Multiple Small Supernumerary Marker Chromosomes Resulting from Maternal Meiosis I or II Errors

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Key Words

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Abstract

We present 2 cases with multiple de novo supernumerary marker chromosomes (sSMCs), each derived from a different chromosome. In a prenatal case, we found mosaicism for an sSMC(4), sSMC(6), sSMC(9), sSMC(14) and sSMC(22), while a postnatal case had an sSMC(4), sSMC(8) and an sSMC(11). SNP-marker segregation indicated that the sSMC(4) resulted from a maternal meiosis II error in the prenatal case. Segregation of short tandem repeat markers on the sSMC(8) was consistent with a maternal meiosis I error in the postnatal case. In the latter, a boy with developmental/psychomotor delay, autism, hyperactivity, speech delay, and hypotonia, the sSMC(8) was present at the highest frequency in blood. By comparison to other patients with a corresponding dupli-

cation, a minimal region of overlap for the phenotype was identified, with *CHRNA3* and *CHRNA6* as dosage-sensitive candidate genes. These genes encode subunits of nicotinic acetylcholine receptors (nAChRs). We propose that overproduction of these subunits leads to perturbed component stoichiometries with dominant negative effects on the function of nAChRs, as was shown by others in vitro. With the limitation that in each case only one sSMC could be studied, our findings demonstrate that different meiotic errors lead to multiple sSMCs. We relate our findings to age-related aneuploidy in female meiosis and propose that predivision sister-chromatid separation during meiosis I or II, or both, may generate multiple sSMCs.

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Additional centric chromosome fragments, which are too small for unambiguous identification by classical cytogenetic techniques alone, are termed small supernu-

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merary marker chromosomes (sSMCs). A so-called 'single sSMC' is detected in about 0.2% of prenatal cases with ultrasound abnormalities and in about 0.3% of postnatal patients with multiple congenital anomalies and/or mental retardation (MCA/MR) [Liehr and Weise, 2007]. Some sSMCs have been associated with specific clinical disorders, for example +i(12)(p10) in Pallister-Killian syndrome, +i(18)(p10) in the iso-18p syndrome, and +inv dup(22)(q11.2) in cat eye syndrome. For the majority of the other single sSMCs, a delineation of the relationship between genotype and clinical phenotype is a work in continuing progress [Crolla et al., 2005; Starke et al., 2003; Liehr et al., 2004, 2006; Liehr, 2012, 2015]. Using morphological criteria, sSMCs can be described as centric minute, ring, iso- or inverted-duplication derivative chromosomes. Most sSMCs are derived from a single chromosome. In rare cases, so-called complex-rearranged sSMCs are formed from 2 different chromosomes [Liehr et al., 2004, 2013; Vetro et al., 2012; Yu et al., 2012; Malvestiti et al., 2014]. Several mechanisms have been proposed to explain the formation of a de novo, single sSMC, including mechanisms involving trisomic rescue [Liehr et al., 2004, 2006, 2011; Crolla et al., 2005; Liehr, 2012].

In contrast to the 0.3% prevalence of carriers of a single sSMC among patients with MCA/MR is the limited number of reports on patients carrying multiple sSMCs. In such patients, more than one sSMC is present in most cells and each sSMC is derived from a different chromosome [Beverstock et al., 2003; Daniel and Malafiej, 2003; Reddy et al., 2003; Liehr, 2012]. Currently, there are 78 cases on record [Liehr, 2015]. All of the 24 different chromosomes can give rise to one of the sSMCs in these patients. In almost all cases with MCA/MR, the sSMCs originated de novo from parents with a normal karyotype, and in most of these, the sSMCs are associated with the patient's clinical phenotype.

Little is known about the mechanisms giving rise to those multiple sSMCs. The mechanism and parental origin have been ascertained by molecular approaches in only 4 patients. In a previous study, we showed that an sSMC(11) and an sSMC(X) in a man with obesity, congenital heart defect and moderate intellectual disability were maternally derived and originated from a meiosis II error [Hochstenbach et al., 2013]. In a boy with developmental delay, autism, verbal apraxia, and mild dysmorphic features, a maternal origin of both sSMC(13) and sSMC(17) was demonstrated as well [Kogan et al., 2009]. In contrast, a paternal origin was demonstrated for both an sSMC(1) and an sSMC(18) in a girl with moderate developmental delay and mild dysmorphic signs [Schwanitz

et al., 2014]. In a boy with Klinefelter-like phenotype, 1 of the 2 sSMCs was a ring derived from the Y chromosome [Weimer et al., 2006]. This is indicative of a paternal origin of both the sSMC(Y) and the other sSMC, a tiny sSMC(8), but this was not proven by molecular studies. These examples show that different mechanisms underlay the origin of multiple de novo sSMCs derived from different chromosomes.

Elucidation of the mechanism of origin is of importance for genetic counseling to estimate the recurrence risk. Here, we describe 2 novel cases with multiple de novo sSMCs, one prenatal, the other one postnatal. Using single nucleotide polymorphism (SNP) markers in the prenatal case and short tandem repeat (STR) markers in the postnatal case, we determined the parental origin and the most likely mechanism leading to the formation of the multiple sSMCs. In addition, *CHRNA6* and *CHRNA3* were identified as dosage-sensitive candidate genes for a MCA/MR phenotype with global developmental/psychomotor delay, speech delay, autism, hyperactivity/attention deficit, and hypotonia in our postnatal case.

Case Reports

Prenatal Case

After she had previously given birth to a healthy boy, the 37-year-old mother decided to have an amniocentesis for her second pregnancy because of advanced maternal age. Paternal age was 36 years. The parents were nonconsanguineous. The pregnancy was uneventful, and repeated ultrasound examinations were inconspicuous. Following the detection of multiple sSMCs in cultured amniotic cells by karyotyping and a partial trisomy 4 by SNP-array investigation, the couple required an induced abortion. This was carried out at 22 weeks of gestation. The parents refused in performing an autopsy to search for any malformations or abnormalities of the female fetus.

Postnatal Case

After 38 weeks of gestation, during which the 32-year-old mother experienced hypoglycemia, a boy with a weight of 3,450 g, length of 54 cm, head circumference of 36 cm (all within the normal range) and an Apgar score of 10 was born. Delivery was spontaneous and uncomplicated. The father was 31 years of age; the couple was nonconsanguineous. At birth, an asymmetric face with retrognathia on the right side, left-sided cryptorchidism and a short frenulum of the foreskin were noted, and the baby needed tube-feeding because of problems with sucking and swallowing. Karyotyping was performed at the age of 2 months because of a ventricular-septum defect. An ultrasound image of the brain was unremarkable. He was operated on his tongue at 6 months of age and on his foreskin at 11 months of age. With time, his face became more symmetric, and his feeding problems diminished. He showed a high serum concentration of calcium, but normal levels of vitamin D₃ and parathyroid hormone. He also had constipation. After

his second year of life, these features normalized spontaneously. Developmental milestones were delayed. He walked at 20 months of age, and his speech development was retarded. He spoke his first words after 1 year of age (regular time), simple sentences at about 3 years of age and more complicated sentences at about 4 years of age. His hearing was normal. Psychiatric assessment revealed autism (according to the Diagnostic and Statistical Manual of Mental Disorders, DSM IV: Autistic Disorder: 299.00), disharmonious development and hyperactivity. He was a pleasant boy, without irritability, hyperexcitability or aggression. At 4 years and 8 months of age, his face was long and dysmorphic, with a straight, high forehead, low-set and wave-shaped eyebrows, straight long eyelashes, synophrys, strabismus divergens, a flat nasal bridge, straight short nose with hypoplastic alae, an open mouth, and coarse hair. The tops of his auricles were flat and thickened. He was hypermetropic (+5D). He had 2 café-au-lait spots on his trunk, a slightly discolored line on the skin under the umbilicus, slight syndactyly of the 2nd and 3rd toes, and hypoplastic nails of the 5th toes. His thumbs were localized proximally. He had joint laxity and hypotonia.

Materials and Methods

Cytogenetic and Molecular Cytogenetic Investigations

Slides containing metaphases from cultured amniocytes (prenatal case) and from phytohemagglutinin-stimulated lymphocytes from peripheral blood (postnatal case) were prepared following standard procedures. Karyotyping using the air-drying method, GTG-banding and fluorescence in situ hybridization (FISH) were performed according to standard methods [Claussen et al., 2002; Liehr and Claussen, 2002]. FISH using combinations of centromere-specific DNA probes were applied to identify the origin of the sSMCs. For the prenatal case, the following DNA probes were used: CEP 4, CEP 6 and CEP 9 (all from Abbott Molecular, Downer's Grove, Ill., USA), SE 14/22 (Kreatech, Amsterdam, The Netherlands), plus the homemade subcentromere-specific M-FISH (subcenM-FISH) probe sets for chromosome 4 (pcp 4p; pcp 4q; RP11-100N21 in 4p12, RP11-535C7 in 4q12), chromosome 6 (pcp 6p; pcp 6q; RP1-61B2 in 6p11.2, RP11-38L5 in 6q12), chromosome 9 (pcp 9p; pcp 9q; RP11-128P23 in 9p12, RP11-430C15 in 9q13, D9Z4), chromosome 14 (=midi54, i.e. microdissection-derived DNA probe for all acrocentric short arms; pcp 14q; RP11-32B11 in 14q11.2; D14/22Z1), and chromosome 22 (=midi54; pcp 22q; RP11-172D7 in 22q11.21; D22Z4) [Nietzel et al., 2001; Starke et al., 2003]. For the postnatal case CEP4, CEP8 and CEP11 were used (all Abbott Molecular).

Genome-Wide Arrays

Genomic DNA isolated from cultured amniocytes of the prenatal case was analyzed with the Affymetrix Genechip 6.0 Mapping SNP-Array (Annotation v.31) for copy number variation using the Chromosome Analysis Suite (V.1.1) and genotyped using the Genotyping Console (Affymetrix, Santa Clara, Calif., USA) according to the instructions of the manufacturer. Genomic DNA isolated from peripheral blood of the postnatal case was analyzed with array comparative genomic hybridization (array CGH) using an 180K oligonucleotide platform (AMADID 026570) from Agilent Technologies (Santa Clara, Calif., USA) and with the HumanOmniExpressExome BeadChip v1.1 SNP array (Illumina, San Diego,

Calif., USA), according to the instructions of the manufacturer. Data were analyzed with the Nexus software package (BioDiscovery, Los Angeles, Calif., USA). All genomic positions were according to the GRCh38/hg38 build of the human reference genome.

SNP Genotyping and STR Marker Analysis

For the prenatal case, all SNP markers with an increased signal for the minor allele within the region of chromosome 4 present in the sSMC(4) were selected by examining the cluster plots of SNP markers (online suppl. fig. 2; for all online suppl. material, see www.karger.com/doi/10.1159/000441408). PCR reactions were performed on 50 ng of genomic DNA. Initial denaturation was at 95°C for 10 min, followed by 33 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, and a final extension at 72°C for 30 min. Primer sequences are available on request. Sequence analyses were performed using BigDye Terminator v1.1 chemistry on an ABI 3500xl DNA analyzer (Applied Biosystems, Foster City, Calif., USA). The sequences obtained were compared with the reference sequence using Sequence Pilot software (JSI Medical Systems, Kippenheim, Germany). For the postnatal case, we selected STR markers that were located within the chromosomal segments contained in the sSMC(8) and in the flanking regions not contained in the sSMC(8). PCR reactions were performed on 50 ng of genomic DNA isolated from a cultured, Epstein-Barr virus (EBV) immortalized lymphoblastoid B-cell line from the patient and each parent. Initial denaturation was at 95°C for 10 min, followed by 33 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, and a final extension at 72°C for 30 min. PCR products were analyzed using an ABI 3130 automated sequencer (Applied Biosystems) with LIZ-500 fluorescent size standards and formamide (Applied Biosystems). GeneMapper software (Applied Biosystems) was used for data collection and analysis.

Results

Prenatal Case

Because fetal tissues could not be investigated after termination of the pregnancy, all molecular cytogenetic investigations had to be performed on amniocytes. Investigation of cultured amniocytes revealed a female karyotype with a minimum of 2 and a maximum of 5 sSMCs per cell in 2 independent amniotic fluid cultures (40 metaphase spreads analyzed) (fig. 1, also see online suppl. fig. 1A). The parents had normal karyotypes in all metaphases analyzed (50 for the mother, 74 for the father). FISH using centromere-specific DNA probes and subcenM probes showed that the sSMCs originated from chromosomes 4, 6, 9, 14, and 22. The different combinations and distribution of the sSMCs are summarized in table 1. The small size of the sSMCs precluded to determine whether they were linear or ring shaped. The largest sSMC originated from chromosome 9 and consisted mainly of the heterochromatic 9q12 region of the long

Fig. 1. FISH results with subcenM probe mixtures for chromosomes 4, 6, 9, 14, and 22 in the prenatal case. For chromosomes 4 and 6, the second homologous chromosome is not shown. The sSMCs originating from chromosomes 6, 14 and 22 were found to consist of centromere-specific DNA repeats only. In contrast, the sSMCs originating from chromosomes 4 and 9 contained euchromatin in addition to centromeric DNA sequences (see text).

Table 1. Distribution of sSMCs in cultured amniocytes of the prenatal case (n = 10 metaphases) and in cultured peripheral blood lymphocytes of the postnatal case (n = 180 metaphases)

Observed combinations of sSMCs	Frequency of each sSMC per cell		
<i>Prenatal case</i>			
+sSMC(4), +sSMC(6), +sSMC(6), +sSMC(14/22), +sSMC(14/22)	20%	sSMC(4)	90%
+sSMC(4), +sSMC(6), +sSMC(6), +sSMC(14/22)	20%	sSMC(6)	60%
+sSMC(4),+sSMC(9),+sSMC(14/22)	10%	sSMC(9)	20%
+sSMC(6),+sSMC(6),+sSMC(14/22)	10%	sSMC(14/22)	80%
+sSMC(4),+sSMC(6),+sSMC(14/22)	10%		
+sSMC(4),+sSMC(14/22)	10%		
+sSMC(4),+sSMC(9)	10%		
+sSMC(4)	10%		
<i>Postnatal case</i>			
+sSMC(4),+sSMC(8),+sSMC(11)	16.1%	+sSMC(4)	43.3%
+sSMC(4),+sSMC(8)	13.9%	+sSMC(8)	73.3%
+sSMC(4),+sSMC(11)	4.4%	+sSMC(11)	43.3%
+sSMC(8),+sSMC(11)	18.4%		
+sSMC(4)	8.9%		
+sSMC(8)	25.0%		
+sSMC(11)	4.4%		
no sSMCs	8.9%		

Based on FISH on metaphases using chromosome-specific DNA probes (usually containing alpha repeat probes, see Materials and Methods). The SE 14/22 probe does not discriminate between chromosomes 14 and 22.

Table 2. Segregation of SNP markers on the sSMC(4) in the prenatal case

Affy-SNP-ID	rs-ID	Chr	Position	MAF	Genotypes			
					fetus ^a , array	fetus ^b , seq	mother, seq	father, seq
SNP_A-4298972	rs989808	4	47,164,764	0.155	GTT	GT(T)	GT	GG
SNP_A-8654229	rs17539326	4	47,212,896	0.062	ATT	AT(T)	AT	AA
SNP_A-1811029	rs4438736	4	47,482,628	0.144	CTT	CT(T)	CT	CC
SNP_A-1960377	rs6447568	4	47,573,655	0.327	CGG	CG(G)	GG	CG
SNP_A-8448627	rs2271036	4	47,677,911	0.237	CCT	TC(C)	TC	TC
SNP_A-2113584	rs2271037	4	47,678,068	0.239	GGT	TG(G)	TG	TG
SNP_A-8476731	rs13129264	4	47,711,962	0.097	ATT	AT(T)	AT	AT
SNP_A-4210151	rs3886429	4	47,737,176	0.097	GAA	GA(A)	GA	GA
SNP_A-8409104	rs2572336	4	48,931,593	0.111	CTT	CT(T)	CT	CC
SNP_A-1805438	rs13143892	4	52,194,358	0.177	AGG	GG(G) ^c	AG	AG

MAF = Minor allele frequency taken from dbSNP. Informative markers are in bold print. Maternal alleles are in red. Paternal alleles are in blue.

^a Allelic intensities taken from cluster plots of genotyping console (compare online suppl. fig. 1).

^b Supernumerary allele in parentheses estimated from the higher allelic peaks of sanger sequences traces.

^c Genotyping error, maybe due to an undetected SNP under one of the primer-binding sites.

arm, and, as shown by subcenM-FISH, it also contained euchromatin from the short arm, with a break in 9p12 (fig. 1). As revealed by SNP-array analysis, the sSMC(6), sSMC(14) and sSMC(22) contained only centromeric alpha-satellite repeats, as no copy number gains were seen around the centromeres of these chromosomes (data not shown). In addition, these sSMCs did not show signals when using the corresponding subcenM-FISH probe combination for FISH (fig. 1). The region of the short arm of the sSMC(9) was not well covered on the SNP array, and a copy number gain was only seen around position 43,550,500 in 9p11.2 (data not shown). Because of the frequent copy number polymorphisms in 9p11.2p12, which are thought to represent euchromatic variants without phenotypic significance [Barber, 2005], the sSMC(9) was predicted to be without clinical consequences. In contrast, SNP-array analysis showed the presence of euchromatic material for the sSMC(4) (data not shown), which according to cytogenetic investigation was present in ~90% of the cells. The sSMC(4) can be described as min or r(4)(::p12→q12::) and has a size of ~5.8 Mb (see online suppl. table 1). It contains 22 protein-coding genes and 1 miRNA gene (see online suppl. table 2) and was therefore predicted to cause phenotypic effects. Based on the SNP data, uniparental disomy of any of the structurally normal homologous chromosomes was excluded (data not shown).

To investigate the parental origin of the sSMC(4), we analyzed the array-SNP cluster plots (online suppl. fig. 2)

and selected 10 SNPs within the sSMC(4) that showed an increased signal for the minor allele for segregation analysis. The 10 SNPs were subsequently genotyped in the fetus and the parents (table 2). Only 4 of the SNPs showed an informative signal distribution. For each of these, the allele on the sSMC(4) was identical to an allele in one of the maternal haplotypes (table 2). These results support an origin of the sSMC(4) through a meiosis II segregation error.

Postnatal Case

Using karyotyping of stimulated peripheral blood lymphocytes and FISH with centromere-specific DNA probes, a male karyotype with 0–3 sSMCs per cell was found (online suppl. fig. 1B). The sSMCs were derived from chromosomes 4, 8 and 11. Due to their small size it could not be determined if they were centric minute or ring-shaped sSMCs. The parents showed normal karyotypes in all 65 metaphases examined each. The different combinations of the sSMCs and their prevalence in lymphocytes are shown in table 1. The sSMC(8) was the most prevalent, being present in about 73% of cells. Array CGH using DNA from peripheral blood allowed delineation of the sizes of each of the 3 sSMCs and their gene content (see online suppl. tables 1, 3). The sSMC(4) had a size of ~7.7 Mb, contained 26 protein-coding genes, 2 miRNA genes, 2 long noncoding RNA genes, and 1 small nucleolar RNA gene. The break in 4q12 disrupted the *SCFD2* gene. The sSMC(11) was about 10.4 Mb in size and con-

Table 3. Segregation of STR markers on the sSMC(8) in the postnatal case

Markers not on sSMC(8)	Markers on sSMC(8)	Cytogenetic band position	Nucleotide position on chromosome 8	Haplotypes					
				mother		proband		father	
D8S1821		8p11.22	38,351,500–38,351,643	146	146	146	165	165	146
D8S2317		8p11.22	38,640,424–38,640,711	280	280	280	280	280	276
D8S255		8p11.21	39,885,306–39,885,468	102	111	111	111	111	123
	D8S1817	8p11.21	40,448,123–40,448,290	175	177	175	177	171	175
	D8S1460	8q11.1	47,035,346–47,035,475	121	133	121	133	121	130
	D8S1133	8q11.21	51,334,709–51,334,887	180	180	180	180	180	180
	D8S1815	8q11.23	52,998,534–52,998,782	234	228	234	228	228	234
D8S601		8q11.23	53,683,335–53,683,559	227	223	223	231	231	231
D8S1737		8q11.23	54,786,663–54,787,054	192	194	194	192	192	192
D8S2332		8q12.1	56,130,223–56,130,389	154	154	154	166	166	154

The table shows one out of 8 possibilities for the reconstruction of the haplotypes (see online suppl. table 4 for all permutations, each being consistent only with a maternal origin through a meiosis I segregation error).

tained 58 protein-coding genes, 4 miRNA genes and 1 long noncoding RNA gene. The break in 11p11.2 disrupted the *TP53I11* gene. SNP-array analysis of DNA extracted from an EBV-immortalized cell line of the patient showed signals for the sSMC(8) only, and allowed us to refine its breakpoints (see online suppl. table 1). The other 2 sSMCs must have been lost during establishment of the cell line or during culturing. The sSMC(8) had a size of ~12.4 Mb and contained 39 protein-coding genes, 3 miRNA genes, one 5S RNA gene, 3 small nucleolar RNA genes, and 1 long noncoding RNA gene. The break in 8q11.23 disrupted the *RB1CC1* gene. Based on the SNP-array data, uniparental disomy of any of the structurally normal homologous chromosomes was excluded (data not shown).

We used STR markers to determine the parental origin of the sSMC(8) (table 3). STR markers flanking the region present in this sSMC showed a normal biallelic segregation pattern in the patient. Of the 4 markers present on the sSMC(8), D8S1133 was not informative. The segregation of the alleles of markers D8S1817 and D8S1815 can be reconciled with either a maternal or paternal origin of

the third allele in the patient. The segregation of the D8S1460 alleles was incompatible with a paternal meiosis I error. Barring hypothetical recombination events in the sSMC8, the segregation of the STR marker alleles agree with a maternal origin. Because D8S1817 showed 3 different alleles in the patient, the sSMC(8) must have resulted from a maternal meiosis I error (also see online suppl. table 4).

Discussion

We describe 2 novel patients with multiple sSMCs and determined the most likely mechanism of their origin. In one case, the sSMCs were detected during karyotyping of amniocytes because of advanced maternal age, in the other case during karyotyping of cultured lymphocytes of a newborn child presenting with asymmetric retrognathia, ventricular septal defect, feeding problems, and cryptorchidism. These cases illustrate the limitations of molecular cytogenetic and molecular investigations in individuals with multiple sSMCs. First the sSMCs are of a limited

size because otherwise they would be incompatible with prenatal development and viability. Therefore, studies of the mechanism of origin are possible only if the euchromatic chromosomal segments present in the sSMCs contain a sufficient number of informative DNA markers. As shown in our prenatal case, this was true for only 1 of the 5 sSMCs. Second, mosaicism, with various combinations of the different sSMCs, occurs in 94% of patients carrying multiple sSMCs [Liehr, 2015], and sSMCs are frequently unstable during mitosis, both in sSMC carriers and during cell culture [Hussein et al., 2014; Spittel et al., 2014]. This is a serious limitation because, as shown in our postnatal case, 2 of the 3 sSMCs were lost in the EBV-immortalized cell line available for parental origin studies. Due to their mitotic instability, the fraction of cells containing a particular sSMC can vary considerably between the different tissues [Fickelscher et al., 2007]. Because in most cases only stimulated T-lymphocytes are studied, we have limited knowledge about the contribution of mosaicism to clinical variability. With these limitations in mind, we discuss the candidate genes for the phenotype and the mechanism of origin of the multiple sSMCs.

Dosage-Sensitive Candidate Genes for the Phenotype in the Prenatal Case

Our prenatal case showed different combinations of 2–5 sSMCs that originated from chromosomes 4, 6, 9, 14, and 22 in cultured amniocytes. We have no indications that the sSMC(6), sSMC(14) and sSMC(22) contained protein-coding genes, and the euchromatic part of chromosome 9 (9p11.2p12) present in the sSMC(9) has been reported as an euchromatic variant in healthy individuals from several families [Barber, 2005]. In order to identify candidate genes, we focused, therefore, on the sSMC(4), which corresponds to the region 46.6–52.4 Mb of chromosome 4, and harbors 22 protein-coding genes (online suppl. tables 1, 2). Of these, 13 are listed in the OMIM database, where 3 of them are considered as disease causing (*CORIN*, *CNGA1*, *SCBG*). Six patients with phenotypic abnormalities (mental retardation, microcephaly, seizures, and dysplastic kidney) carrying duplications ranging from 0.22 to 5.5 Mb within this region were listed in DECIPHER [Bragin et al., 2014] and ClinGen databases [Rehm et al., 2015]. Based on this knowledge, the pregnancy was terminated. Because of the lack of phenotypic data on our fetus, it is not possible to attribute any clinical phenotype to the genes on the sSMC(4). Meanwhile, even larger sSMCs derived from chromosome 4 have been reported in apparently normal, liveborn individuals in 2 cases. A single sSMC(4) corresponding to

44.1–62.8 Mb of chromosome 4 has been found in 95% of cultured amniotic fluid cells during prenatal chromosome investigation; after birth, the boy showed normal development at the age of 1 year [Li et al., 2011, case 2]. A male with asthenoteratozoospermia, but who was otherwise normal, had a single sSMC(4) containing 45.5–61.3 Mb in 46% of cultured lymphocytes [Castronovo et al., 2013, patient 3]. Given the reported data, a final conclusion that the sSMC(4) in our case would provoke phenotypic effects cannot be drawn.

Dosage-Sensitive Candidate Genes for the Phenotype in the Postnatal Case

Our postnatal case was mosaic for 3 sSMCs that were derived from chromosomes 4, 8 and 11. There is no evidence that any of the interrupted genes on the sSMCs contribute to the phenotype. Transcription of the interrupted *SCFD2* gene on the sSMC(4) or of the interrupted *RBCC1* gene on the sSMC(8) is highly unlikely because the promoter of each of these genes is not present on the corresponding sSMC (data not shown). Also the interrupted *TP53I11* gene is unlikely to be of significance because only the promoter and exon 1 are retained on the sSMC(11), which would lead to nonsense-mediated decay (data not shown). We focused on the sSMC(8) for the identification of dosage-sensitive genes. This sSMC is most likely of phenotypic impact because it was present at the highest frequency in stimulated lymphocytes (73%) and was the only remaining sSMC after prolonged culturing of an EBV-immortalized cell line. It contained 39 protein-coding genes (online suppl. table 3). To systematically identify candidate genes for the phenotype, we searched in PubMed, DECIPHER [Bragin et al., 2014] and the sSMC Database [Liehr, 2015] for patients with an overlapping pericentromeric chromosome 8 duplication or single sSMC(8). In figure 2, the sSMC(8) of our patient is compared to 6 other such patients, all of whom were reported to have developmental or psychomotor delay and different combinations of the neuropsychiatric disorders seen in our patient (autism, speech delay, hyperactivity/short attention span, and hypotonia/feeding problems during infancy). The minimal region of overlap shared between these patients contains 10 protein-coding genes. The *CHRNA6* and *CHRNA3* genes are the most likely dosage-sensitive candidate genes for the clinical symptoms of these patients because they are expressed in the central nervous system (CNS) and, by physical interaction with other proteins, can cause a dominant negative effect, as explained below. In addition, both genes are members of the nicotinic acetylcholine receptor (nAChRs)

Fig. 2. Comparison of the gene content in the sSMC(8) of the post-natal case with that of other patients selected from the literature, DECIPHER and the sSMC Database (see text). The blue box shows the genes in the minimal region of overlap shared between the patients.

gene family that each encode an α or a β subunit of nAChRs, and several other members of this gene family are known to be involved in diverse neuronal disorders [Dineley et al., 2015]. These ligand-gated cation channels participate in dopaminergic neurotransmission at synapses in various signaling cascades throughout the CNS that are essential for normal behavior, ranging from muscle contraction to cognitive functions. In mammals, at least 9 different α subunit and 3 different β subunit genes

are expressed in the CNS [Lukas et al., 1999; Scofield and Gardner, 2014; Fasoli and Gotti, 2015]. In the CNS, heteromeric nAChRs exist as pentamers, each composed of a unique combination of 5 α and β subunits, with either a 2 α /3 β or a 3 α /2 β stoichiometry. The β 3 subunit can only be incorporated into functional nAChRs as a fifth subunit, together with at least one other α and β subunit [Wu and Lukas, 2011; Scofield and Gardner, 2014; Yakel, 2014]. Both the α 6 and β 3 subunits are coassembled with many other subunits, forming a wide range of nAChRs [Gotti et al., 2006; Yakel, 2014; Fasoli and Gotti, 2015]. In *Xenopus* oocytes coexpression of equimolar amounts of β 3 with other subunits affects the function of nAChRs that are composed of α 2, α 3 or α 4 plus β 2 or β 4 [Dash et al., 2012]. In particular, α 4 β 2-containing nAChRs may become stable but functionless intermediates in the presence of β 3 [Kuryatov et al., 2008; Dash et al., 2012]. The nAChRs containing α 4 and β 2 are widely and strongly expressed in the CNS [Gotti et al., 2006] and are implicated in cognitive decline and autism [Dineley et al., 2015]. In addition, β 3 incorporation abolishes the function of nAChRs composed of α 6 plus β 4 [Dash et al., 2011]. Thus, the additional copy of the *CHRNA6* gene has a dominant negative effect on nAChR function, with the number of available β 3 subunits controlling the number of functional nAChRs. This is in agreement with the gene dosage balance hypothesis, which states that alterations in the stoichiometry of macromolecular complexes or cellular networks are responsible for dominant phenotypes [Veitia, 2003, 2004, 2010; Veitia and Birchler, 2010; Poot et al., 2011]. Taken together, it is conceivable that the additional copies of *CHRNA6* and *CHRNA6* on the sSMC(8) affect the function of the nAChRs in the CNS of our and other patients, leading to developmental/psychomotor delay, in different combinations with speech delay, autism, attention deficit, and hypotonia.

However, the relationship between such copy number gains and the phenotype is not straightforward because carriers of a single sSMC(8) containing *CHRNA6* and *CHRNA6* have been described with an apparently normal phenotype [Liehr, 2015]. This suggests that, in addition to differences in the frequency and distribution of the sSMCs, the severity of the phenotype is modulated by other genetic factors. A 2-locus mechanism has been proposed for patients with either intellectual delay or autism and may explain the clinical variability of patients and healthy individuals with recurrent and unique deletions, duplications and sSMCs [Girirajan and Eichler, 2010; Girirajan et al., 2010, 2012; Poot et al., 2011; Leblond et al., 2012]. Indeed, in our patient, one of these modifiers may be the ad-

ditional dosage of genes on the sSMC(11) because 2 patients with overlapping gains of chromosome 11 have been described, each with developmental delay, and one with speech delay [Guichet, 2005; DECIPHER patient 291037].

Different Meiotic Segregation Errors Contribute to Multiple sSMCs

Previously suggested models to explain the presence of multiple sSMCs, each derived from a different chromosome, are based on fertilization of the oocyte by 2 spermatozoa concomitantly [Beverstock et al., 2003], or on the non-extrusion of the second polar body during meiosis II [Daniel and Malafiej, 2003]. In both scenarios, such an event was assumed to be followed by partial degradation of the third chromosome complement, with only centromere-containing fragments remaining as multiple sSMCs in the zygote. Our marker segregation data are compatible with a maternal origin of the multiple sSMCs in both the prenatal and the postnatal case. We propose, based on the cases described here and previously [Kogan et al., 2009; Hochstenbach et al., 2013], that multiple sSMCs arise through different types of subsequent segregation errors that affect multiple, different chromosomes during maternal meiosis I, II or both. A postzygotic origin of the multiple sSMCs during the cleavage stage divisions of the embryo cannot be completely excluded in the cases in which the segregation of the markers is consistent with a maternal meiosis II error. However, a postzygotic origin is unlikely because the major type of mitotic segregation error during early embryogenesis is anaphase lagging [Coonen et al., 2004; Daphnis et al., 2005; Ioannou et al., 2012; Capalbo et al., 2013; McCoy et al., 2015]. Anaphase lagging leads to loss of chromosomes, not gains.

Studies on chromosome segregation based on comparative genomic hybridization of DNA from polar body I, polar body II and the zygote from stimulated ovarian cycles have shown that gains of multiple, nonhomologous, single chromatids result from segregation errors during meiosis I, followed by errors affecting other chromosomes during meiosis II [reviewed by Fragouli and Wells, 2014]. This may also be the case in naturally cycling women <36 years [Verpoest et al., 2008; Labarta et al., 2012]. Maternal ages were 37 and 32 years in the prenatal and postnatal case, respectively, and both conceived during a natural cycle. For example, Handyside et al. [2012] describe a zygote containing a gain of chromosomes 9 and 18 as a result of meiosis I errors and gains of chromosomes 4, 10, 15, and 21 by meiosis II errors. The meiosis I gain of chromosome 9 resulted from premature

sister chromatid separation, that of chromosome 18 from nondisjunction. This example illustrates that different, subsequent meiotic errors lead to the presence of multiple supernumerary chromosomes in the zygote, and that up to 6 supernumerary chromosomes are possible. This fits with the maximum number of 6–7 multiple sSMCs, each derived from a different chromosome, that has been reported in the literature [Liehr, 2015]. Also, any of the 24 different chromosomes can be implicated in these meiotic errors [Fragouli et al., 2011; Handyside et al., 2012], explaining why any chromosome can be found as one of the multiple sSMCs [Liehr, 2015]. It can be predicted that most of the meiosis I errors are due to premature sister chromatid separation, which has been shown to happen much more frequently than nondisjunction of both homologous chromosomes, the latter accounting only for 3–9% of all maternal meiosis I segregation errors [Fragouli et al., 2011; Gabriel et al., 2011; Forman et al., 2013; Handyside et al., 2012; Capalbo et al., 2013; Christopikou et al., 2013]. In addition, as again shown by the segregation studies in polar bodies and zygotes, it can also be predicted that meiosis II errors occur more frequently than meiosis I errors [Handyside et al. 2012]. In the prenatal case, we found 2:1 allelic ratios for 4 of the SNP markers on the sSMC(4) (table 2), indicative of a meiosis II error, and in the postnatal case, we found 1:1:1 or 2:1 allelic ratios for the STR markers on the sSMC(8) (table 3), compatible with a meiosis I error. The limitation in each of these cases is that only for one of the multiple sSMCs informative markers were available for segregation analysis. Cases in which some of the multiple sSMCs originate from errors in meiosis I and others from errors in meiosis II have not yet been described, but are likely to be identified as additional cases are studied. It is also possible that all sSMCs are the result of misdivision during meiosis I or meiosis II. In a previously reported case, we also found 2:1 ratios for the STR markers on a maternally derived sSMC(11) and sSMC(X), indicating that both resulted from a meiosis II error. There were no informative markers on an sSMC(8) in this case [Hochstenbach et al., 2013]. Additional cases must be investigated to confirm the proposed model and its predictions.

It has been firmly established that ovarian ageing is the most important risk factor for aneuploidies in human zygotes [reviewed by Vogt et al., 2008; Nagaoka et al., 2012]. Several lines of evidence show that this is also true for sSMC formation. First, by examining 143,000 prenatal chromosome investigations, it could be shown that the incidence of a single, de novo sSMC is significantly increased in aged women [Malvestiti et al., 2014]. In addi-

tion, array CGH shows that in women aged 43–45 years, 87% of zygotes have multiple aneuploidies compared to 20% in women aged 33–36 years [Handyside et al., 2012], and direct microscopic observation of meiosis II spindles in women aged 40–45 years shows that displaced chromosomes occur in ~80% of oocytes [Battaglia et al., 1996]. The largest age-dependent increase in error rate is seen for misdivision of sister chromatids [Fragouli et al. 2011]. The predominance of predivision separation of sister chromatids suggests that loss of cohesin during ageing is a significant contributor to meiotic segregation errors [Duncan et al., 2012; Nagaoka et al., 2012]. In addition, studies in ageing mice indicate that decreased expression of components of the spindle assembly checkpoint, a higher sensitivity to disturbances in spindle structure and a reduced energy production for spindle operation by mitochondrial dysfunction also contribute to oocyte aneuploidy [Vogt et al., 2008].

We further propose that aberrant kinetochore-microtubule interactions underlay these segregation errors and lead to DNA breaks within or immediately adjacent to the centromere, thereby reducing complete chromosomes to the different types of sSMCs (such as rings or centric minutes). This is based on studies of the DNA content of single sSMCs by array CGH [Baldwin et al., 2008], demonstrating that ~80% of nonacrocentric, ring-shaped sSMCs have one or both breaks within the centromeric alpha repeat array. The other 20% of sSMCs have breaks in p and q arms, each within a few Mb from the centromere. These different types of sSMCs are seen in the sSMCs of our cases and in other patients with multiple sSMCs [Liehr, 2015]. Following investigations of DNA breaks generated by spindle defects during tumorigenesis [Martínez-A and van Wely, 2010] and in cell lines with defective spindles [Guerrero et al., 2010a, b], we propose that kinetochores of prematurely separated sister chromatids attach to both spindle poles. The resulting opposite pulling forces exerted by the microtubules on the kinetochore lead to

DNA double-strand breaks within or in close proximity to the centromere. This may activate DNA damage repair, generating small ring-shaped sSMCs by the joining of both ends, or to centric minute sSMCs by recombination-mediated telomere capture or de novo TTAGGG repeat synthesis. This model is supported by the finding that, in mouse oocytes, experimentally induced double-strand breaks delay the extrusion of the first polar body due to a disrupted attachment of microtubules to kinetochores and spindle assembly checkpoint activation [Ma et al., 2013; Lin et al., 2014]. Metaphase spreads at meiosis I and II revealed fragmented chromosomes and misaligned chromosome fragments [Ma et al., 2013; Lin et al., 2014]. We conclude that ageing-related stochastic, rather than mutation-dependent deterministic processes, seem to underlie the formation of (multiple) sSMCs. This makes estimates of the recurrence risk for sSMCs difficult. These hypotheses need further investigations, which will become feasible when more patients with multiple sSMCs are studied.

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Statement of Ethics

For both cases, informed consent for publication of the data has been obtained according to the regulations of the respective institutions.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

- Baldwin EL, May LF, Justice AN, Martin CL, Ledbetter DH: Mechanisms and consequences of small supernumerary marker chromosomes: from Barbara McClintock to modern genetic-counseling issues. *Am J Hum Genet* 82:398–410 (2008).
- Barber JCK: Directly transmitted unbalanced chromosome abnormalities and euchromatic variants. *J Med Genet* 42:609–629 (2005).
- Battaglia DE, Goodwin P, Klein NA, Soules MR: Influence of maternal age on spindle assembly in oocytes from naturally cycling women. *Hum Reprod* 11:2217–2222 (1996).
- Beverstock GC, Bezrookove V, Mollevanger P, van de Kamp JJP, Pearson P, et al: Multiple supernumerary ring chromosomes of different origin in a patient: a clinical report and review of the literature. *Am J Med Genet A* 122A:168–173 (2003).
- Bragin E, Chatzimichali EA, Wright CF, Hurles ME, Firth HV, et al: DECIPHER: database for the interpretation of phenotype-linked plausibly pathogenic sequence and copy-number variation. *Nucleic Acids Res* 42(Database issue): D993–D1000 (2014).

- Capalbo A, Bono S, Spizzichino L, Biricik A, Baldi M, et al: Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblasts: insights into female meiotic errors and the chromosomal segregation in the preimplantation window of embryo development. *Hum Reprod* 28:509–518 (2013).
- Castronovo C, Valtorta E, Crippa M, Tedoldi S, Romitti L, et al: Design and validation of a pericentromeric BAC clone set aimed at improving diagnosis and phenotype prediction of supernumerary marker chromosomes. *Mol Cytogenet* 6:45 (2013).
- Christopikou D, Tsorva E, Economou K, Shelley P, Davies S, et al: Polar body analysis by array comparative genomic hybridization accurately predicts aneuploidies of maternal meiotic origin in cleavage stage embryos of women of advanced maternal age. *Hum Reprod* 28:1426–1434 (2013).
- Claussen U, Michel S, Mühlig P, Westermann M, Grummt UW, et al: Demystifying chromosome preparation and the implications for the concept of chromosome condensation during mitosis. *Cytogenet Genome Res* 98:136–146 (2002).
- Coonen E, Derhaag JG, Dumoulin JCM, van Wissen LCP, Bras M, et al: Anaphase lagging mainly explains chromosomal mosaicism in human preimplantation embryos. *Hum Reprod* 19:316–324 (2004).
- Crolla JA, Youings SA, Ennis S, Jacobs PA: Supernumerary marker chromosomes in man: parental origin, mosaicism and maternal age revisited. *Eur J Hum Genet* 13:154–160 (2005).
- Daniel A, Malafiej P: A series of supernumerary small ring marker autosomes identified by FISH with chromosome probe arrays and literature review excluding chromosome 15. *Am J Med Genet A* 117A:212–222 (2003).
- Daphnis DD, Delhanty JDA, Jerkovic S, Geyer J, Craft I, Harper JC: Detailed FISH analysis of day 5 human embryos reveals the mechanism leading to mosaic aneuploidy. *Hum Reprod* 20:129–137 (2005).
- Dash B, Bhakta M, Chang Y, Lukas RJ: Identification of N-terminal extracellular domain determinants in nicotinic acetylcholine receptor (nAChR) $\alpha 6$ subunits can influence effects of wild-type or mutant $\beta 3$ subunits on function of $\alpha 6\beta 2^*$ - or $\alpha 6\beta 4^*$ -nAChR*. *J Biol Chem* 286:37976–39989 (2011).
- Dash B, Bhakta M, Chang Y, Lukas RJ: Modulation of recombinant, $\alpha 2^*$, $\alpha 3^*$ or $\alpha 4^*$ -nicotinic acetylcholine receptor (nAChR) function by nAChR $\beta 3$ subunits. *J Neurochem* 121:349–361 (2012).
- Dineley KT, Pandya AA, Yakel JL: Nicotinic ACh receptors as therapeutic targets in CNS disorders. *Trends Pharmacol Sci* 36:96–108 (2015).
- Duncan FE, Hornick JE, Lampson MA, Schultz RM, Shea LD, Woodruff TK: Chromosome cohesion decreases in human eggs with advanced maternal age. *Ageing Cell* 11:1121–1124 (2012).
- Fasoli F, Gotti C: Structure of neuronal nicotinic receptors, in Balfour DJK, Munafo MR (eds): *The Neurobiology and Genetics of Nicotine and Tobacco. Current Topics in Behavioral Neurosciences* 23, pp 1–17 (Springer International Publishing, Cham 2015).
- Fickelscher I, Starke H, Schulze E, Ernst G, Kosyakova N, et al: A further case with a small supernumerary marker chromosome (sSMC) derived from chromosome 1 – evidence for high variability in different tissues of sSMC carriers. *Prenat Diagn* 27:783–785 (2007).
- Forman EJ, Treff NR, Stevens JM, Garnsey HM, Katz-Jaffe MG, et al: Embryos whose polar bodies contain isolated reciprocal chromosome aneuploidy are almost always euploid. *Hum Reprod* 28:502–508 (2013).
- Fragouli E, Wells D: Human embryonic aneuploidy. eLS DOI:10.1002/9780470015902.a0025706 (2014).
- Fragouli E, Alfarawati S, Goodall NN, Sánchez-García JF, Colls P, Wells D: The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. *Mol Hum Reprod* 17:286–295 (2011).
- Gabriel AS, Thornhill AR, Ottolini CS, Gordon A, Browns APC, et al: Array comparative genomic hybridisation on first polar bodies suggests that non-disjunction is not the predominant mechanism leading to aneuploidy in humans. *J Med Genet* 48:433–437 (2011).
- Girirajan S, Eichler EE: Phenotypic variability and genetic susceptibility to genomic disorders. *Hum Mol Genet* 19(R2):R176–R187 (2010).
- Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, et al: A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet* 42:203–209 (2010).
- Girirajan S, Rosenfeld JA, Coe BP, Parikh S, Friedman N, et al: Phenotypic heterogeneity of genomic disorders and rare copy-number variants. *N Engl J Med* 367:1321–1331 (2012).
- Gotti C, Zoli M, Clementi F: Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci* 27:482–491 (2006).
- Guerrero AA, Gamero MC, Trachana V, Fütterer A, Pacios-Bras C, et al: Centromere-localized breaks indicate the generation of DNA damage by the mitotic spindle. *Proc Natl Acad Sci USA* 107:4159–4164 (2010a).
- Guerrero AA, Martínez-A C, van Wely KHM: Merotelic attachments and non-homologous end joining are the basis of chromosomal instability. *Cell Division* 5:13 (2010b).
- Guichet A: Pericentromeric 11p11 duplication in a girl with isolated learning disability, particularly dyscalculia (abstract 1.39-P). *Chromosome Res* 13 Suppl 1:32 (2005).
- Handyside AH, Montag M, Magli MC, Repping S, Harper J, et al: Multiple meiotic errors caused by predivision of chromatids in women of advanced maternal age undergoing in vitro fertilization. *Eur J Hum Genet* 20:742–747 (2012).
- Hochstenbach R, van Gijn M, Krijtenburg PJ, Raemakers R, van 't Slot R, et al: Gain of *FAM123B* and *ARHGEF9* in an obese man with intellectual disability, congenital heart defects and multiple supernumerary ring chromosomes. *Mol Syndromol* 3:274–283 (2013).
- Hussein SS, Kreskowski K, Ziegler M, Klein E, Hamid AB, et al: Mitotic stability of small supernumerary marker chromosomes depends on their shape and telomeres – a long term in vitro study. *Gene* 552:246–248 (2014).
- Ioannou D, Fonseka KGL, Meershoek EJ, Thornhill AR, Abogrein A, et al: Twenty-four chromosome FISH in human IVF embryos reveals patterns of post-zygotic chromosome segregation and nuclear organization. *Chromosome Res* 20:447–460 (2012).
- Kogan JM, Miller E, Ware SM: High resolution SNP based microarray mapping of mosaic supernumerary marker chromosomes 13 and 17: delineating novel loci for apraxia. *Am J Med Genet Part A* 149A:887–893 (2009).
- Kuryatov A, Onksen J, Lindstrom J: Roles of accessory subunits in $\alpha 4\beta 2^*$ nicotinic receptors. *Mol Pharmacol* 74:132–143 (2008).
- Labarta E, Bosch E, Alamá P, Rubio C, Rodrigo L, Pellicer A: Moderate ovarian stimulation does not increase the incidence of human embryo chromosomal abnormalities in in vitro fertilization cycles. *J Clin Endocrinol Metab* 97:E1987–E1994 (2012).
- Leblond CS, Heinrich J, Delorme R, Proepper C, Betancur C, et al: Genetic and functional analyses of *SHANK2* mutations suggest a multiple hit model of autism spectrum disorders. *PLoS Genet* 8:e1002521 (2012).
- Li P, Pomianowski P, DiMaio MS, Florio JR, Rossi MR, et al: Genomic characterization of prenatally detected chromosomal structural abnormalities using oligonucleotide array comparative genomic hybridization. *Am J Med Genet Part A* 155A:1605–1615 (2011).
- Liehr T: Small Supernumerary Marker Chromosomes (sSMC). A Guide for Human Geneticists and Clinicians. (Springer, Berlin 2012).
- Liehr T: Small supernumerary marker chromosomes. <http://ssmc-tl.com/sSMC.html>, accessed April 11, 2015.
- Liehr T, Claussen U: FISH on chromosome preparations of peripheral blood, in Rautenstrauss B, Liehr T (eds): *FISH Technology*, pp 73–81 (Springer, Berlin 2002).
- Liehr T, Weise A: Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics. *Int J Mol Med* 19:719–731 (2007).
- Liehr T, Claussen U, Starke H: Small supernumerary marker chromosomes (sSMC) in humans. *Cytogenet Genome Res* 107:55–67 (2004).
- Liehr T, Mrasek K, Weise A, Dufke A, Rodríguez L, et al: Small supernumerary marker chromosomes – progress towards a genotype-phenotype correlation. *Cytogenet Genome Res* 112:23–34 (2006).

- Liehr T, Ewers E, Hamid AB, Kosyakova N, Voigt M, et al: Small supernumerary marker chromosomes and uniparental disomy have a story to tell. *J Histochem Cytochem* 59:842–848 (2011).
- Liehr T, Cirkovic S, Lalic T, Gus-Scekic M, de Almeida C, et al: Complex small supernumerary marker chromosomes – an update. *Mol Cytogenet* 6:46 (2013).
- Lin F, Ma XS, Wang ZB, Wang ZW, Luo YB, et al: Different fates of oocytes with DNA double-strand breaks in vitro and in vivo. *Cell Cycle* 13:2674–2680 (2014).
- Lukas RJ, Changeux JP, Le Novère N, Albuquerque EX, Balfour DJK, et al: International Union of Pharmacology. XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. *Pharmacol Rev* 51:397–401 (1999).
- Ma JY, Ou Yang YC, Wang ZW, Wang ZB, Jiang ZZ, et al: The effects of DNA double-strand breaks on mouse oocyte meiotic maturation. *Cell Cycle* 12:1233–1241 (2013).
- Malvestiti F, De Toffol S, Grimi B, Cinetti S, Marcato L, et al: De novo small supernumerary marker chromosomes detected on 143,000 consecutive prenatal diagnoses: chromosomal distribution, frequencies, and characterization combining molecular cytogenetics approaches. *Prenat Diagn* 34:460–468 (2014).
- Martínez-A C, van Wely KHM: Are aneuploidy and chromosome breakage caused by a CINGLe mechanism? *Cell Cycle* 9:2275–2280 (2010).
- McCoy RC, Demko Z, Ryan A, Banjevic M, Hill M, et al: Common variants spanning *PLK4* are associated with mitotic-origin aneuploidy in human embryos. *Science* 348:235–238 (2015).
- Nagaoka SI, Hassold TJ, Hunt PA: Human aneuploidy: mechanisms and new insights into an age-old problem. *Nat Rev Genet* 13:493–504 (2012).
- Nietzel A, Rocchi M, Starke H, Heller A, Fiedler W, et al: A new multicolor-FISH approach for the characterization of marker chromosomes: centromere-specific multicolor-FISH (cenM-FISH). *Hum Genet* 108:199–204 (2001).
- Poot M, van der Smagt JJ, Brilstra EH, Bourgeron T: Disentangling the myriad genomics of complex disorders, specifically focusing on autism, epilepsy, and schizophrenia. *Cytogenet Genome Res* 135:228–240 (2011).
- Reddy KS, Wang S, Groh S, Gonatos S: SKY assessment of two karyotypes with 0–6 supernumerary marker/ring chromosomes and review of previously reported cases with two or more markers. *Am J Med Genet A* 118A:156–171 (2003).
- Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, et al: ClinGen – the Clinical Genome Resource. *New Engl J Med* 372:2235–2242 (2015).
- Schwanitz G, Hagh JK, Rad IA, Omrani MD, Gamerding U, et al: Patient with three euchromatic supernumerary marker chromosomes derived from chromosomes 1, 12, and 18: characterization and evaluation of the aberrations. *Am J Med Genet A* 164A:736–740 (2014).
- Scofield MD, Gardner PD: Molecular underpinnings of neuronal nicotinic acetylcholine receptor expression, in Lester RAJ (ed): *Nicotinic Receptors, The Receptors* 26, pp 39–60 (Springer Science+Business Media, New York 2014).
- Spittel H, Kubek F, Kreskowski K, Ziegler M, Klein E, et al: Mitotic stability of small supernumerary marker chromosomes: a study based on 93 immortalized cell lines. *Cytogenet Genome Res* 142:151–160 (2014).
- Starke H, Nietzel A, Weise A, Heller A, Mrasek K, et al: Small supernumerary marker chromosomes (SMCs): genotype-phenotype correlation and classification. *Hum Genet* 114:51–67 (2003).
- Veitia R: Nonlinear effects in macromolecular assembly and dosage sensitivity. *J Theor Biol* 220:19–25 (2003).
- Veitia R: Gene dosage balance in cellular pathways: implications for dominance and gene duplicability. *Genetics* 168:569–574 (2004).
- Veitia R: A generalized model of gene dosage and dominant negative effects in macromolecular complexes. *FASEB J* 24:994–1002 (2010).
- Veitia RA, Birchler JA: Dominance and gene dosage balance in health and disease: why levels matter! *J Pathol* 220:174–185 (2010).
- Verpoest W, Fauser BC, Papanikolaou E, Staessen C, Van Landuyt L, et al: Chromosomal aneuploidy in embryos conceived with unstimulated cycle IVF. *Hum Reprod* 23:2369–2371 (2008).
- Vetro A, Manolakos E, Petersen MB, Thomaidis L, Liehr T, et al: Unexpected results in the constitution of small supernumerary marker chromosomes. *Eur J Med Genet* 55:185–190 (2012).
- Vogt E, Kirsch-Volders M, Parry J, Eichenlaub-Ritter U: Spindle formation, chromosome segregation and the spindle checkpoint in mammalian oocytes and susceptibility to meiotic error. *Mutat Res* 651:14–29 (2008).
- Weimer J, Metzke-Heidemann S, Plendl H, Caliebe A, Grunewald R, et al: Characterization of two supernumerary marker chromosomes in a patient with signs of Klinefelter syndrome, mild facial anomalies and severe speech delay. *Am J Med Genet A* 140:488–495 (2006).
- Wu J, Lukas RJ: Naturally-expressed nicotinic acetylcholine receptor subtypes. *Biochem Pharmacol* 82:800–807 (2011).
- Yakel JL: Functional distribution and regulation of neuronal nicotinic ACh receptors in the mammalian brain, in Lester RAJ (ed): *Nicotinic Receptors, The Receptors* 26, pp 93–114 (Springer Science+Business Media, New York 2014).
- Yu S, Fiedler SD, Brawner SJ, Joyce JM, Zhou XG, Liu HY: Characterizing small supernumerary marker chromosomes with combination of multiple techniques. *Cytogenet Genome Res* 136:6–14 (2012).