Small Supernumerary Marker Chromosome May Provide Information on Dosage-insensitive Pericentric Regions in Human

Ahmed B. Hamid Al-Rikabi¹, Sona Peková², Xiaoabo Fan¹, Tereza Jančušková² and Thomas Liehr¹,*

¹Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Am Klinikum 1, 07747 Jena, Germany; ²Synlab Genetics s.r.o., Evropska 176/16, 16000 Prague 6, Czech Republic

Abstract: Background: Cytogenetically visible chromosomal imbalances in humans are deleterious and adverse in the majority of the cases. However, healthy persons living with chromosomal imbalances in the range of several megabasepairs (Mbps) in size, like carriers of small supernumerary marker chromosomes (sSMCs) exist.

Material & Methods: The identification of healthy sSMC carriers with euchromatic centromere-near (ECN) imbalances led to the following proposal: ECN-regions do not contain any dosage sensitive genes. Due to own previous work, dosage-insensitive pericentric ECN-regions were already determined with an accuracy of 0.3 and 5 Mbp. Based on this data we established 43 new pericentromeric probe sets spanning about 3-5 Mbp of each euchromatic human chromosome arm starting from the known insensitive regions towards distal. Such so called pericentromeric-critical region fluorescence in situ hybridization (PeCR-FISH) probe sets were applied exemplarily and successful here in 15 sSMC cases as available from the Else Kröner-Fresenius-sSMC-cellbank <http://ssmc-eli.com/ekf-cellbank.html>.

Conclusion: Most of the involved sSMC breakpoints could be characterized as a higher resolution than before. An unexpected result was that in 5/15 cases cryptic mosaicism was characterized. The latter is also to be considered to have potentially an influence on the clinical outcome in these so-called discontinuous sSMCs. Overall, the suitability of PeCR-FISH to characterize sSMCs was proven; the potential of this probe set to further delineate sizes of dosage insensitive pericentric regions is obvious but dependent on suited cases. Furthermore, discontinuous sSMCs can be identified by this approach and this new subtype of sSMC needs to be studied in more detail in future.

Keywords: Molecular cytogenetics, Small supernumerary marker chromosomes (sSMCs), Discontinuous sSMCs, Pericentromeric-critical region fluorescence in situ hybridization (PeCR-FISH), Dosage insensitive genomic regions, Euchromatic centromere-near imbalances.

1. INTRODUCTION

For several decades chromosomes were analyzed exclusively by karyotyping, and banding cytogenetics has been and still is a very powerful tool to scan the whole human genome for aberrations in one simple approach. However, the major disadvantage of cytogenetic banding techniques is their limited resolution. Particular problematic are the analysis of extensively rearranged chromosomes, of marker chromosome identification and their detailed characterization [1]. However, nowadays fluorescence in situ hybridization (FISH) including array-comparative genomic hybridization (aCGH) can be applied to achieve higher resolution in such cases [2-4].

1.1. Submicroscopic Copy Number Variants

Cytogenetically visible chromosomal imbalances are in the size of several megabasepairs (Mbps) and thus traditionally thought to be always deleterious and adverse in humans.

The same was suggested initially for submicroscopic euchromatic imbalances, however, soon after introduction of aCGH approach it became obvious that there are many, non-deleterious so-called submicroscopic, but relatively large (in the range of 0.1 to 1 Mbps) Copy Number Variants (CNVs) [5, 6]. In general it is true for CNVs, that the larger they are the more likely an adverse effect on the phenotype must be suggested for the carrier. Still, during last decades more and more probands were reported where this was not the case [7]. Apart from the meanwhile in the minds of cytogeneticists well anchored so-called euchromatic variants in 4p16, 8p23, 9p12, 9q13, 15q11.2 and 16p11.2 [8], cytogenetically visible CNVs were reported as single case reports along all human chromosomes [7].

1.2. Small Supernumerary Marker Chromosomes

Furthermore, carriers of small supernumerary marker chromosomes (sSMCs) represent the largest subgroup among healthy persons living with chromosomal imbalances [7, 9]. The identification of such healthy sSMC carriers with centromere-near imbalances in the range of several Mbps led to the proposal, that these euchromatic regions being present
in three or more copies do not contain any dosage sensitive genes [9, 10]. Thus, those sSMC-carriers can be regarded as ideal models to characterize the dosage-insensitive stretches in human pericentric regions. Besides the fact that asymptomatic carriers of euchromatic sSMCs are the best source for such studies, also patients with clinical symptoms and with well-defined sSMCs are necessary for the identification of potentially disease-causing, dosage sensitive genes, and thus for clear definition where a dosage-insensitive region ends and a dosage sensitive region begins.

Here 43 new pericentromeric probe sets spanning about 3-5 Mbp of each euchromatic human chromosome arm were established; their localization was based on previous own work which narrowed those regions already with an accuracy of 0.3 and 5 Mbp [9-11] (Table 1).

Selected ones of these so called pericentromeric-critical region fluoroscence in situ hybridization (PeCR-FISH) probe sets were applied here in 15 sSMC cases from the Else Kröner-Fresenius-sSMC-celldbank [11, 12]. These probe sets can be applied in future to characterize the borders of dosage-insensitive pericentric regions in more detail for each of the 24 human chromosomes. Additionally, sSMC, are known to be present in low mosaics [13] and/or cryptic mosaics [14]. Patients carrying two or more populations of sSMCs, different in size and shape, cannot be resolved by aCGH [4]; thus, PeCR-FISH may be an elegant possibility to perform such kind of studies.

2. MATERIAL AND METHODS

2.1. Cell Lines

Fifteen cell lines with sSMC derived from 1, 7, 8, 20, 21, 22, and Y-chromosome were selected for this study from the Else Kröner-Fresenius-sSMC-celldbank [11, 12]. Details on the clinical data of the sSMC carriers and the sSMC content as characterized previously by molecular cytogenetics and/or aCGH [11] can be found in Tables 2 and 3. Cytogenetic preparations were obtained from all 15 cell lines according to standard procedures [1].

2.2. Molecular Cytogenetics

FISH was done as previously reported [15]. For establishing the here reported PeCR-FISH probesets (Fig. 1A; Table 3 and Table S1) 208 Bacterial Artificial Chromosome (BAC) clones were selected from the publically available whole human genome sequence [16]. Due to facts (i) that several of the probes available to the authors are only mapped in assembly NCBI 36 (hg18), and (ii) the sSMC-webpage is also mainly based on this assembly, NCBI 36 (hg18) has been used also in the present paper as a basis for (Table 3 and Table S1).

Five different fluorochromes were applied to label each chromosome-arm specific PeCR-FISH-probeset: Spectrum Green-dUTP (SG), Spectrum Orange-dUTP (SO), Texas Red-dUTP (TR), Diethylaminocoumarin (DEAC) and Cyanin 5 (Cy.5 - coupled to avidin to detect biotin-16-dUTP). Besides, the probesets can be combined with a corresponding homemade centromeric probes [17] labeled in DEAC and Cy 5. BAC and centromeric DNA were amplified and labeled as previously reported by degenerated oligonucleotide primed polymerase chain reaction (DOP-PCR) [18]. Hybridization, post-hybridization washes and signal detection of the probe set mix was carried out following standard protocols [15]. Twenty metaphase spreads were analyzed using an Axioplan II microscope (Carl Zeiss, Jena GmbH, Oberkochen, Germany) equipped with filters set for fluorochromes FITC, SO, TR, Cy.5, DEAC and 4’-6-diamidino-2-phenylindole (DAPI) a general DNA counterstain (Chroma Technology). Images were captured and processed using the isis/mFISH imaging system (MetaSystems GmbH, Alt- lusheim, Germany).

3. RESULTS

43 chromosome-arm specific probesets were established according to Fig. (1A). Five BACs were selected in a ~1Mbp distance to each other spanning the yet known borders of dosage-independent and dosage dependent regions of the corresponding chromosome-arm. As the acrocentric chromosome all carry a heterochromatic p-arm for chromosomes 13, 14, 15, 21, and 22 only q-arm specific probe-sets were established. Furthermore, as for chromosome-arms 1p, 6q and 10p the critical regions were already <0.5 Mbp only two to three instead of five BACs were selected for these regions.

All 43 PeCR-FISH probe sets were tested on chromosome spreads of at least two normal carriers (results not shown). No relevant cross-hybridizations or background could be observed. The idea for how to establish this kind of probeset was previously stated in a comment for a paper published by Castronovo and colleagues in 2013 [19, 20].

The probe sets specific for chromosome arms 1p and 1q (case 1), 4p and 4q (case 6), 7p and 7q (cases 2 and 3), 8p and 8q (cases 4, 5, and 6), 11p and 11q (case 6), 20p and 20q (cases 7 and 8), 21q (case 9), 22q (cases 10, 11, and 12), Yp and Yq (cases 13-15) were applied in the 15 selected sSMC from the Else Kröner-Fresenius-sSMC-celldbank (Table 3, Fig. 1B-1D).

The cases 3, 5, 8 and 15 were known to be cryptic mosaic ones. After analyses with PeCR-FISH these results were confirmed but also the cases 4, 6, 13 and 14 were identified as being cryptic mosaic, indeed.

Four sSMC (cases 6 (sSMCs 4 and 11), 9 and 11) carried only centromeric material and/or euchromatic material not covered not covered by PeCR-FISH-probe sets. Case 5 was known to have a discontinuous sSMC, which however could not be found by PeCR-FISH. In cases 6, 13, 14, and 15 discontinuous sSMC were identified.

In the present study, it could be demonstrated that PeCR-FISH can be used to characterize within one single step chromosomal rearrangements in pericentromeric region with involvement of euchromatic material in sSMC which derived from this region. Table 4 summarizes the meaning of the results for the narrowing down of dosage sensitive and insensitive pericentric tested regions.

5. DISCUSSION

It is widely accepted that the phenotypes of patients carrying sSMCs are mainly determined by the euchromatic material involved in the sSMCs and additionally influenced by
the degree of sSMC-mosaicism and a possible uniparental disomy of the sSMC’s sister chromosomes [9, 21]. Over the years, many FISH-based approaches have been developed and applied to improve sSMC characterization, such as multicolor-FISH using whole chromosome paints as probes, centromere-specific multicolor FISH probes sets, or others like FISH-banding approaches (for review see [3]). Besides aCGH is a good tool to characterize sSMC breakpoints in detail [22] and the potential of next-generation sequencing [23] for sSMC characterization has to be determined.

As only FISH-based methods can easily characterize also sSMC with cryptic mosaics, i.e. sSMCs of different sizes present in the same patient [14, 24], previously, we established already a probe sets called pericentric-ladder-FISH (PCL-FISH) with a resolution of ~10 Mbps [25]. Also two similar probe sets were published before in 2007 and 2013 [19, 26]. However, a probe set for the pericentric region for narrowing down the dosage insensitive regions of all human chromosome arms was not available yet.

The here presented chromosome-arm specific PeCR-FISH probe sets can be applied after the origin of an sSMC has been determined to (1) check for exact size of the sSMC, (2) narrow down the breakpoints, (3) to uncover cryptic mosaics, and (4) as this study showed to characterize presence of discontinuous sSMCs. (1) is necessary to provide for a better genotype-phenotype correlation of sSMC, (2) can provide information if sSMC breakpoints are identical in different independent cases; if so new insights into genomic architecture may be gained as well as breakpoint prone regions in human genome defined. (3) and (4) can also help explain unclear genotype phenotype correlations in single cases. Also, the presence of discontinuous sSMCs may be interpreted as a hint on the involvement of chromothripsis [27] in postzygotic evolution of sSMC and in trisomic rescue processes [21].

Table 1. Dosage-insensitive pericentric regions in human acc. to [11]. UCSC / hg18 genome browser.

| Chromosome No. | Dosage Sensitive Region p-arm | Dosage Insensitive Region p-arm | Heterochromatic/ Centromeric Region | Dosage Insensitive Region q-arm | Dosage Sensitive Region q-arm |
|----------------|-------------------------------|-------------------------------|-----------------------------------|-------------------------------|-------------------------------|
| 1              | 115.30                        | 115.80                        | 121.10 - 142.40                   | n.a.                          | n.a.                          |
| 2              | 80.70                         | 85.62                         | 91.00 - 95.70                     | 101.58                        | 103.47                        |
| 3              | n.a.                          | 74.67                         | 89.40 - 93.20                     | 104.78                        | n.a.                          |
| 4              | 39.80                         | 44.03                         | 48.70 - 52.40                     | 62.63                         | n.a.                          |
| 5              | 30.64                         | 37.21                         | 45.80 - 50.50                     | 55.27                         | 61.21                         |
| 6              | n.a.                          | 57.35                         | 58.40 - 63.40                     | 66.40                         | 65.20                         |
| 7              | 55.42                         | 56.45                         | 57.40 - 61.10                     | 67.00                         | 74.00                         |
| 8              | 40.35                         | 42.50                         | 43.20 - 48.10                     | 61.33                         | n.a.                          |
| 9              | 37.88                         | 42.96                         | 46.70 - 70.00                     | 70.50                         | n.a.                          |
| 10             | 34.46                         | 34.75                         | 38.80 - 42.10                     | 43.82                         | n.a.                          |
| 11             | 50.47                         | 50.95                         | 51.40 - 56.40                     | 60.23                         | 65.02                         |
| 12             | n.a.                          | 28.47                         | 33.20 - 36.50                     | 39.90                         | 40.20                         |
| 13             | -                             | -                             | 0 - 18.40                         | 19.31                         | 22.29                         |
| 14             | -                             | -                             | 0 - 19.10                         | 20.24                         | 20.17                         |
| 15             | -                             | -                             | 0 - 18.40                         | 21.18                         | 22.80                         |
| 16             | n.a.                          | 28.86                         | 34.40 - 45.50                     | 46.02                         | 46.16                         |
| 17             | 17.75                         | 18.68                         | 22.10 - 23.20                     | 23.32                         | 24.75                         |
| 18             | 12.59                         | 12.80                         | 15.40 - 17.30                     | 18.12                         | 19.34                         |
| 19             | n.a.                          | 17.50                         | 26.70 - 30.20                     | 43.88                         | n.a.                          |
| 20             | n.a.                          | 22.83                         | 25.70 - 28.40                     | 29.93                         | 30.12                         |
| 21             | -                             | -                             | 0 - 13.20                         | 16.90                         | 18.10                         |
| 22             | -                             | -                             | 0 - 16.30                         | 16.37                         | 17.00                         |
| X              | 50.90                         | n.a.                          | 56.60 - 65.10                     | n.a.                          | n.a.                          |
| Y              | n.a.                          | n.a.                          | 11.20 - 12.50                     | n.a.                          | n.a.                          |
Fig. (1). A) Schematic depiction of metacentric / submetacentric and acrocentric chromosomes shows the principle of CR-FISH probe set for each of these chromosomes. The locus-specific probes (BAC clones) covered the pericentric region in specific for uncritical / critical regions of the chromosome in distance between each other ~1Mbp and labeled with five different fluorochromes (SG/TR/DEAC/SO/Biotin-Cy.5), in addition to centromeric probe labeled with dual-color (DEAC and Biotin-Cy.5).43 probe set was established to be specific for p- and q-chromosome arms. B) PeCR-FISH set 7p used to characterize the sSMC of case 3, revealing that the breakpoint is distal from probe 7p5 (for probe specification, see Table S1). C) Application of PeCR-FISH set 8q showed that the breakpoint in the sSMC(8) of case 5 is distal from 8p1 and proximal of probe 8p2 (for probe specification see Table S1). D) The same probe set as used in Fig. 1C applied in case 6 revealed a discontinuous structure of one of the two sSMC(8) present here (for probe specification see Table S1).

Table 2. List of clinical manifestations of sSMC carriers; the clinical details are documented in [11].

| Case No. | Case No. sSMC Homepage | Gender | GTG Banding / Mosaicism | Origin of Marker | Clinical Details |
|----------|------------------------|--------|-------------------------|-----------------|-----------------|
| 1        | 01-W-p11.1/3-1         | M      | 47,XY, r[3]/46,XY[47]   | de novo         | Cardiac anomalies and horseshoe kidney. |
| 2        | 07-U-14                | F      | 47,XX, r[53]/46,XX[47]  | de novo         | Pre- and postnatal growth-retardation, macrocephalus, macro cornea and Silver-Russell Syndrome (SRS) |
| 3        | 07-W-p11.2/1-2         | F      | 47,XX, +mar[25]/46,XX[75] | de novo         | Statomotoric retardation, developmental delay and OFC at 75th centile. |
| 4        | 08-W-p11.21–11.22/1-1  | F      | 47,XX, +mar[17]/46,XX[34] | de novo         | Autism; Physically normal to somewhat late on most milestones, lost some cognitive skills, little language acquisition, Vineland Adaptive Behavior Scales, physically normal and full puberty |
| 5        | 08-U-6                 | F      | 47,XX, +mar[17]/46,XX[34] | de novo         | Advanced maternal age and no further information available |
| 6        | mult 3.5               | M      | 48,XY, +2mar[1]/47,XY, +mar[41]/46,XY[9] | de novo         | Pierre-Robin-sequence, ventricular septum defect, patent foramen ovale, cryptochism, flaccid joints, gothic palate, umbilical hernia, at birth urinary tract infection |

(Table 2 contd....)
| Case No. | Case No. sSMC Homepage | Gender | GTG Banding / Mosaicism | Origin of Marker | Clinical Details |
|---------|-------------------------|--------|------------------------|-----------------|-----------------|
| 7       | 20-W-p11.22/1-2        | F      | 47,XX.+mar[7]/46,XX[45] | de novo         | Hypochondroplasia, Breathing through mouth, speech is slurred, and poor in vocabulary. Macrocephaly, slightly reclined neck, short long bones, strong suborbital skin fold and flat nasal root. |
| 8       | 20-W-p11.1/1-1         | F      | 47,XX.+mar[1]/46,XX[55] | de novo         | Retardation in speech development, weak ear dysmorphism and slight clinodactyly of 5th finger. |
| 9       | 21-O-q11.2/2-1         | M      | 47,XY.+mar[149]/46,XY[1] | de novo         | Advanced maternal age, normal development and no dysmorphic signs. |
| 10      | 22-Wces-5-93           | F      | 47,XX.+mar[48]/46,XY[2] | na              | Cat-eye syndrome (CES) |
| 11      | 22-O-q11.21/2-1        | M      | 47,XY.+mar[26]/46,XY[28] | Maternal        | Normal |
| 12      | 22-W-q12.1/1-1         | M      | 47,XY.+mar[41]/46,XY[9] | de novo         | Morbus Hirschsprung, muscular hypotonia and statomotoric retardation, macrocephaly, head circumference, adipositas, antimongoloid palpebral fissures, long philtrum and deep sitting ears. |
| 13      | f-iY-q11.21/1-1        | F      | 45.X[13]/46,X,der(Y)[43] | n.a.            | Short stature |
| 14      | m-iY-q11.22/1-3        | M      | 45.X[19]/46,X,der(Y)[45] | de novo         | Lack of puberty, (develop) mental retardation and short stature |
| 15      | m-rY-p11.32/5-1        | M      | 45.X[5]/46,XY[10]/46,X,der(Y)[34] | n.a.            | Developmental delay and mental retardation |

Table 3. Summarizing of CR-FISH probe set results of 15 sSMC, specific probe set used for p- or q-arm according to the sSMC origin, molecular cytogenetic characterization and aCGH used from previous available data; the breakpoints positions are identified according to NCBI 36 / hg18 genome browser.
| Case | PeCR probe-set | Size acc. to Previous FISH Results and/or aCGH (NCBI36/hg18) [Mbp] | Breakpoints p- & q-arm / Position of Breakpoints (NCBI36/hg18) [Mbp] | Final Karyotype Including Data from Previous Studies and New Data After PeCR-FISH (bold) | Discontinuous sSMC acc. to PeCR-FISH | Cryptic mosaic sSMC acc. to PeCR-FISH |
|------|----------------|-------------------------------------------------|------------------------|---------------------------------------------------------------------------------|------------------------|------------------------|
| 5    | 8p 8q          | 42.50-48.37                                     | - distal from 8p2 = between 40.19 and 41.42  
- proximal of 8q1 = >61.36  
- min(8)(p11.21→q11.21::)[8]  
- min(8)(p11.21→q11.1::)[3]  
- min(8)(p11.21→q11.1::)[1]  
- r(8)(p11.21→q11.1::)q11.1→p11.21;q11.21→q11.21::)j2 | no                      | yes                     |
| 6    | 8p 8q          | 42.50-48.37                                     | - distal from 8p2 [6/20] = between 40.19 and 41.42  
- distal from 8p5 to proximal of 8p5; distal from 8p2 to proximal of 8p1 [14/20] = <38.29 to between 38.49 to 39.21; between 40.19 and 41.42 to proximal of 42.67  
- proximal of 8q1 = >61.36  
- min(8)(p11.21→q11.21::)[6]  
- min(8)(p21.1→p12::)p11.21→q11.21::)j14 | yes                      | yes                     |
| 7    | 20p 20q        | 25.47-29.93                                     | - distal from 20p4 = between 19.75 and 20.71  
- distal from 20q5 = >33.39  
- min(20)(p11.23→q11.22::) | no                       | no                      |
| 8    | 20p 20q        | 25.7-29.93                                      | - proximal of 20p1 = <23.60  
- distal from 20q5 = >33.39  
- min(20)(p11.1→q11.23::)[18]  
- min(20)(q11.23→p11.1→q11.23::)[7]  
- r(20)(::p11.1→q11.23::)p11.1→q11.23::)j3 | no                       | no                      |
| 9    | 21q            | 0-14.85                                         | - proximal of 21q1 = <16.73  
- inv dup(21)(q11.2::) | no                       | no                      |
| 10   | 22q            | 0-16.35                                         | - distal from 22q5 = >20.75  
- inv dup(22)(q11.21::) | no                       | no                      |
| 11   | 22q            | 0-16.35                                         | - distal from 22q1 = between 16.35 and 17.10  
- inv dup(22)(q11.21::) | no                       | no                      |
| 12   | 22q            | 0-24.60                                         | - distal from 22q5 = >20.75  
- min(22)(p21.1::q2.1::) | no                       | no                      |
| 13   | Yp Yq          | 0-15.80                                         | - distal from Yp5 [15] = <7.23  
- distal from Yq5 [103/122] = >17.23  
- distal from Yq2 [3/122] = between 14.26 and 15.27  
- distal from Yq2; proximal of Yq4; distal from Yq5 [16/122] = between 14.26 and 15.27; between 15.45 and 16.11; >17.23  
- idic(Y)(q11.21::)[103]  
- del(Y)(q11.21→q11.21::)[3]  
- der(Y)(p11.1→q11.21→q11.22::)q11.21→q11.21::)j15  
- breakpoint in Yq not in 15.80 but distal from 17.23 Mbp  
- discontinuous sSMC | yes                      | yes                     |
| 14   | Yp Yq          | 0-15.80                                         | - distal from Yp5 [15] = <7.23  
- distal from Yq5 [89/121] = >17.23  
- distal from Yq5 [8/121] 2x = >17.23  
- proximal of Yq1; proximal of Yq2 to distal from Yq2; proximal of Yq4 to proximal of Yq5 [24/121] = proximal of 13.04; between 13.17 and 14.16 and between 14.26 and 15.26; between 15.45 and 16.11 and distal from 17.23 (signals in double)  
- inv dup(Y)(q11.221::)[89]  
- inv dup(Y)(q11.221::s2[8])  
- der(Y)(p11.1→q11.21→q11.21::)q11.221→q11.221::q11.221→q11.21→q11.21::)j24  
- breakpoint in Yq not in 15.80 but distal from 17.23 Mbp  
- discontinuous sSMC | yes                      | yes                     |

(Table 3 contd....)
Table 4. PeCR-FISH probe sets in to better characterization of breakpoints (12/15 cases) and mosaic status (5/15).

| Case No. | By PeCR-FISH Better Characterization of | | |
|----------|-----------------------------------------|-------------------------------------------------|-----------------|
|          | Breakingpoint                             | of Mosaic State                                  | of Dosage Inensitive Region |
| 1        | -                                       | -                                               | -                |
| 2        | +                                       | -                                               | -                |
| 3        | +                                       | +                                               | -                |
| 4        | +                                       | +                                               | -                |
| 5        | +                                       | -                                               | -                |
| 6        | +                                       | +                                               | -                |
| 7        | +                                       | -                                               | -                |
| 8        | +                                       | -                                               | -                |
| 9        | -                                       | -                                               | -                |
| 10       | +                                       | -                                               | -                |
| 11       | +                                       | -                                               | -                |
| 12       | -                                       | -                                               | -                |
| 13       | +                                       | +                                               | n.a.             |
| 14       | +                                       | +                                               | n.a.             |
| 15       | +                                       | +                                               | n.a.             |

CONCLUSION

PeCR-FISH provides an additional important tool for a straightforward and comprehensive sSMC characterization and also for sSMC-research. Especially it is well suited to identify cryptic mosaics and/or discontinuous sSMC.

LIST OF ABBREVIATIONS

aCGH = Array-comparative genomic hybridization
BAC = Bacterial artificial chromosomes
CNV = Copy number variant
Cy5 = Cyanin 5
DAPI = 4’-6-diamidino-2-phenylindole
DEAC = Diethylaminocoumarin
DOP-PCR = Degenerated oligonucleotide primed polymerase chain reaction
FISH = Fluorescence in situ hybridization
Mbps = Megabasepairs
PCL-FISH = Pericentric-ladder-fluorescence in situ hybridization
PeCR-FISH = Pericentromeric-critical region-fluorescence in situ hybridization
SG = Spectrum Green
SO = Spectrum Orange
sSMCs = Small supernumerary marker chromosome
TR = Texas Red

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.
CONSENT FOR PUBLICATION
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
Supported in parts by the DAAD and the China Scholarship Council. It was also supported by a short term fellowship of the postgraduate academy of the FSU Jena.

SUPPLEMENTARY MATERIAL
Supplementary material is available on the publisher’s web site along with the published article.

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