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Supernumerary marker chromosomes in man: parental origin, mosaicism and maternal age revisited

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The details of all cytogenetic abnormalities diagnosed in the Wessex Regional Genetics Laboratory (WRGL) since 1967 to the present day have been recorded in the Salisbury Treasury of Interesting Chromosomes (STOIC). From this resource, we identified 137 patients with constitutional autosomal supernumerary marker chromosomes (SMC) ascertained in four principal groups: (i) 37% with abnormal phenotypes; (ii) 7% couples with reproductive difficulties; (iii) 47% antenatal diagnoses and (iv) 9% miscellaneous. Overall, 81 (59%) SMCs were mosaics and 56 (41%) nonmosaics. Of the 109 cases with known parental origins, 70% were *de novo*, 19% maternally and 11% paternally inherited. The chromosomal origins of 112/137 (82%) of the SMCs have been determined by fluorescence *in situ* hybridization (FISH). In all, 36/112 (32%) were derived from nonacrocentric autosomes, and 76/112 (68%) from the acrocentric autosomes 13/21, 14, 15 and 22. Of these acrocentric SMCs, 39 (51%) were derived from chromosome 15, so that SMC(15) constituted 39/112 (35%) of all SMCs with known chromosomal origins. The frequencies with which mosaicism was observed varied considerably according to the chromosomal origin of the SMCs and accounted for 8/39 (20%) SMC(15), 13/37 (35%) SMCs from other acrocentrics and 25/36 (69%) of nonacrocentric SMCs. The data were analysed for parental age effects, and only *de novo* SMC(15)s were found to be associated with a significantly increased maternal age.

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Introduction

Over the past 30 years, a large number of reports have been directed towards the identification, classification and clinical significance of human constitutional supernumerary marker chromosomes (SMC). Historically, Buckton *et al*¹ first described a correlation between SMCs and their possible phenotypic effects when they showed that the

ascertainment of SMCs among individuals in psychiatric institutions was 3.27/1000 compared to their frequency of only 0.24/1000 among consecutive newborns.

Population studies on antenatally ascertained cases show that SMCs occur with a frequency ranging from 0.4/1000² to 0.8/1000.^{3,4} Hook and Cross³ also published data suggesting that antenatally ascertained SMCs were associated with advanced maternal age. Among these early studies, the reported overall frequencies of mosaicism involving SMCs ranged from 27 to 66%.^{1–3} These early studies using conventional cytogenetic techniques also divided the SMCs based on whether they had recognizable satellites and therefore assumed to be derived from the

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acrocentric autosomes, and whether they were without satellites and were assumed to be comprised of the nonacrocentric chromosomes. From these early data, it was clear that SMCs derived from the acrocentric autosomes comprised the majority population, for example, 64% acrocentrics vs 36% nonacrocentrics.²

Studies of the cytogenetic and phenotypic effects of SMCs gained a fresh impetus when fluorescence *in situ* hybridization (FISH) was utilized to identify their chromosomal origins.^{5–10} From these studies further insights were obtained into karyotype/phenotype correlations, particularly with respect to SMCs derived from chromosomes 15^{11–13} and 22.^{14–16} Delineations of possible phenotypic effects in SMC derived from the other autosomes, however, remains more problematic (reviewed by Crolla¹⁷).

From the combined conventional and molecular cytogenetic studies of autosomal SMCs, a number of trends have emerged from the literature. Principally, these include: (a) SMCs overall are associated with advanced maternal age, (b) among acrocentric SMCs, the majority are derived from chromosome 15 and (c) mosaic and nonmosaic cells lines are observed with variable frequencies depending on whether or not the SMCs have satellites.^{2–4}

We have re-examined the above parameters using a population of autosomal SMCs diagnosed and characterized in a contiguous geographical area in the UK over a continuous period of 30 years. The results of these analyses in the context of previous studies of SMCs are presented.

Materials and methods

All the cases presented were referred to the WRGL for routine conventional chromosome analysis. Analyses were carried out on either PHA-stimulated peripheral blood and/or amniotic fluid or CVS fibroblasts following standard culture and chromosome preparation protocols used to produce conventional cytogenetic preparations. Chromosome analyses were carried out following standard G-banding to a minimum of 550 bands, and in mosaic cases, 30 metaphases were scored.

FISH was carried out using standard techniques based on a modification of Pinkel *et al*,¹⁸ and the probes used have been reported previously (eg Crolla¹⁹ and Crolla *et al*²⁰).

Retrieval and analyses of the SMC data were facilitated by the Salisbury Treasury of Interesting Chromosomes (STOIC) database. For the purpose of these studies, the populations have been subdivided into four groups, *viz* nonacrocentric SMC; acrocentric SMCs excluding chromosome 15 (ie 13/21, 14 and 22); chromosome 15-derived SMCs and SMCs with unknown chromosomal origins.

Statistical analyses

Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA) for regression, logistic regression

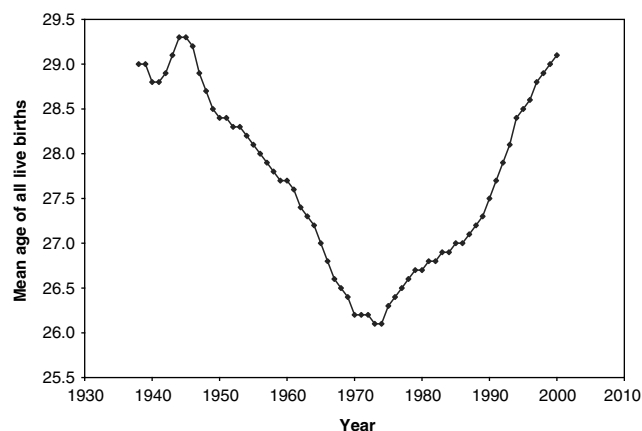


Figure 1 Mean maternal age in England and Wales from 1938 to 2000. Notes: (1) Mean maternal age data for England and Wales may not be the most accurate control for our sample. The available data suggest that mean maternal age is approximately 1 year earlier for mothers in Wales compared to mothers in England and this could have an anticonservative effect on our analysis. (2) Mean maternal age data for England and Wales is given irrespective of birth order. Our Wessex data may be enriched for non-firstborn individuals. (3) Despite concerns outlined in 1 and 2, all our results from our regression show the intercept as not being significantly different from zero, which suggests the control population is appropriate. It is possible that individuals in the marker chromosome database have been ascertained after reproductive difficulties and so maternal age may be advanced compared to the mean.

means tests and χ^2 statistics in frequency tables. Data analysis was carried out using date of birth as well as the maternal age at the birth of each patient. Paternal age at birth is included where given, but these data are sparse. As dates of birth for patients range from 1928 to 2003, changing trends of mean maternal age over this time period were accounted for (Figure 1). This was achieved by taking the mean maternal age for England and Wales for the relevant years and calculating the deviation from this mean for each of the mothers of individuals with marker chromosomes. This derived variable was labelled 'deviate' and used as the dependent variable for a number of the regression analyses examining the effect(s) of maternal age.

Results

Ascertainment and study population

The 137 patients in this study group with autosomal SMCs were derived from four principal ascertainment groups: (1) 51 (37%) with abnormal phenotypes; (2) nine (7%) presenting with reproductive difficulties, principally multiple miscarriages; (3) 64 (47%) antenatal diagnoses and (4) 13 (9%) with other referral reasons (Table 1). These patterns of ascertainment were the same irrespective of whether the

SMCs were acrocentric or nonacrocentric in structure. This study has excluded SMCs that are resolvable by conventional light microscopy and associated with a defined clinical phenotype, that is, i(12p), i(9p), i(18p).

Chromosomal origins of the SMCs

A total of 112 of the 137 SMCs were further characterized by FISH and the distribution of chromosomal origins is shown in Table 2. In all, 36 (32%) of the SMCs with known chromosomal origins were nonacrocentrics and 76 (68%) were derived from the acrocentric autosomes. Of the 76 acrocentrics, 14 (18%) were SMC(13/21), 13 (17%) were SMC(14), 10 (13%) were SMC(22) and 39 (51%) were SMC(15). Overall therefore, where the chromosomal origin was known, SMC(15) comprised 51% of all acrocentric SMCs and 35% of the SMC population overall.

Parental origin

The parental origins of 109/137 (79%) cases were known, of which 76 (70%) were *de novo*, 21 (19%) maternally and 12 (11%) paternally inherited (Table 3). However, there were marked differences in parental origin among the groups: 27 of the 30 nonacrocentric SMCs (90%) were *de novo*, and three (10%) maternally transmitted, whereas of the 62 acrocentric SMCs with known parental origins, 37 (60%) were *de novo*, 15 (24%) maternally and 10 (16%) paternally inherited (Table 3).

Mosaicism

A total of 81 (59%) of the 137 cases were nonmosaic and 56 (41%) mosaic (Table 4). In all, 25 nonacrocentric SMCs (69%) were mosaics, with the remaining 11 (31%) nonmosaic. By comparison, 21 (28%) of all the acrocentric SMCs were mosaics so that the frequency of mosaicism

seen among nonacrocentric SMCs was twice that seen in SMCs derived from the acrocentric chromosomes. However, within the acrocentric SMCs, mosaicism was observed considerably less frequently among SMC(15)s, that is, (20%) compared with the SMCs derived from 13/21, 14 and 22 (38%) (see Table 5).

Analyses of parental age effects

For the analyses of parental ages, the marker chromosomes were also categorized into four classes according to their chromosomal composition, *viz*: (A) unknown, $n=25$; (B) nonacrocentrics, $n=36$; (C) acrocentrics excluding chromosome 15, $n=37$ and (D) chromosome 15, $n=39$.

A number of the patients in the database are known to have been ascertained through advanced maternal age. These individuals fall into the antenatal diagnosis ascertainment groups representing serum increased risk, nuchal translucency risk and maternal age, respectively. We also expected that individuals in the ascertainment group representing a familial marker chromosome may have older mothers. We tested this expectation by regressing the dependent variable 'deviate' against these groups.

Table 3 Parental origins of SMCs with known chromosomal origins ($n=109$)

Category	De novo		Mat		Pat	
	n	%	n	%	n	%
Nonacrocentric	27	90	3	10	0	0
All acrocentric	37	60	15	24	10	16
Acrocentric excluding SMC(15)	19	63	7	24	4	13
SMC(15)	18	56	8	25	6	19
Overall	76	70	21	19	12	11

Table 1 Ascertainment and distribution of mosaicism in total population ($n=137$)

Ascertainment	Nonmosaic	Mosaic	Total
Abnormal phenotype	33	18	51 (37%)
Reproductive problems	5	4	9 (7%)
Prenatal diagnosis	31	33	64 (47%)
Other	12	1	13 (9%)
Total	81	56	137

Table 4 Parental origins and distribution of mosaicism in total population ($n=137$)

Origin	Nonmosaic	Mosaic	Total
De novo	36 (47%)	40 (53%)	76 (56%)
Mat	18 (86%)	3 (14%)	21 (15%)
Pat	12 (100%)	0	12 (9%)
Unknown	15 (54%)	13 (46%)	28 (20%)
Total	81 (59%)	56 (41%)	137 (100%)

Table 2 Supernumerary marker chromosomes. Chromosome assignment by FISH

Chromosome	1	2	3	4	5/19	6	7	8	9	10	11	12	13/21	14	15	16	17	18	19	20	21	22	?	Total
Nonmosaic	1	1	0	0	1	0	0	2	0	0	1	0	7	9	31	2	0	2	1	0	0	8	15	81
Mosaic	0	0	1	2	3	2	0	1	3	0	0	7	7	4	8	5	0	0	1	0	0	2	10	56
Total	1	1	1	2	4	2	0	3	3	0	1	7	14	13	39	7	0	2	2	0	0	10	25	137

Table 5 Distribution of mosaicism in SMC with known chromosomal origins ($n = 112$)

Category	Mosaic (%)	Nonmosaic (%)	Total (%)
Nonacrocentric	25 (69%)	11 (31%)	36 (32%)
Acrocentric excl SMC(15)	13 (35%)	24 (65%)	37 (33%)
SMC(15) only	8 (20%)	31 (80%)	39 (35%)
Total	46 (41%)	66 (59%)	112 (100%)

Table 6 Mean deviate from maternal age

	N_{obs}	$N_{missing}$	$N_{nonmissing}$	Mean	SE	Min	Max	Origin
A Chromosome class								
A – Unknown	15	9	6	3.2167	1.5217	-1.8	8.3	
B – Nonacrocentrics	17	6	11	-0.9364	1.3287	-7.9	5.6	
C – Acrocentrics excluding chromosome 15	22	8	14	1.3214	1.6586	-12.0	9.3	
D – Chromosome 15	24	6	18	7.2944	1.4817	-4.6	21.6	
D group parental origin+all others	61	35	26	1.1971	0.9017	-12.0	9.3	0
D group unknown origin	6	3	3	4.9333	2.0169	0.9	7	1
D group <i>de novo</i>	11	11	0	9.2818	1.9709	0.4	21.6	2
	<i>Estimate</i>		<i>SE</i>		<i>F</i>	<i>P</i>		
B Regression of deviation on X								
Intercept	1.1851		0.9198		1.66	0.2039		
D group <i>de novo</i>	4.0292		0.9391		18.41	0.0001		

As expected, mothers of patients ascertained through advanced maternal age were significantly older than the mothers of the rest of the cohort ($F = 16.04$, $P < 0.0001$). The same was true for mothers of patients ascertained through a familial marker chromosome, although the effect here (as a partial regression after allowance for maternal age ascertainment) was less striking ($F = 4.44$, $P = 0.04$). These results confirmed that individuals ascertained in these ways should be excluded from further analyses. No individuals were recorded as ascertained through the nuchal translucency test. Surprisingly, we found no significant increase in maternal age in the group ascertained through serum increased risk; however, we also excluded this group on the basis of theoretical bias. A total of 59 observations were therefore excluded from further analyses, leaving a cohort of 78 individuals for our study (Table 6).

Regression analysis, again using 'deviate' as the dependent variable, identified class D [SMC(15)] as having a significantly advanced maternal age ($F = 14.57$, $P = 0.0004$), whereas classes A, B and C showed no significant increase in maternal age (see Table 6A and B). We then included the origin of the marker chromosome into our analysis and found that the significance of the advanced maternal age effect found in class D was increased in those patients with *de novo* marker

chromosomes ($F = 18.4072$, $P < 0.0001$). We also found that the increase in maternal age was linear when those with either maternally or paternally inherited chromosomes were coded as 'Origin' = 0, those whose marker chromosome origin was unknown (and so presumably a mix of inherited and *de novo*) were coded 'Origin' = 1 and those with *de novo* chromosomes were coded 'Origin' = 2. This finding was expected and confirmed the heterogeneity of those marker chromosomes of unassigned origin.

To test for any additive effect of mosaicism, we recoded the Origin variable. Assuming mosaicism arises mitotically and nonmosaicism meiotically, group D *de novo* mosaic individuals were recoded as 'Origin' = 0. We had 11 informative observations for individuals in group D of *de novo* origin, two of which are mosaics. Regressing this recoded Origin variable against the dependent variable 'deviate' did not result in any increase of significance. This would suggest that there is no evidence for any effect of mosaicism with regard to maternal age; however, the data are few and perhaps more data are needed to observe a small effect.

We have examined the distribution of the Prader-Willi/Angelman Syndrome Critical region (PWACR) in group D with regard its origin. We find a significant deficiency of observations for SMC(15)s containing two copies of the PWACR (CR++) (Fisher's exact test; $p_{two-tailed} = 0.018$) in

the maternally and paternally derived markers with a relative abundance of CR⁻ chromosomes (ie SMCs not containing the PWACR) when compared with those observations of *de novo* origin. Observations of unknown origin appear to have a mixed CR⁺⁺ and CR⁻ composition.

Discussion

Total population and methods of ascertainment

The data used for this study are derived from the STOIC database, which records all chromosome abnormalities reported following routine clinical ascertainment in the Wessex Regional Genetics Laboratory (WRGL) since 1967. We have therefore excluded for the purposes of the present study any cases that were recruited and analysed as part of defined SMC research protocols.^{11,16,19,20} The population served by the WRGL, approximately 2.5 million, over the period of the study has been stable and has not witnessed significant changes in migration patterns as seen in other parts of the UK.

The WRGL reports constitutional cytogenetic chromosome results from both perinatal and postnatal patient groups. The postnatal ascertainment patterns have remained relatively unchanged consisting largely of dysmorphic perinates and infants, patients with congenital abnormalities, developmental delay and/or idiopathic mental retardation and couples with reproductive difficulties (Table 1). By contrast, over the same time period, the prenatal referral pattern has changed from predominantly maternal age referrals to ascertainment following either serum screening and/or ultrasound measurements of nuchal thickness and congenital abnormality scans (data not shown – see below). Overall, 47% of all the SMCs were ascertained during prenatal diagnosis, and among those where the chromosomal origin was determined, 29% were SMC(15)s, 33% SMC(other acrocentrics) and 37% non-acrocentric SMCs.

The distribution of SMCs by chromosomal origin and ascertainment

Using FISH, we were able to identify the autosomal chromosomal origins of 112/137 (82%) of the SMCs in our study population, of which 76 (68%) were derived from the acrocentric autosomes with all other autosomes represented at least once except for chromosomes 7, 10, 17, 20 and 21 (Table 3). SMCs derived from chromosome 15 were predominantly of the type usually referred to as inv dup(15)s and accounted for 39/112 (35%) of all the chromosomally identified SMCs and for approximately one-half of all the acrocentric SMCs observed. The results of the present study confirm that SMC(15)s are the most frequently diagnosed SMCs in man. Some previous reports have suggested that SMC(15)s account for over half of all SMCs detected.²¹ However, more recently, FISH-based

studies have shown that the distribution of antenatally ascertained acrocentric SMCs varies from 77 to 81%,^{4,22,23} and within these studies SMC(15)s accounted for 76, 45 and 33% of all the acrocentric markers identified. By combining the data from the present study with the three studies quoted above, a frequency overall of 36% SMC(15) encountered during prenatal diagnosis is obtained.

Mosaicism and the chromosomal origins of SMCs

Our data confirm previous reports showing striking differences in the distribution of mosaicism when the SMCs are classified according to their chromosomal origins. Overall, 59% of SMCs were found in association with a normal cell line, but the distribution was markedly asymmetric with 28% of the acrocentric SMCs compared with 69% of nonacrocentric SMCs being mosaic. Previous larger studies have also reported differences in the levels of mosaicism depending on whether the SMC was satellited or nonsatellited (pre-FISH)^{1,2} or acrocentric or nonacrocentric (post-FISH). In these studies, the level of mosaicism overall was reported to range from 13 to 50% (ave. 46%), but with ~30% of the acrocentric SMCs compared with ~70% of the nonacrocentric SMCs being classified as mosaics.^{2,4,10,22,23}

Further analyses of our data show that within the acrocentric SMCs, 20% of the SMC(15) and SMC(22) were mosaic compared with 31% for SMC(14) and 50% for SMC(13/21) respectively. Previous reports on SMC(15) have shown that on average ~85% of SMC(15)s present as nonmosaics^{13,24,28} and the majority of SMC(22) reported are also nonmosaics.^{25,26}

The fact that the majority of SMC(15) are nonmosaics suggests a meiotic origin of these markers and this is supported by the observations that all *de novo* SMC(15)s characterized molecularly have been shown to be maternal in origin,^{11,24,27–29} and in a proportion of those containing additional copies of the PWACR, molecular studies show that they are divided equally between being inter- and intrachromosomal in origin.³⁰ As far as we are aware, no comparable and detailed molecular studies have been carried out on SMCs derived from chromosome 13/21, 14 and 22, and so it is not clear if the mechanisms responsible for the formation of SMC(15) are unique to this chromosomal class.

Parental age effects in SMCs

Hook and Cross³ reported a significant maternal age effect in association with *de novo* SMCs in a study population of 75 000 prenatal cytogenetic diagnoses. This study was carried out before the advent of FISH so that the chromosomal subclasses of SMC were not analysed independently as in the present study. As far as we are aware, this original observation with respect to parental age effects associated with SMCs has not been re-examined, so it is generally assumed that all SMCs, irrespective of

chromosomal origin, are associated with an increased maternal age effect.

Our results have shown that although there is no overall maternal age effect associated with SMCs, *de novo* SMCs derived from chromosome 15 are associated with a significantly increased maternal age. It has previously been shown that there is an exponential increase with maternal age associated with maternal meiosis I nondisjunction involving chromosome 15³¹ and with uniparental disomy (UPD) for chromosome 15.³² There have also been a number of hypotheses to account for the relatively high frequency of SMC(15)s in humans, and it is true that all of the SMC(15)s studied molecularly have been found to have arisen following female meiotic errors. The maternal age effect associated with *de novo* SMC(15)s would suggest therefore a common origin for both trisomy 15 following nondisjunction at meiosis I with subsequent complete trisomy rescue leading to UPD(15) or, incomplete trisomy rescue resulting in the formation of a SMC(15). In either event, the common origin appears to be a maternal age-related disruption of normal disjunction of the chromosome 15 bivalent during female meiosis. As discussed above, lack of comparable data concerning SMCs from the other acrocentric chromosomes does not allow a conclusion to be drawn on the uniqueness or otherwise of the observations concerning chromosome 15.

Our previous studies classified the larger SMC(15)s, which predominantly contained two copies of the PWACR into two broad groups based on their most distal breakpoint. The larger group (~70%) were generally asymmetrical with two distal breakpoints, both located distal to the common PWACR distal breakpoint (BP3). The remaining ~30% of SMC(15)s by comparison reveal a more heterogeneous distribution of breakpoints most of which had occurred within BP3.³³ Both groups of SMC(15)s were also shown to have asymmetrical breakpoints and, furthermore, the two main breakpoint regions occur at sites of duplicated genomic segments,³⁴ suggesting that these duplicons are responsible, at least in part, for the increased instability in this region, and the most popular theory for the formation of SMC(15)s is a U-type exchange between homologous chromosomes during meiosis I followed by illegitimate fusion of the chromatids and nondisjunction.^{35,36} A combination of these events, with maternal age related nondisjunction as the primary mechanism, may go some way to explain the relatively high frequency with which SMC(15)s are ascertained in the human population.

In conclusion, this study has shown that autosomal SMCs derived from acrocentric and nonacrocentric chromosomes differ in the proportion, which have arisen *de novo*, and also in the frequency with which the SMC is seen associated with a normal cell line. Furthermore, we have shown that a significant maternal age effect is restricted to *de novo* SMCs derived from chromosome 15.

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