cytogenetic and FISH analysis is reported. The majority of the SMCs originated from chromosome 15 (61.5%), followed by chromosome 18 (15.3%), 13 (7.6%) and 22 (3.8%). We believe that this is the order in which the SMCs should be tested in order to determine their chromosomal origin.

In the majority of cases, the SMCs were *de novo* and only in two cases were they inherited. In familial cases, the identification of a normal phenotype in the carrier parent is reassuring and usually indicates a favorable prognosis (6). Nevertheless, even in familial cases, FISH analysis was performed to fully characterize every SMC. It is interesting to note that in the familial case No. 1 the SMC[15] was mosaic in conjunction with an euploid cell line, possibly as a result of mitotic instability or anaphase lag of the SMC (7). The same SMC[15] was identified in a lower percentage in the phenotypically normal father. The mild phenotypic effect on the child was possibly due to the presence of the SMC in the abnormal cell line in over 50% of the metaphases analyzed. To the best of our knowledge, this is the first time that a paternally-inherited mosaic SMC[15] has been reported, since the two SMC[15] familial cases previously reported were of maternal origin (8, 9). This number of reported cases, however, is too small to permit any conclusions about the SMC phenotypic effect and parental origin.

Patients with an SMC[15] are at increased risk of developing PW/AS syndrome if the paternal or maternal chromosome is imprinted. They may also exhibit developmental delay due to additional copies of genes located within the 15q11-q13 critical region (9). In our study, patients No. 10 and 14, with SMC[15]s containing the SNRPN gene, were referred for cytogenetic investigation at the age of 8 and 8.5 months, respectively, because of hypotonia and seizures. Both had mild dysmorphic features with hypertelorism, low-set ears and epicanthic folds. Microsatellite analysis of the 15q11-q13 critical region in both patients confirmed the presence of three alleles and demonstrated that the marker chromosome was of maternal origin. It is interesting to note that, in both cases, the maternal age was >35 years and the formation of the SMC

was possibly due to meiosis I non-disjunction associated with advanced maternal age (10). The diagnosis of PW/AS due to imprinting in both patients was excluded by a methylation-specific PCR.

Of special interest was patient No. 11, referred for cytogenetic analysis at the age of two years because of slight dysmorphic features, including bilateral blepharoptosis, a small nose, long philtrum, large low-set ears and symptoms of idiopathic arthritis. By the age of four, multiple relapses were noted, mainly affecting the distal phalangeal joints of his fingers and toes, resulting in flexion deformity. Poor response to anti-inflammatory, corticosteroid and immunosuppressive treatments in different combinations was also noted. This case outlines a probable association between specific chromosomal regions of chromosome 15 and polyarthritis. To the best of our knowledge, this is the first reported case associating partial trisomy of chromosome 15 with idiopathic polyarthritis and further investigation is needed in order to clarify a possible role of chromosome 15 in the pathogenesis of idiopathic arthritis.

In cases No. 17-21, FISH analysis with a WCP 18 probe, as well as with a PCP 18p probe, showed that the marker chromosome was an i(18p). The majority of cases with i(18p) reported to date were *de novo*, as was the case in our patients. Our patients shared most of the clinical manifestations of i(18p) syndrome including dysmorphic features, various congenital malformations and developmental delay (11). Formation of a non-mosaic *de novo* tetrasomy 18p, due to an additional monocentric i(18p), requires two events: non-disjunction and centromeric misdivision (mitotic centromeric fission with fusion of sister p-arms). Molecular studies in cases with SMC[18]s have shown that the majority arise from a maternal MII error (12). This is in agreement with the most common model proposed, invoking MII non-disjunction as the first step in the formation of tetrasomy 18p. Tetrasomy 18 is associated with advanced maternal age, as in all five cases included in our study. Fisher *et al.* (12) reported that the maternal age effect was significant in the MII error group. To date, no association between advanced parental age and tetrasomy 18p has been demonstrated. In 1978, Nielsen *et al.* (13) found a mean maternal age of 30 years for the 25 cases of tetrasomy 18p, but none of these previously reported papers explained the mechanisms involved in i(18p) formation.

In case No. 22, the SMC was identified during prenatal diagnosis and contained the proximal 22q euchromatin, which did not extend beyond the DiGeorge/VCFS critical region. SMC[22] containing euchromatin was identified in patients with cat-eye syndrome, but the extent of the additional material did not directly correlate with the severity of the malformations (14). Although ultrasonographic examination of the fetus did not reveal congenital anomalies and the fetal size was consistent with the gestational age, the parents decided to terminate the pregnancy.

In cases No. 23 and 24, an SMC[13] was determined. Patient No. 23 presented with mild phenotypic abnormalities, as did all five cases so far reported (15). In case No. 24, the SMC was identified during prenatal diagnosis and the pregnancy was terminated.

In two unrelated patients (No. 25 and 26), cytogenetic analysis showed a 47, XX+der(22)t(11;22)(q23; q11) karyotype in all cells analyzed, and a distinct phenotype with multiple anomalies and severe mental retardation was noted. Translocation 11;22 is the most frequently identified familial reciprocal translocation in humans. In translocation carriers, a 3:1 meiotic segregation with tertiary trisomy can occur resulting in abnormal progeny with the der(22) as SMC (16, 17). In both patients, as in the majority of cases so far reported (16), the mothers were carriers of a t (11;22) (q23;q11). This was attributed either to the higher incidence of t (11;22) in females, or to their higher risk of meiotic non-disjunction compared to males.

In conclusion, molecular cytogenetics in combination with other molecular studies can provide valuable information on the chromosomal origin and the composition of SMCs. Accurate phenotype-genotype correlation can be established in cases such as SMC[15] and SMC[18]. However, the low frequency of SMCs derived from other chromosomes in the general population limits our ability to estimate the phenotypic consequences. FISH has greatly facilitated the characterization of marker chromosomes, and, as more SMCs are classified and more data are collected, better genetic counseling and risk evaluation can be offered.

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