Small supernumerary marker chromosomes: A legacy of trisomy rescue?

Small supernumerary marker chromosomes: a legacy of trisomy rescue?

Authors and Affiliations

Nehir Edibe Kurtas[1*], Luciano Xumerle[2], Lorena Leonardelli[2¶], Massimo Delledonne[2], Alfredo Brusco[3], Krystyna Chrzanowska[4], Albert Schinzel[5], Daniela Larizza[6], Silvana Guerneri[7], Federica Natacci[7], Maria Clara Bonaglia[8], Paolo Reho[9], Emmanouil Manolakos[10], Teresa Mattina[11], Fiorenza Soli[12], Aldesia Provenzano[9,13], Ahmed H. Al-Rikabi[14], Edoardo Errichiello[1], Lusine Nazaryan-Petersen[15], Sabrina Giglio[9,13], Niels Tommerup[15], Thomas Liehr[14], Orsetta Zuffardi[1*]

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy,

[2]Department of Biotechnology, University of Verona, Verona, Italy,

[3]Department of Medical Sciences, University of Turin, Torino, Italy,

[4]Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland,

[5]Institute of Medical Genetics, University of Zurich, Zurich, Switzerland,

[6]Pediatrics and Adolescentology Unit, University of Pavia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

[7]Laboratory of Medical Genetics, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy,

[8]Cytogenetics Laboratory, Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy

[9] Biomedical Experimental and Clinical Sciences "Mario Serio", University of Florence, Firenze, Italy,

[10]Laboratory of Genetics, Access to genome P.C., Thessaloniki, Greece,

[11]Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

[12] Department of Genetics, Santa Chiara Hospital, Trento, Italy

[13]Azienda Ospedaliero-Universitaria Meyer, Firenze, Italy,

[14]Institute of Human Genetics, Jena University Hospital, Jena, Germany

[15]Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

*Corresponding Authors

Nehir Edibe Kurtas

e-mail:nehirkurtas@gmail.com

Orsetta Zuffardi

e-mail: orsetta.zuffardi@unipv.it

¶present address: The Microsoft Research, University of Trento Centre for Computational and Systems Biology (COSBI), Rovereto, Italy.

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

i(12p), idic(15), i(18p), and idic(22), *de novo* SMCs are private rearrangements that may be more complex than previously estimated. Most of them, either recurrent or non-recurrent, are characterized by: (i) increased maternal age at conception, and (ii) a mosaic condition with a normal cell line and a second one with the sSMC (Malvestiti et al., 2014). Seldom, segmental uniparental disomy (UPD) or UPD for the chromosome by which the *de novo* sSMC is derived has also been reported (see for a review Kotzot, 2001; Liehr et al., 2015). Even more rarely, fluorescence in situ hybridization (FISH) or array comparative genomic hybridization (array-CGH) have documented some sSMCs as constituted by non-contiguous regions of the same chromosome or the terminal regions of two different chromosomes (Rothlisberger, 2000; Vetro et al., 2012). Moreover, at least in some of the recurrent sSMCs, trios genotyping supported the presence of three genotypes with two being of maternal origin (Conlin et al., 2012; Roberts et al., 2003; Wandstrat & Schwartz, 2000).

Our study, approved by the institutional review board of Meyer Hospital in Florence, on 12 *de novo* non-recurrent sSMCs (Table 1 and Supp. Table S1), all but one associated with developmental delay and/or phenotypic abnormalities (Supp. Table S1), brings together all previous observations, demonstrating by a whole cytogenomics approach that the primary driver for *de novo* SMCs is a non-disjunction at the maternal meiosis followed by a partial trisomy rescue of the supernumerary chromosome present in the trisomic zygote, through chromothripsis-like processes. Trisomy, which is the most frequent chromosomal abnormality in humans and the leading cause of spontaneous abortions, is essentially linked to chromosome mis-segregation at the maternal meiosis with the risk for a trisomic conceptus increasing with the increase of maternal age (Franasiak et al., 2014; Nagaoka et al., 2012). Trisomy rescue, reported in no less than 1-2% of first trimester invasive prenatal diagnosis (Hahnemann & Vejerslev, 1997; Kalousek & Vekemans, 1996) and considered responsible for most false positive results by non-invasive prenatal screening (Hartwig et al., 2017; Van

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

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

the paternal chromosome 8, followed by chromothripsis of the supernumerary 8 and recovery of its pericentromeric region.

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

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

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

could solve 8 out of the 12 novel fusions. A ring chromosome constitution was suggested by the absence of telomere sequence on sSMC11. Remarkably, we detected Alu-Alu mediated recombination at six fusion junctions (Supp. Figure S5). Involvement of Alu elements in constitutional chromothripsis was recently reported in a family (Nazaryan-Petersen et al., 2016).

Gene disruptions were detected in 29 out of 60 breakpoints (Supp. Table S6), 28 of them occurring within introns while one was exonic. Only in case sSMC11, a possible fusion gene was predicted as a result of the fusion of two truncated genes (*PHF21A-SLC39A13*).

As a whole, our data show that the trigger for the formation of *de novo* non-recurrent sSMCs is a maternal meiotic non-disjunction followed by a post-zygotic chromothripsis event, due to anaphase lagging and repositioning of one of the trisomic chromosomes within a micronucleus. It seems likely that the formation of the new chromosome after the massive shattering that occurred following anaphase lagging, depends on stochastic events, in the context however of some main limitations such as the propensity of the broken ends of the various fragments to integrate with each other, and the selection of more capable cells to survive and multiply in the presence of supernumerary chromosomal portions. Centric fragments (b and dbe in Figure 1) should be easily preserved as sSMC, provided that they assume a ring conformation to compensate for the absence of telomeric sequences at both ends. Indeed FISH analysis in sSMC18, sSMC2.b, sSMC7.a, sSMC8.a, sSMC7.b, sSMC11, sSMC7.c, and sSMC8.c, whose small size made it impossible to understand if they were linear or circular structures, demonstrated the absence of the telomeric sequences, thus supporting their ring conformation. In contrast, chromothripsed fragments equipped with both centromeric and telomeric sequences at one end only (ab in Figure 1), may be stabilized provided that they capture a telomeric region from another chromosome, thus forming a linear *de novo* derivative supernumerary marker chromosome (cases 3 and 4 in Vetro et al., 2012).

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

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

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

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

Acknowledgments

We are grateful to all of the families and patients for their participation to this study.

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.

References

Ahram, D. F., Stambouli, D., Syrogianni, A., Al-Sarraj, Y., Gerou, S., El-Shanti, H., & Kambouris, M. (2016). Mosaic partial pericentromeric trisomy 8 and maternal uniparental disomy in a male patient with autism spectrum disorder. *Clinical Case Reports*, *4*(12), 1125–1131. https://doi.org/10.1002/ccr3.705

Blyth, M., Maloney, V., Beal, S., Collinson, M., Huang, S., Crolla, J., … Baralle, D. (2015). Pallister-Killian syndrome: a study of 22 British patients. *Journal of Medical Genetics*, *52*(7), 454–464. https://doi.org/10.1136/jmedgenet-2014-102877

Bonaglia, M. C., Kurtas, N. E., Errichiello, E., Bertuzzo, S., Beri, S., Mehrjouy, M. M., … Zuffardi, O. (2018). De novo unbalanced translocations have a complex history/aetiology. *Human Genetics*, *137*(10), 817–829. https://doi.org/10.1007/s00439-018-1941-9

Butler, M. G., Hartin, S. N., Hossain, W. A., Manzardo, A. M., Kimonis, V., Dykens, E., … Driscoll, D. J. (2018). Molecular genetic classification in Prader-Willi syndrome: a multisite cohort study. *Journal of Medical Genetics*.

Carvalho, C. M. B., & Lupski, J. R. (2016). Mechanisms underlying structural variant

formation in genomic disorders. *Nature Reviews Genetics*, *17*(4), 224–238. https://doi.org/10.1038/nrg.2015.25

Chantot-Bastaraud, S., Stratmann, S., Brioude, F., Begemann, M., Elbracht, M., Graul-Neumann, L., … Eggermann, T. (2017). Formation of upd(7)mat by trisomic rescue: SNP array typing provides new insights in chromosomal nondisjunction. *Molecular Cytogenetics*, *10*(1), 1–7. https://doi.org/10.1186/s13039-017-0329-1

Conlin, L. K., Kaur, M., Izumi, K., Campbell, L., Wilkens, A., Clark, D., … Krantz, I. D. (2012). Utility of SNP arrays in detecting, quantifying, and determining meiotic origin of tetrasomy 12p in blood from individuals with Pallister-Killian syndrome. *American Journal of Medical Genetics, Part A*, *158 A*(12), 3046–3053. https://doi.org/10.1002/ajmg.a.35726

Franasiak, J. M., Forman, E. J., Hong, K. H., Werner, M. D., Upham, K. M., Treff, N. R., & Scott, R. T. J. (2014). The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertility and Sterility*, *101*(3), 656–663.e1. https://doi.org/10.1016/j.fertnstert.2013.11.004

Grochowski, C. M., Gu, S., Yuan, B., Tcw, J., Brennand, K. J., Sebat, J., … Carvalho, C. M. B. (2018). Marker chromosome genomic structure and temporal origin implicate a chromoanasynthesis event in a family with pleiotropic psychiatric phenotypes. *Human Mutation*, (December 2017), 939–946. https://doi.org/10.1002/humu.23537

Hahnemann, J. M., & Vejerslev, L. O. (1997). European collaborative research on mosaicism in CVS (EUCROMIC)--fetal and extrafetal cell lineages in 192 gestations with CVS mosaicism involving single autosomal trisomy. *American Journal of Medical Genetics*, *70*(2), 179–187.

Hartwig, T. S., Ambye, L., Sorensen, S., & Jorgensen, F. S. (2017). Discordant non-invasive prenatal testing (NIPT) - a systematic review. *Prenatal Diagnosis*, *37*(6), 527–539. https://doi.org/10.1002/pd.5049

Janssen, A., van der Burg, M., Szuhai, K., Kops, G. J. P. L., & Medema, R. H. (2011). Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science (New York, N.Y.)*, *333*(6051), 1895–1898. https://doi.org/10.1126/science.1210214

Kalousek, D. K., & Vekemans, M. (1996). Confined placental mosaicism. *Journal of Medical Genetics*, *33*(7), 529–533.

Kato, T., Ouchi, Y., Inagaki, H., Makita, Y., Mizuno, S., Kajita, M., … Kurahashi, H. (2017). Genomic Characterization of Chromosomal Insertions: Insights into the Mechanisms Underlying Chromothripsis. *Cytogenetic and Genome Research*, *1192*, 1–9. https://doi.org/10.1159/000481586

King, D. A., Fitzgerald, T. W., Miller, R., Canham, N., Clayton-Smith, J., Johnson, D., … Hurles, M. E. (2014). A novel method for detecting uniparental disomy from trio genotypes identifies a significant excess in children with developmental disorders. *Genome Research*, *24*(4), 673–687. https://doi.org/10.1101/gr.160465.113

Klein, E., Rocchi, M., Ovens-Raeder, A., Kosyakova, N., Weise, A., Ziegler, M., … Liehr, T.

(2012). Five novel locations of Neocentromeres in human: 18q22.1, Xq27.1 approximately 27.2, Acro p13, Acro p12, and heterochromatin of unknown origin. *Cytogenetic and Genome Research*, *136*(3), 163–166. https://doi.org/10.1159/000336648

Kotzot, D. (2001). Complex and segmental uniparental disomy (UPD): review and lessons from rare chromosomal complements. *J Med Genet*, *38*, 497–507.

Liehr, T., Ewers, E., Hamid, A. B., Kosyakova, N., Voigt, M., Weise, A., & Manvelyan, M. (2015). Small Supernumerary Marker Chromosomes and Uniparental Disomy Have a Story to Tell. *Journal of Histochemistry & Cytochemistry 59(9) 842 –848*.

Liu, S., Kwon, M., Mannino, M., Yang, N., Renda, F., Khodjakov, A., & Pellman, D. (2018). Nuclear envelope assembly defects link mitotic errors to chromothripsis. *Nature*, *561*(7724), 551–555. https://doi.org/10.1038/s41586-018-0534-z

Ly, P., & Cleveland, D. W. (2017). Interrogating cell division errors using random and chromosome-specific missegregation approaches. *Cell Cycle (Georgetown, Tex.)*, *16*(13), 1252–1258. https://doi.org/10.1080/15384101.2017.1325047

Ly, P., Teitz, L. S., Kim, D. H., Shoshani, O., Skaletsky, H., Fachinetti, D., … Cleveland, D. W. (2016). Selective Y centromere inactivation triggers chromosome shattering in micronuclei and repair by non-homologous end joining. *Nature Cell Biology*, *1*(April). https://doi.org/10.1038/ncb3450

Malvestiti, F., De Toffol, S., Grimi, B., Chinetti, S., Marcato, L., Agrati, C., … Grati, F. R. (2014). De novo small supernumerary marker chromosomes detected on 143000 consecutive prenatal diagnoses: Chromosomal distribution, frequencies, and characterization combining molecular cytogenetics approaches. *Prenatal Diagnosis*, *34*(5), 460–468. https://doi.org/10.1002/pd.4330

Melo, B. C. S., Portocarrero, A., Alves, C., Sampaio, A., & Mota-Vieira, L. (2015). Paternal Transmission of Small Supernumerary Marker Chromosome 15 Identified in Prenatal Diagnosis Due to Advanced Maternal Age. *Clinical Medicine Insights. Case Reports*, *8*, 93–96. https://doi.org/10.4137/CCRep.S31958

Nagaoka, S. I., Hassold, T. J., & Hunt, P. A. (2012). Human aneuploidy: Mechanisms and new insights into an age-old problem. *Nature Reviews Genetics*. https://doi.org/10.1038/nrg3245

Nazaryan-Petersen, L., Bertelsen, B., Bak, M., Jønson, L., Tommerup, N., Hancks, D. C., & Tümer, Z. (2016). Germline Chromothripsis Driven by L1-Mediated Retrotransposition and Alu/Alu Homologous Recombination. *Human Mutation*, *37*(4), 385–395. https://doi.org/10.1002/humu.22953

Nicholson, J. M., Macedo, J. C., Mattingly, A. J., Wangsa, D., Camps, J., Lima, V., … Cimini, D. (2015). Chromosome mis-segregation and cytokinesis failure in trisomic human cells. *ELife*, *4*(MAY), 1–23. https://doi.org/10.7554/eLife.05068

Roberts, S. E., Maggouta, F., Thomas, N. S., Jacobs, P. A., & Crolla, J. A. (2003). Molecular and fluorescence in situ hybridization characterization of the breakpoints in 46 large supernumerary marker 15 chromosomes reveals an unexpected level of complexity. *American Journal of Human Genetics*, *73*(5), 1061–1072. https://doi.org/10.1086/379155

Robinson, W. P. (2000). Mechanisms leading to uniparental disomy and their clinical consequences. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology*, *22*(5), 452–459. https://doi.org/10.1002/(SICI)1521-1878(200005)22:5<452::AID-BIES7>3.0.CO;2-K

Rothlisberger, B. (2000). A supernumerary marker chromosome originating from two different regions of chromosome 18. *Journal of Medical Genetics*, *37*, 121–124. https://doi.org/10.1136/jmg.37.2.121

Spielmann, M., Lupiáñez, D. G., & Mundlos, S. (2018). Structural variation in the 3D genome. *Nature Reviews Genetics*, *19*(7), 453–467. https://doi.org/10.1038/s41576-018-0007-0

Srebniak, M., Boter, M., Oudesluijs, G., Joosten, M., Govaerts, L., Van Opstal, D., & Galjaard, R. J. H. (2011). Application of SNP array for rapid prenatal diagnosis: Implementation, genetic counselling and diagnostic flow. *European Journal of Human Genetics*, *19*(12), 1230–1237. https://doi.org/10.1038/ejhg.2011.119

Trinkaus, E. (2011). Late Pleistocene adult mortality patterns and modern human establishment. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(4), 1267–1271. https://doi.org/10.1073/pnas.1018700108

Van Opstal, D., Diderich, K. E. M., Joosten, M., Govaerts, L. C. P., Polak, J., Boter, M., … Srebniak, M. I. (2018). Unexpected finding of uniparental disomy mosaicism in term placentas: is it a common feature in trisomic placentas? *Prenatal Diagnosis*, (March), 1–9. https://doi.org/10.1002/pd.5354

Van Opstal, D., van Maarle, M. C., Lichtenbelt, K., Weiss, M. M., Schuring-Blom, H., Bhola, S. L., … Sistermans, E. A. (2018). Origin and clinical relevance of chromosomal aberrations other than the common trisomies detected by genome-wide NIPS: results of the TRIDENT study. *Genetics in Medicine : Official Journal of the American College of Medical Genetics*, *20*(5), 480–485. https://doi.org/10.1038/gim.2017.132

Vetro, A., Manolakos, E., Petersen, M. B., Thomaidis, L., Liehr, T., Croci, G., … Zuffardi, O. (2012). Unexpected results in the constitution of small supernumerary marker chromosomes. *European Journal of Medical Genetics*, *55*(3), 185–190. https://doi.org/10.1016/j.ejmg.2012.01.010

Vialard, F., Molina-Gomes, D., Quarello, E., Leroy, B., Ville, Y., & Selva, J. (2009). Partial chromosome deletion: a new trisomy rescue mechanism? *Fetal Diagnosis and Therapy*, *25*(1), 111–114. https://doi.org/10.1159/000203400

Wandstrat, A. E., & Schwartz, S. (2000). Isolation and molecular analysis of inv dup(15) and construction of a physical map of a common breakpoint in order to elucidate their mechanism of formation. *Chromosoma*, *109*(7), 498–505.

Wang, S., Hassold, T., Hunt, P., White, M. A., Zickler, D., Kleckner, N., & Zhang, L. (2017). Inefficient Crossover Maturation Underlies Elevated Aneuploidy in Human Female Meiosis. *Cell*, *168*(6), 977–989.e17. https://doi.org/10.1016/j.cell.2017.02.002

Warburton, D. (1987, January). Reproductive loss: how much is preventable? *The New England Journal of Medicine*. United States. https://doi.org/10.1056/NEJM198701153160308

Zhang, C.-Z., Spektor, A., Cornils, H., Francis, J. M., Jackson, E. K., Liu, S., … Pellman, D. (2015). Chromothripsis from DNA damage in micronuclei. *Nature*, *522*, 179–184. https://doi.org/10.1038/nature14493

Figure Legends

Figure 1: Fate of the supernumerary chromosome undergoing chromothripsis

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

undergo the circularization and acquisition of a neocentromere, resulting in a sSMC (see above).

Notably, the pathogenic consequences for these rearrangements may be exacerbated if the partial rescue of the trisomy is borne by the chromosome inherited from the father, leading to maternal hetero / isodisomy for the remaining two chromosomes.