BJMG 10/1 (2007) 33–37
10.2478/v10034-007-0006-5

ORIGINAL ARTICLE

CHARACTERIZATION OF SMALL SUPERNUMERARY MARKER CHROMOSOMES BY A SIMPLE MOLECULAR AND MOLECULAR CYTOGENETICS APPROACH

Liehr T[1,*], Trifonov V[1,2], Polityko A[1,3], Brecevic L[1,4], Mrasek K[1], Weise A[1], Ewers E[1], Reich D[1], Iourov I[1,5], Mkrtchyan H[1,6], Manvelyan M[1,6], Kosyakova N[1,7]

***Corresponding Author:** Dr. Thomas Liehr, Institut für Humangenetik und Anthropologie, Kollegiengasse 10, D-07743 Jena, Germany; Tel.: +49-3641-935533; Fax: +49-3641-935582; E-mail: i8lith@mti.uni-jena.de

ABSTRACT

Small supernumerary marker chromosomes (sSMC) are still a major problem especially in prenatal cytogenetic diagnostics and counseling. These structurally abnormal chromosomes cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and are generally about the size of or smaller than a chromosome 20 in the same metaphase spread. We describe a straightforward algorithm, based on data from 2,211 reported cases (http://www.markerchromosomes.ag.vu) to quickly characterize the sSMC's chromosomal origin.

Key words: Small supernumerary marker chromosomes (sSMC); Cytogenetic(s); Fluorescence *in situ* hybridization (FISH); Metaphase FISH, Molecular cytogenetics; Prenatal diagnostics.

[1] Institute of Human Genetics and Anthropology, Friedrich-Schiller-University Jena, Germany
[2] Institute of Cytology and Genetics, Novosibirsk, Russia
[3] Institute of Hereditary Diseases, Minsk, Belarus
[4] School of Medicine, Croatian Institute for Brain Research, Zagreb, Croatia
[5] National Research Center of Mental Health, Russian Academy of Medical Sciences, Moscow, Russia
[6] Department of Genetic and Laboratory of Cytogenetics, State University, Jerewan, Armenia
[7] Research Centre for Medical Genetics, Russian Academy of Medical Sciences, Moscow, Russian Federation

INTRODUCTION

Small supernumerary marker chromosomes (sSMC) are present in ~2.7 million people worldwide [1]. They are defined as structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone; they are generally equal in size or smaller than a chromosome 20 in the same metaphase spread. Additionally, sSMC can be present in an otherwise normal karyotype, in a numerically abnormal karyotype (*e.g.,* Turner's or Down's syndromes) or in a structurally abnormal but balanced karyotype with or without ring chromosome formation. If detected in banding cytogenetics, they are a major problem as they are too small to be characterized for their chromosomal origin or content by traditional banding techniques. Molecular cytogenetic techniques are necessary for their characterization [2]. Cases with a *de novo* sSMC, particularly those that are prenatally ascertained, are not easy to correlate with a clinical outcome [3]. It has been established that substantial parts of sSMC lead to four specific syndromes, *i.e.,* Pallister-Killian [= i(12p)], isochromosome 18p [i(18p)], cat-eye [i(22p~q)] and derivative chromosome 22 [der(22) t(11;22)] syndromes [2]. In general, the risk for an abnormal phenotype in prenatally ascertained *de novo* cases with sSMC is considered to be ~13% [4]. This has been refined to 7% (for sSMC from chromosomes 13, 14, 21 or 22) and 28% (for all non-acrocentric autosomes) [5] and has recently been suggested to be 26-30% [1,6]. Also,

generally speaking, sSMC transmitted by normal sSMC carriers to their progeny are not correlated with clinical problems [7], although exceptions have been described [8].

MATERIALS AND METHODS

By reviewing the literature, all available reported sSMC cases (total 2,211) were collected on a regularly updated sSMC homepage [9]. All cases were included when at least the chromosomal origin of an sSMC was reported in an article listed at the National Center for Biotechnology Information webpage (or If available, gender of the carrier, age at diagnosis, the cytogenetically studied material (blood, amniocytes, fibroblasts), parental origin of sSMC, the GTG-banding result including mosaicism of sSMC, final fluorescence *in situ* hybridization (FISH) results of sSMC, applied FISH methods, exclusion of uniparental disomy (UPD) for the sSMC's sister-chromosomes and the clinical symptoms are also listed. Thus, according to this data [9], the distribution of the chromosomal origin of sSMC is summarized as available from the literature (Fig. 1). Even though this data is biased by the fact that not all but mainly 'the interesting sSMC cases' are published, it provides the only available insight on the chromosomes most frequently involved in sSMC-formation.

According to that data, an algorithm for a straightforward characterization of sSMC origins was worked out (as presented in Results). According to the chromosome-specific frequency of sSMC, the application of commercially available centromere specific probes is suggested to quickly achieve information on the chromosomal origin of a centric sSMC [2]. Commercially available whole chromosome painting probes can be used for the characterization of neocentric sSMC. Neocentric, also called analphoid sSMC, "carry newly derived centromeres (or "neocentromeres") that are apparently formed within interstitial chromosomal sites that have not previously been known to express centromere function" [10].

RESULTS

The chromosomal distribution of sSMC from 2,211 reported cases is shown in Fig. 1. The four syndromes Pallister-Killian, isochromosome 18p, cat-eye and derivative chromosome 22 syndromes, accounted for 31% of cases. Overall, chromosome #15 was the most frequent participant (30%) and was followed by chromosomes #14/#22 (26%), #12 (9%), #18 (7%), #13/#21 (5%), #8 (4%), #1 (~2%), #16 (~2%), #9 (~1%), #3 (~1%), #20 (~1%), X (~1%), and the remainder (~11%). Interestingly, a very similar distribution is observed in neocentric

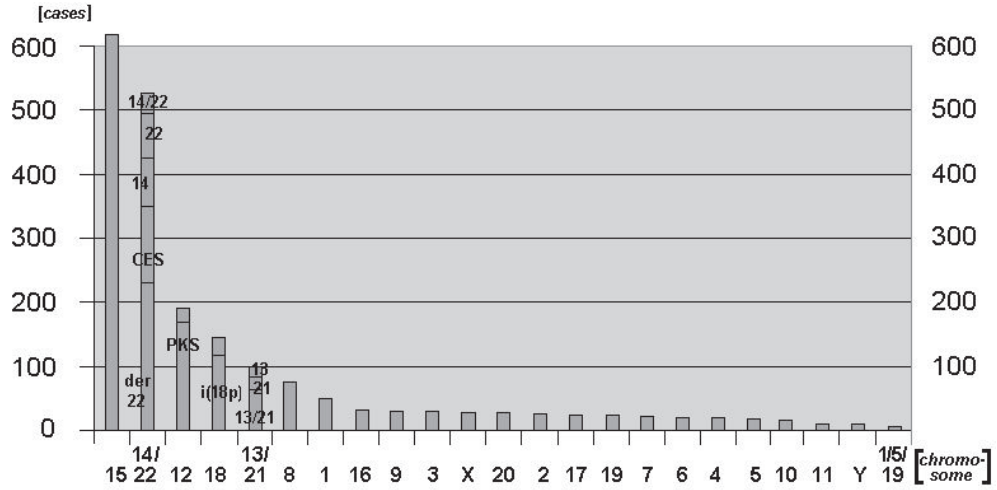

Figure 1. Frequency of sSMC according to their chromosomal origin: Chromosomal origin of 2,211 sSMC cases collected from the literature [9]. From left to right the most frequent to the most rare. Abbreviations: CES: cat-eye-syndrome; der 22: derivative chromosome 22 syndrome; i(18p): isochromosome 18p syndrome; PKS: Pallister Killian syndrome.

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sSMC: chromosome #15 (24%), chromosomes #8 (15%), #13 (15%), #8 (15%), #3 (9%),#1 (7%), #12 (4%), which could be in connection with the mechanisms of sSMC-formation (U-type exchange, summarized in [2]).

Comprehensive characterization of marker chromosomes is often hampered by the lack of available multicolor-FISH (M-FISH) approaches (such as micro dissection and reverse FISH) [11], M-FISH applying whole chromosome painting probes (*i.e.,* M-FISH [12], spectral karyotyping (SKY) [13], or (sub)centromere-specific probes (cenM-FISH) [14] and subcenM-FISH) [15]). Thus, an algorithm was developed that allows determination of the chromosomal origin of an sSMC in a straightforward and effective manner. The chromosomal origin of an sSMC provides better risk assessment of the clinical outcome based on similar cases summarized on the sSMC homepage [9]. A first attempt at genotype-phenotype correlation for sSMC has been reported [16].

The following algorithm provides a practical method for determining the chromosomal origin of an sSMC for diagnostic laboratories which do not have sophisticated molecular cytogenetic possibilities:

1) Clarify by conventional chromosome-banding analysis of parental peripheral blood if sSMC is *de novo*. If the sSMC is inherited go to **2)**; if it is *de novo* or its parental origin cannot be determined go to **3)**.

2) If the sSMC is inherited from one clinically normal parent, the identification of the sSMC origin may be replaced by genetic counseling with close monitoring of the pregnancy by high resolution ultrasound examinations. However, an inherited sSMC may be connected with clinical abnormalities in exceptional cases ([8] case I). If origin of the sSMC is to be clarified, go to **4)**.

3) If the *de novo* sSMC is almost the size of chromosome 20 in the same metaphase spread, the presence of a large inverted duplication chromosome 15 (inv dup15), an isochromosome 18 [i(18p)] or an [i(12p)] should be excluded by the appropriate centromeric and/or whole chromosome painting probes. If, as happens in about one-third of cases, the origin of the sSMC is clarified in that way, go to **9)**. If the origin of the sSMC was not determined in this way, go to **4)**.

4) If a clear and positive nucleolus organization region (NOR) silver staining result is obtained [17] for the sSMC, its origin can be determined by hybridizing commercially available centromeric probes for all acrocentric chromosomes, *i.e.,* #13/#21, #14/#22, #15. If, as happens in ~75% of cases, the origin of the sSMC is clarified in this way, go to **9)**. If the origin of the sSMC was not determined in this way, go to **5)**.

5) To determine the origin of the sSMC use commercially available centromeric probes, testing sequentially for #8, #1, #20, X, #18, #3 and #12. Even if sSMC was NOR-negative, test for #14/#22, #15 and #13/#21, as cases have been reported with sSMC derived from acrocentric chromosomes, but without NOR [9]. If, as happens in ~90% of cases, the origin of the sSMC is clarified in this way, go to **9)**. If the origin of the sSMC was not determined in this way, go to **6)**.

6) To determine if the case is a rare one with a neocentric sSMC, a commercially available pan centromeric probe should be used. This test is important since neocentric sSMC nearly always have a clinically adverse prognosis [2,9,16]. In ~4% of the cases no α-satellite DNA is present on the sSMC. If the sSMC has α-satellite DNA, go to **7)**; if the sSMC has no α-satellite DNA, go to **8)**.

7) An sSMC with α-satellite DNA can still arise from 12 different human chromosomes. If there is enough material to continue the analysis, proceed in the following sequence (applying centromeric probes if nothing else is mentioned): #19 (whole chromosome painting probe), #9, #16, #17, #7, #6, #2, #4, #5 (whole chromosome painting probe), #11 and Y. If, as happens in ~100% of cases, the origin of a centric sSMC is clarified, go to **9)**.

8) To characterize the origin of a neocentric sSMC use FISH, applying the following sequence of whole chromosome painting probes: #15, #8, #13, #3, #1 and #12. If, as happens in ~75% of cases, the origin of the neocentric sSMC is clarified, go to **9)**.

9) In ~10% of sSMC cases, UPD of the cytogenetically normal sSMC's sister chromosome has been reported [2]. Because of this, it has been recommended [2,18-19] that, after identification of the origin of the sSMC, the normal sister chromosomes should be tested for their parental origin to exclude possible UPD. This can be tested by molecular genetic approaches, such as

microsatellite analysis [19] or methylation-specific polymerase chain reaction (PCR) [20] and should be done for every sSMC case in which parental cell material is available. Apart from the chromosomes known to be connected with imprinting (#6, #7, #14, #15, #20), other chromosomes should be tested as uniparental isodisomy can lead to homozygotization of an otherwise recessive, disease-causing gene (*e.g.,* see [21]).

DISCUSSION

Our straightforward algorithms for characterization of the origin of an sSMC has the advantages that it can be performed without the use of sophisticated or highly specialized equipment, except for one- or two-color-FISH and that it provides the chromosomal origin of a sSMC. Moreover, if molecular genetic approaches such as micro satellite analysis are available, a possible disease-causing UPD can be excluded. However, only subcenM-FISH [15] or array-CGH (comparative genomic hybridization) [22] can exclude or detect a small partial tri- or polysomy of the centromere-near region of the sSMC. In most cases, after exclusion of an UPD of an sSMC's sister chromosome, such an imbalance determines the clinical effect of the marker chromosome [16]. When possibilities for determination of the euchromatic contents of an sSMC are not available, all reported sSMC cases (sorted by chromosomal origin) can be found on the sSMC homepage [9]. The authors welcome the opportunity to characterize sSMC cases by subcenM-FISH or other approaches.

ACKNOWLEDGMENTS

This study was supported in part by funds from the DFG (436 RUS 17/135/03; 436 RUS 17/109/04, 436 RUS 17/22/06, 436 WER 17/1/04, 436 WER 17/5/05, WE 3617/2-1), the Schering Foundation, the Boehringer Ingelheim Fonds and the Evangelische Studienwerk e.V. Villigst.

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