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Small supernumerary marker chromosomes: a legacy of trisomy rescue?

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Abstract

We studied by a whole genomic approach and trios genotyping, 12 *de novo*, non-recurrent small supernumerary marker chromosomes (sSMC), detected as mosaics during pre- or postnatal diagnosis and associated with increased maternal age. Four sSMCs contained pericentromeric portions only, whereas eight had additional non-contiguous portions of the same chromosome, assembled together in a disordered fashion by repair-based mechanisms in a chromothriptic event. Maternal hetero/isodisomy was detected with a paternal origin of the sSMC in some cases, whereas in others two maternal alleles in the sSMC region and biparental haplotypes of the homologs were detected. In other cases the homologs were biparental while the sSMC had the same haplotype of the maternally inherited chromosome. These findings strongly suggest that most sSMCs are the result of a multiple-step mechanism, initiated by maternal meiotic non-disjunction followed by post-zygotic anaphase lagging of the supernumerary chromosome and its subsequent chromothripsis.

Keywords

chromothripsis, small supernumerary marker chromosome (sSMC), whole genome paired-end sequencing (WGS), maternal meiotic non-disjunction, evolutionary trade-off

Main Text

For a long time de novo non-recurrent small supernumerary marker chromosomes (sSMC) have been considered pieces of chromosomes predominantly derived from the pericentromeric regions or, in rare cases, from acentric portions that have acquired a neocentromere. Accordingly, in terms of genetic counseling, these sSMCs were handled as copy number gains, with genotype-phenotype correlations based on the presence/absence of dosage-sensitive genes, although a prognosis remained challenging in prenatal diagnosis even if no known disease-genes were present. However, over time evidences accumulated showing that, except for the recurrent sSMCs with mirror duplicated genomic regions, including

66 i(12p), idic(15), i(18p), and idic(22), *de novo* SMCs are private rearrangements that may be
67 more complex than previously estimated. Most of them, either recurrent or non-recurrent, are
68 characterized by: (i) increased maternal age at conception, and (ii) a mosaic condition with a
69 normal cell line and a second one with the sSMC (Malvestiti et al., 2014). Seldom, segmental
70 uniparental disomy (UPD) or UPD for the chromosome by which the *de novo* sSMC is
71 derived has also been reported (see for a review Kotzot, 2001; Liehr et al., 2015). Even more
72 rarely, fluorescence in situ hybridization (FISH) or array comparative genomic hybridization
73 (array-CGH) have documented some sSMCs as constituted by non-contiguous regions of the
74 same chromosome or the terminal regions of two different chromosomes (Rothlisberger,
75 2000; Vetro et al., 2012). Moreover, at least in some of the recurrent sSMCs, trios genotyping
76 supported the presence of three genotypes with two being of maternal origin (Conlin et al.,
77 2012; Roberts et al., 2003; Wandstrat & Schwartz, 2000).
78 Our study, approved by the institutional review board of Meyer Hospital in Florence, on 12 *de*
79 *novo* non-recurrent sSMCs (Table 1 and Supp. Table S1), all but one associated with
80 developmental delay and/or phenotypic abnormalities (Supp. Table S1), brings together all
81 previous observations, demonstrating by a whole cytogenomics approach that the primary
82 driver for *de novo* SMCs is a non-disjunction at the maternal meiosis followed by a partial
83 trisomy rescue of the supernumerary chromosome present in the trisomic zygote, through
84 chromothripsis-like processes. Trisomy, which is the most frequent chromosomal abnormality
85 in humans and the leading cause of spontaneous abortions, is essentially linked to
86 chromosome mis-segregation at the maternal meiosis with the risk for a trisomic conceptus
87 increasing with the increase of maternal age (Franasiak et al., 2014; Nagaoka et al., 2012).
88 Trisomy rescue, reported in no less than 1-2% of first trimester invasive prenatal diagnosis
89 (Hahnemann & Vejerslev, 1997; Kalousek & Vekemans, 1996) and considered responsible
90 for most false positive results by non-invasive prenatal screening (Hartwig et al., 2017; Van

91 Opstal et al., 2018) may save some of the embryos otherwise fated to be spontaneously
92 aborted, leading to confined placental mosaicism where the abnormal cell line theoretically is
93 isolated to the placenta and missing from amniotic cells or other fetal tissues. A probably less
94 frequent phenomenon is a partial trisomy rescue in which only a part of the original trisomic
95 chromosome is eliminated while a part remains, more often in the form of a supernumerary
96 marker, in mosaic with a normal cell line. Cases in which the initial full trisomy could be
97 documented by direct villus analysis with the subsequent partial correction leading to the
98 presence of a sSMC are few (Srebniak et al., 2011; Vialard et al., 2009). More numerous are
99 the cases in which the presence of the *de novo* sSMC is accompanied by maternal
100 hetero/isodisomy of the homologous chromosomes (Ahram et al., 2016; Liehr et al., 2015;
101 Melo et al., 2015), a situation that can only be explained by a partial trisomic rescue of the
102 supernumerary chromosome of paternal origin, after a non-disjunction event at the maternal
103 MI. The same applies to those sSMCs in which three different haplotypes at the level of the
104 marker chromosome and biparental origin of the single nucleotide polymorphisms (SNPs)
105 along the normal homologs are detected, with the only difference that the trisomic rescue
106 occurred on one of the two chromosomes of maternal origin. It is well known that anaphase
107 lagging accounts for trisomy rescue of the supernumerary chromosome (Ly & Cleveland,
108 2017; Nicholson et al., 2015) which is then trapped within a micronucleus where massive
109 shattering occurs after disruption of the nuclear envelope exposing DNA to the cytoplasm
110 (Liu et al., 2018; Zhang et al., 2015). As a consequence, the supernumerary chromosome is
111 eliminated in one daughter cell, thus explaining the presence of the normal cell line. After the
112 re-embedding of the micronuclear material into the main nucleus where DNA repair occurs
113 (Ly et al., 2016), a second cell line containing a supernumerary chromothripsed chromosome
114 would form, composed of only parts of the original supernumerary chromosome stitched
115 together in a non-contiguous order. Depending on which of the three homologs undergo

116 anaphase lagging, the remaining two may be in maternal hetero/isodisomy (loss of the
117 paternal one) or of biparental origin (loss of one of the maternal ones). Trios genotyping
118 (Supp. Tables S2, S3 and S4) in cases sSMC2.b, sSMC7.a, sSMC7.b, and sSMC1 detected
119 maternal hetero/isodisomy of the normal homologs while the paternal origin of the sSMC
120 could be demonstrated only in cases sSMC2.b, sSMC7.b, but was inconclusive in cases
121 sSMC1 and sSMC7.a. This condition fits with a maternal meiosis I (mat-MI) non-disjunction,
122 followed by chromothripsis of the supernumerary chromosome of paternal origin. Case
123 sSMC8.a, with two different maternal haplotypes and a paternal one within the chromosome
124 8-derived sSMC region, and biparental SNPs along the two normal chromosomes 8, also
125 indicates a mat-MI non-disjunction as the first event, in this case followed by chromothripsis
126 of one of the chromosomes of maternal origin. In contrast, in cases sSMC18, sSMC2.a,
127 sSMC17, and sSMC11, the marker region has the same haplotype as the intact maternally
128 inherited chromosome, with biparental origin of the SNPs and/or microsatellites along the two
129 homologous chromosomes (Table 1, Supp. Tables S2, S3 and S4). Since the markers we
130 studied are from the pericentromeric regions of the respective chromosomes of origin, where
131 cross-overs are not expected to occur, this finding indicates either a previous maternal meiosis
132 II (mat-MII) nondisjunction or a postzygotic event. Indeed, in a number of cases of trisomy
133 rescue (Butler et al., 2018; Chantot-Bastaraud et al., 2017) a mat-MII error has been
134 documented. Similarly, the mechanism leading to the formation of the supernumerary i(12p),
135 associated with Pallister-Killian syndrome, has been proven to be prezygotic and of maternal
136 origin, presumably occurring at MII as demonstrated by the presence of three genotypes at the
137 distal 12p region and only two at the pericentromeric one (Blyth et al., 2015; Conlin et al.,
138 2012). The only case not compatible with a maternal meiotic non-disjunction is sSMC8.b,
139 whose haplotype was paternal while the normal homologs were biparental (Table 1, Supp.
140 Tables S2, S3 and S4). Thus, in this case we have to assume a postzygotic non-disjunction of

141 the paternal chromosome 8, followed by chromothripsis of the supernumerary 8 and recovery
142 of its pericentromeric region.
143 Overall, we can conclude that the origin of the sSMC from a trisomy caused by maternal non-
144 disjunction error at meiosis I, was directly demonstrated in four cases with hetero/iso UPD
145 (sSMC2.b, sSMC7.a, sSMC7.b and sSMC1) and in one case (sSMC8.a) with two maternal
146 alleles on the marker region, while in five cases (sSMC18, sSMC2.a, sSMC17, sSMC11,
147 sSMC8.c), the demonstration of a maternal meiotic error was indirect (Table 1). Remarkably,
148 in all of these cases except for sSMC18 the maternal age at birth (Table 1) was increased
149 (37.4 years on average), in agreement with a triggering event of maternal meiotic non-
150 disjunction. To get further insight into the sSMCs structure and their breakpoint
151 characteristics, we performed paired-end whole genome sequencing (WGS) (Supp. Table S5)
152 in 10 out of the 12 cases, using Illumina TruSeq DNA PCR Free library, with DNA isolated
153 from blood in 8 cases, abortive tissue in 1 case (sSMC2.b) and amniotic fluid in 1 case
154 (sSMC11), and try to confirm all possible breakpoints by PCR and Sanger Sequencing.
155 Indeed, a full reconstruction of the sSMCs with Sanger confirmation of all the WGS
156 breakpoints was successful only for sSMC18, while we failed to confirm 22 out of the total 60
157 WGS breakpoints. Anyway this analysis (Table 1, Supp. Table S6 and Supp. Figures S1-S13)
158 revealed that the sSMCs in 7 out of 10 cases, in addition to the pericentromeric region,
159 contained one or more additional segments from their corresponding chromosomes, which
160 were disordered assembled, a finding highly suggestive of a chromothripsis event. Notably,
161 previous CGH or SNP+CGH array investigations had highlighted a non-contiguous
162 constitution only in 4 of these cases (Supp. Table S1 and S6). Among the 60 WGS
163 breakpoints we identified within the duplicated regions (4 in sSMC18, 7 in sSMC2.a, 4 in
164 sSMC2.b, 5 in sSMC7.a, 6 in sSMC17, 6 in sSMC8.a, 2 in sSMC8.b, 2 in sSMC7.b, 2 in
165 sSMC1, 22 in sSMC11), we could fully characterize 19 fusion junctions (Supp. Table S6),

166 which showed chromothripsis signatures such as blunt fusions (4: one in sSMC2.b and
167 sSMC7.a, two in sSMC11), 2 to 8 bp microhomology (7: one in sSMC2.a, sSMC8.a, and
168 SMC8.b, two in sSMC11 and sSMC18), and 2 to 36 bp insertions (12: one in sSMC2.a,
169 sSMC7.a and SMC17, three in sSMC8.a, and six in sSMC11), indicating predominantly
170 repair-based (NHEJ or alt-NHEJ) mechanism (Table 1). Similar sequence signature has been
171 observed in rearrangements proposed to be formed by a replicative-repair mechanism,
172 MMBIR (Carvalho & Lupski, 2016), which uses microhomology to restore a collapsed
173 replication fork. On the other hand, in most of our cases, genotyping analysis on whole
174 chromosome and not only on the duplication region showed that the duplication was the
175 residual portion of the third chromosome rather than emerging through a microhomology-
176 driven DNA synthesis. Among the insertions, two were Line-1 elements (sSMC7a and
177 sSMC17) and two were small insertions coming from distal portions of the same chromosome
178 (sSMC11), while the remaining ones were non-templated. Approximately 62% of the
179 breakpoints detected by WGS were located in repeated regions and 20% of these repeats were
180 LINE elements. Based on the Sanger sequencing data covering 400bp downstream and
181 upstream of the fusion junction we did not observe further *de novo* point mutations. In all but
182 two cases (sSMC1 and sSMC7.b) the sSMC had one of the breakpoints falling within the
183 centromeric alphoid sequences, which impaired the complete characterization of the
184 breakpoint sequences. Only in case sSMC18 (Supp. Figure S1), in which the sSMC was
185 constituted by the fusion of the two non-contiguous duplicated segments, 18b and 18d, we
186 were able to identify both the two novel fusion junctions in spite one involved the alphoid
187 sequences: BPJ_18b(+)_18d(+) (chr18:18594804::chr18:41472065) and ring closure junction
188 RingJ_18d(+)_Alphoid (chr18:49040431::Alphoid DNA L1.84 of chromosome 18). Absence
189 of telomere sequences, as demonstrated by metaphase FISH analysis using telomere specific
190 (TTAGGG) PNA probes, supported its ring constitution. In case sSMC8.a (Supp. Figure S2),

191 the initial SNP+CGH array indicated the marker as constituted by a single copy number gain
192 at 8p11.21p11.1, while NGS data showed that the discordant reads, at the edge of the
193 chr8:40082798-53561524 pericentromeric region, mapped also at two distally located
194 additional copy number gains (fragments 8f at chr8:60002688-60002774 and 8d at
195 chr8:55759348-55759565). Sanger confirmation allowed imputing the exact closure junction,
196 thus indicating a ring structure, also supported by the TTAGGG FISH analysis. In sSM2.a
197 (Supp. Figure S3), we identified four separate copy number gain regions with different levels
198 of coverage, indicating triplication of fragment 2b (chr2:95326241-98026880), showing a
199 3~4x relative coverage, duplication of a fragment 2c (chr2:98058590-102613162), suggested
200 by its 3x relative coverage, and mosaic duplications of fragments 2d (chr2:102613,162-
201 102867861) and 2f (chr2:106555286-107260062), both having 2~3x relative coverage.
202 Although discordant reads were detected only at the end of fragment 2c, a novel fusion
203 junction was highlighted by Sanger, between fragments 2c and 2f
204 (chr2:102613162::chr2:106555286), thus demonstrating their disordered orientation. In this
205 case, the presence of duplication and triplication copy number gains, suggested the
206 involvement of a chromoanasynthesis event as recently reported for a maternally inherited
207 sSMC9 (Grochowski et al., 2018). In case sSMC11 (Supp. Figure S4), NGS analysis revealed
208 an unexpected complexity compared to the initial CGH-array data in which a single *de novo*
209 9,1Mb pericentric duplication between 11p11.2 and 11q12.1 was detected. A second
210 duplication at distal 11p (Supp. Figure S4) is a false, possibly related to the control DNA.
211 Indeed the same duplication was shown in all the DNAs analyzed by array-CGH using this
212 specific control DNA kit, including those of the mother and her partner. Coverage analysis
213 after WGS revealed a series of duplicated portions spanning the entire 11p up to 11q12.1.
214 Discordant reads at the breakpoints of each copy number gain region, revealed a total of 14
215 fragments, where 13 were stitched together in a disordered pattern. By Sanger sequencing we

216 could solve 8 out of the 12 novel fusions. A ring chromosome constitution was suggested by
217 the absence of telomere sequence on sSMC11. Remarkably, we detected Alu-Alu mediated
218 recombination at six fusion junctions (Supp. Figure S5). Involvement of Alu elements in
219 constitutional chromothripsis was recently reported in a family (Nazaryan-Petersen et al.,
220 2016).
221 Gene disruptions were detected in 29 out of 60 breakpoints (Supp. Table S6), 28 of them
222 occurring within introns while one was exonic. Only in case sSMC11, a possible fusion gene
223 was predicted as a result of the fusion of two truncated genes (*PHF21A-SLC39A13*).
224 As a whole, our data show that the trigger for the formation of *de novo* non-recurrent sSMCs
225 is a maternal meiotic non-disjunction followed by a post-zygotic chromothripsis event, due to
226 anaphase lagging and repositioning of one of the trisomic chromosomes within a
227 micronucleus. It seems likely that the formation of the new chromosome after the massive
228 shattering that occurred following anaphase lagging, depends on stochastic events, in the
229 context however of some main limitations such as the propensity of the broken ends of the
230 various fragments to integrate with each other, and the selection of more capable cells to
231 survive and multiply in the presence of supernumerary chromosomal portions. Centric
232 fragments (b and dbe in Figure 1) should be easily preserved as sSMC, provided that they
233 assume a ring conformation to compensate for the absence of telomeric sequences at both
234 ends. Indeed FISH analysis in sSMC18, sSMC2.b, sSMC7.a, sSMC8.a, sSMC7.b, sSMC11,
235 sSMC7.c, and sSMC8.c, whose small size made it impossible to understand if they were
236 linear or circular structures, demonstrated the absence of the telomeric sequences, thus
237 supporting their ring conformation. In contrast, chromothripsed fragments equipped with both
238 centromeric and telomeric sequences at one end only (ab in Figure 1), may be stabilized
239 provided that they capture a telomeric region from another chromosome, thus forming a linear
240 *de novo* derivative supernumerary marker chromosome (cases 3 and 4 in Vetro et al., 2012).

241 Instead, the preservation of supernumerary interstitial acentric fragments (de in Figure 1)
242 would require a neocentromerization event as indeed demonstrated in some sSMCs (Klein et
243 al., 2012) and their circularization (Figure 1). The case reported by Kato et al., 2017 of a de
244 novo interstitial translocation derived by chromothripsis of a supernumerary chromosome
245 present in a trisomic zygote, demonstrates that acentric interstitial fragments may also be
246 captured by another chromosome (Figure 1). In contrast, chromothripsed fragments equipped
247 with telomeric sequences but without centromere (f in Figure 1) may be captured by a non-
248 chromothripsed chromosome which, by losing its distal portion, generates a de novo
249 unbalanced translocation, as recently demonstrated for a number of them (Bonaglia et al.,
250 2018).

251 In conclusion our findings give account of all the peculiarities associated with de novo sSMC:
252 maternal meiotic non-disjunction, which is the prelude to the formation of the sSMC, explains
253 the increased maternal age reported in most *de novo* cases; anaphase lagging of the
254 supernumerary chromosome and its subsequent insertion within a micronucleus that
255 segregates to one of the two daughter cells, accounts for the mosaic condition with a normal
256 cell line and a second one containing the sSMC; maternal (segmental) UPD occurs whenever
257 the partial trisomy rescue affects the chromosome of paternal origin; chromothripsis explains
258 why some sSMCs are formed by non-contiguous regions of a given chromosome. This
259 multiple-step mechanism underlying the formation of most non-recurrent *de novo* sSMCs
260 identifies a link between numerical and structural chromosomal anomalies and indeed
261 suggests investigating how frequently other structural anomalies such as some unbalanced *de*
262 *novo* translocations and insertions may be the final result of a mechanism initiated by a
263 trisomy (Bonaglia et al., 2018; Kato et al., 2017), passing through the elimination of the
264 supernumerary chromosome by anaphase lagging and subsequent chromothripsis, as already
265 anticipated (Janssen et al., 2011). On the other hand, from the point of view of genetic

266 counseling, the discovery of such a multiple-step mechanism reveals a bitter truth, that is that
267 the prognosis for those sSMCs identified in prenatal diagnosis will be infeasible. Indeed
268 within a chromosome formed by multiple pieces, disruption of higher-order chromatin
269 organization such as topologically associating domains (Spielmann et al., 2018) will occur.
270 The final effect of altered gene dosage, potential for dysregulation and for formation of new
271 genes by gene fusion (Spielmann et al., 2018), all in a mosaic state, will be a highly
272 problematic cocktail.

273 Trisomy rescue is likely to be the evolutionary trade-off to compensate for the massive loss of
274 embryos caused by the high level of aneuploidy of human female gametes. The push towards
275 elimination of the supernumerary chromosome must be elevated at least in the early stages of
276 early embryogenesis, as suggested by the demonstration of multiple rescue events in 3 out of
277 10 placentas from newborns with autosomal trisomy at the NIPT (Van Opstal et al., 2018).
278 However, the rarity with which the loss of the supernumerary chromosome is estimated to
279 occur in healthy people (King et al., 2014; Robinson, 2000) indicates that this event, although
280 providing a rescue from deleterious conditions, has no evolutionary advantage and reinforces
281 the idea that meiotic non-disjunction in human females and the consequent aneuploidy
282 leading to implantation failure and early miscarriage, is under Darwinian pressure. Indeed, by
283 increasing the time between subsequent pregnancies, thus preserving the maternal resources,
284 and by decreasing the likelihood of pregnancy in women too old to raise children (Wang et
285 al., 2017; Warburton, 1987), the immense failure of aneuploidy pregnancies appears an
286 optimal strategy to ensure the offspring of the attention and nourishment necessary for their
287 survival and, not last, reduce the risk of dying from delivery haemorrhage. Noteworthy, the
288 human life span from prehistory until 300 years ago was much shorter (Trinkaus, 2011), so
289 women did not reach the menopause age and remained fertile until their death. On the other
290 hand, most of the embryos carrying genetic defects secondary to total/partial trisomy rescue,

either imprinting disorders, autosomal recessive diseases due to UPD, and supernumerary marker chromosomes for which a negative outcome is reported in 14-30% of the cases, appear able to get to the postnatal life, thus dissipating the benefits provided by the early loss of the conceptus. This may account for the limited evolutionary success of this mechanism.

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Declaration of Interests

The authors declare no conflict of interest.

Accession Numbers

Reconstruction of sSMC and clinical data have been archived in publically available database for sSMC (http://ssmc-tl.com/sSMC.html). The accession numbers for the cases sSMC1, sSMC2.a, sSMC2.b, sSMC7.a, sSMC7.b, sSMC7.c, sSMC8.a, sSMC8.b, sSMC8.c and sSMC11 reported in this paper are: 01-Uu-3, 02-Ud-1, 02-Ud-2, 12L0080, 07-Uu-10, 07-Uu-9, 2010B110, 08-W-p23.1/2-1, 08-Ud-6 and 11-Ud-2.

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452 **Figure Legends**

453 **Figure 1: Fate of the supernumerary chromosome undergoing chromothripsis**

454 On the left, the hypothethical supernumerary chromosome shattered in a number of fragments
455 (a, b, c, d, e, f); telomeres are in red, centromere in light brown. Depending on which
456 fragments of the original in-trisomy chromosome that are preserved and lost after
457 chromothripsis, different types of rearrangements may be formed. **Top box**: Partial rescue of
458 trisomy leading to constitution of a supernumerary marker chromosome (sSMC). **Centric**
459 **fragment**: when at least a centric fragment (centromere in light brown) without telomeric
460 sequences is preserved, the sSMC is a ring chromosome formed either by the single
461 centromeric region or also by other non-contiguous portions of the original supernumerary
462 chromosome. A single fragment ring and a complex one, formed by non-contiguous
463 fragments, are depicted. If both a centric and one telomeric portion (in red) are preserved, the
464 chromothripsed chromosome may acquire a second stabilizing telomeric region (in dark
465 brown) from another chromosome, generating a derivative supernumerary chromosome, as
466 reported in Vetro et al., 2012. **Acentric fragment**: when the preserved fragment(s) does not
467 contain either a centromeric or telomeric sequence, the acquisition of a neocentromere and the
468 circularization of the fragment(s) may result in a stable sSMC. **Lower box**: Partial trisomy
469 rescue leading to the formation of unbalanced translocation or insertion. Left: an acentric
470 fragment equipped with one telomeric portion is donated to a recipient chromosome that loses
471 one of its distal regions, leading to an unbalanced translocation within a 46 chromosome
472 karyotype (Bonaglia et al., 2018). Right: acentric fragment(s) devoid of telomeric sequences,
473 may be inserted within another chromosome leading to an unbalanced insertion within a 46
474 chromosome karyotype, as reported in Kato et al., 2017. As an alternative pathway, it can

475 undergo the circularization and acquisition of a neocentromere, resulting in a sSMC (see
476 above).
477 Notably, the pathogenic consequences for these rearrangements may be exacerbated if the
478 partial rescue of the trisomy is borne by the chromosome inherited from the father, leading to
479 maternal hetero / isodisomy for the remaining two chromosomes.
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