

Frequency of small supernumerary marker chromosomes in prenatal, newborn, developmentally retarded and infertility diagnostics

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Abstract. In this study the substantial and in part contradictory data available in the literature was collected concerning the frequency of small supernumerary marker chromosomes (sSMC) in the human population in general, and in special subpopulations. One hundred and thirty-two studies on sSMC were reviewed. In summary 1,288,693 cytogenetically studied cases detecting 980 sSMC were compiled. In 132 international surveys there were no ethnic effects detected in the sSMC frequency. sSMC were present in 0.075% of unselected prenatal cases but only in 0.044% of consecutively studied postnatal ones. In infertile subjects, 0.125% were sSMC carriers, distinguishing male from female subjects by a 7.5:1 difference in sSMC frequency for this special group. In developmentally retarded patients the sSMC rate was elevated to 0.288%, similar to prenatal cases with ultrasound abnormalities (0.204%). No increased risk for the presence of sSMC was detected in ICSI-induced pregnancies. Worldwide there are $\sim 2.7 \times 10^6$ living sSMC carriers; 1.8×10^6 have a *de novo* sSMC and $\sim 70\%$ of those are clinically normal. Strikingly, 30-50% of pregnancies diagnosed with an sSMC fetus are terminated. This may be connected with the empirical risk that $\sim 30\%$ of sSMC carriers manifest clinical abnormalities. Thus, in summary there is a strong need for a better genotype-phenotype correlation enabling better genetic counseling.

Introduction

Small supernumerary marker chromosomes (sSMC) were recently defined as structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone; they are generally equal in size or smaller than a chromosome 20 of the same metaphase spread. If detected in banding cytogenetics they

are still a major problem as they are too small to be considered for their chromosomal origin by traditional banding techniques; molecular cytogenetic techniques are needed for their characterization (1). The risk for an abnormal phenotype in prenatally ascertained *de novo* cases with sSMC is given as $\sim 13\%$ (2). This has been refined to 7% (for sSMC from chromosome 13, 14, 21 or 22) and 28% (for all non-acrocentric autosomes) (3) and recently has been suggested to be 26% (4). Thus, the statement of Paoloni-Giacobino *et al* (5) is still valid, i.e. cases with a *de novo* sSMC, particularly prenatally ascertained ones, are not easily correlated with a clinical outcome, even though first approaches in that direction were recently attempted (6). With respect to current technical developments in molecular cytogenetics, such as cenM-FISH techniques (7-9) and molecular genetic approaches as array-CGH, (10), further progress in this clinically important field is expected.

However, an important, but not yet thoroughly discussed and understood basic issue is the frequency of sSMC in prenatal as well as in postnatal cases and in patients with infertility or with developmental and/or mental retardation. Here we address this question by reviewing 132 suited datasets derived from the literature as well as from our own laboratory.

Materials and methods

We recently collected all the literature on sSMC and made it available on the sSMC homepage (11). Based on this collection we presented the available literature from which conclusions on sSMC frequency in different human subpopulations can be drawn. Data from 132 studies with a total of 1,288,693 cytogenetically studied cases detecting 980 sSMC were assembled (Tables I-IV).

According to the cytogenetic definition of an sSMC (1) also cases *i*(18p), *der*(22), *i*(12p) and *inv dup*(22) were counted as sSMC, if listed separately in any of the 132 included studies.

In summary, 1,074,421 prenatal cases were included in Tables IA and B. In Table IA only unselected prenatal cases (688,030) and in Table IB three types of pre-selected cases were incorporated, i.e. 386,391 cases reporting only *de novo* aberrations, 4,409 cases selected due to ultrasound abnormalities and 4,625 cases born after ICSI treatment.

In Table IIA, 121,694 consecutive newborn individuals from 10 studies were summarized. Table IIB shows the only available study on 1,405 unselected normal adult cases.

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Table I. sSMC frequency in prenatal cases.

No. of study	Reference	Country study performed in	No. of centers involved	Studied cases	Cases with sSMC	
					Absolute	(%)
1	Jotterand-Bellomo <i>et al</i> , 1988 (32)	Switzerland	1	551	0	0.000
2	Fortuny <i>et al</i> , 1988 (33)	Spain	1	600	0	0.000
3	Crandall <i>et al</i> , 1980 (34)	USA	1	2,500	0	0.000
4	Hsieh <i>et al</i> , 1992 (35)	P.R. China	1	2,975	0	0.000
5	Boue <i>et al</i> , 1982 (36)	France	1	5,315	0	0.000
6	Yaegashi <i>et al</i> , 1998 (37)	Japan	4	5,484	0	0.000
7	Eydoux <i>et al</i> , 1989 (13)	France	4	6,515	0	0.000
8	Park <i>et al</i> , 2003 (38)	Korea	1	5,501	2	0.036
9	Caron <i>et al</i> , 1999 (15)	Canada	1	35,131	13	0.037
10	Stengel-Rutkowski and Nummermann, 1991 (39)	Germany	1	7,124	3	0.042
11	Tabor and Philip, 1987 (40)	Denmark	1	2,264	1	0.044
12	Shaffer <i>et al</i> , 2004 (31)	USA	2	45,000	22	0.048
13	Squire <i>et al</i> , 1982 (41)	UK	1	1,687	1	0.049
14	Vejerslev and Mikkelsen, 1989 (42)	Europe (14 countries)	36	7,800	4	0.051
15	Association of Clinical Cytogeneticists, 1994 (43)	UK	n.a. (>1)	7,415	4	0.054
16	Stengel-Rutkowski <i>et al</i> , 1978 (44)	Germany	n.a. (>1)	5,165	3	0.058
17	Thein <i>et al</i> , 2000 (45)	UK	1	1,687	1	0.059
18	Ferguson-Smith and Yates, 1984 (21)	UK	58	52,965	32	0.060
19	Djalali, 1990 (46)	Germany	1	20,370	13	0.064
20	Hsu <i>et al</i> , 1996 (20)	USA	12	179,663	113	0.063
21	Woo <i>et al</i> , 2003 (47)	Korea	1	1,541	10	0.065
22	Daniel <i>et al</i> , 1982 (48)	Australia	1	3,000	2	0.067
23	Grati <i>et al</i> , 2006 (49)	Italy	85	15,109	11	0.073
24	Golbus <i>et al</i> , 1979 (50)	USA	1	2,699	2	0.074
25	Al-Kouatly <i>et al</i> , 2002 (51)	USA	3	9,199	7	0.076
26	Ledbetter <i>et al</i> , 1992 (52)	USA	9	11,436	9	0.078
27	Hook and Cross, 1987 (16)	USA	40	78,567	62	0.079
28	Blennow <i>et al</i> , 1994 (28)	Sweden	5	39,105	31	0.079
29	Bartsch <i>et al</i> , 2005 (53)	Germany	1	43,273	42	0.097
30	Brondum-Nielsen and Mikkelsen M, 1995 (19)	Denmark	1	12,699	14	0.110
31	Li <i>et al</i> , 2000 (54)	USA	1	15,781	18	0.114
32	Benn and Hsu, 1984 (55)	USA	1	6,500	8	0.123
33	Karaman <i>et al</i> , 2006 (27)	Turkey	2	15,792	20	0.127
34	Sachs <i>et al</i> , 1987 (18)	The Netherlands	1	10,000	15	0.150
35	Hume <i>et al</i> , 1995 (26)	USA	1	12,454	19	0.153
36	Kaluzewski <i>et al</i> , 2001 (56)	USA	1	5,955	10	0.168
37	Lippman <i>et al</i> , 1992 (57)	Canada	7	2,888	5	0.173
38	Carrasco Juan <i>et al</i> , 1990 (58)	Spain	1	1,000	2	0.200
39	Authors' laboratory 2000-2005	Germany	1	2,671	6	0.225
40	Dahoun-Hadorn and Delozier-Blanchet, 1990 (59)	Switzerland	1	811	2	0.247
41	Van den Berg <i>et al</i> , 2000 (60)	The Netherlands	1	1,838	7	0.381
Sum			~240	688,030	514	0.075

^aForty-one studies on prenatal cases detecting 514 sSMC in 688,030 cytogenetic cases are summarized here. n.a., not available for all sSMC cases reported.

B, Pre-selected prenatal cases.^b

No. of study	Reference	Country study performed in	No. of centers involved	Studied cases	Cases with sSMC	
					Absolute	(%)
Only <i>de novo</i> sSMC						
42	Warburton, 1991 (2)	USA, Canada	92	377,357	162	0.043
Only in ultrasound aberrant cases						
43	Wilson <i>et al</i> , 1992 (61)	Canada	1	151	0	0.000
44	Palmer <i>et al</i> , 1992 (62)	USA	1	147	0	0.000
45	Van Zalen-Sprock <i>et al</i> , 1991 (63)	The Netherlands	1	288	0	0.000
46	Staebler <i>et al</i> , 2005 (64)	Belgium	6	428	0	0.000
47	Daniel <i>et al</i> , 2003 (65)	Australia	8	2,143	3	0.140
48	Rizzo <i>et al</i> , 1990 (66)	Italy	1	237	1	0.422
7	Eydoux <i>et al</i> , 1989 (13)	France	4	875	4	0.457
49	Hentemann <i>et al</i> , 1989 (67)	Canada	1	140	1	0.714
Sum			23	4,409	9	0.204
ICSI cases						
50	Lam <i>et al</i> , 2001 (68)	Canada	1	43	0	0.000
51	Van Golde <i>et al</i> , 1999 (69)	Spain	1	56	0	0.000
52	Causio <i>et al</i> , 1999 (70)	Italy	1	63	0	0.000
53	Van Opstal <i>et al</i> , 1997 (71)	The Netherlands	1	71	0	0.000
54	Testart <i>et al</i> , 1996 (72)	France	1	108	0	0.000
55	Samli <i>et al</i> , 2003 (73)	Turkey	1	142	0	0.000
56	Szigeti <i>et al</i> , 2004 (74)	Hungary	1	146	0	0.000
57	Wennerholm <i>et al</i> , 1999 (75)	Sweden	1	149	0	0.000
58	Loft <i>et al</i> , 1999 (76)	Denmark	13	209	0	0.000
59	Jozwiak <i>et al</i> , 2004 (77)	Turkey	1	1,136	0	0.000
60	Bonduelle <i>et al</i> , 2002 (78)	Belgium	1	1,586	0	0.000
61	Wisanto <i>et al</i> , 1996 (79)	Belgium	1	486	1 ^c	0.205
62	Aboulghar <i>et al</i> , 2001 (80)	Egypt	1	430	1	0.233
Sum			25	4,625	2	0.043

^bIn summary 22 studies on three pre-selected subpopulations of prenatal diagnostics detecting only *de novo* sSMC or looking for sSMC in ICSI or in ultrasound abnormal prenatal cases. ^cTwin pregnancy, both twins had sSMC - familial.

Tables III and IV summarize 69,332 developmentally and/or mentally retarded patients and 30,510 patients with infertility problems, respectively.

In Table V cases already listed in Table I and II were analyzed for the frequency of *de novo* and inherited sSMC.

Results

The goal of this paper was to give an approximate rate of sSMC cases expected in four main groups of patients: prenatal, postnatal, developmentally and/or mentally retarded and infertile people.

Apparent from Tables I-V the study sizes as well as the detection rates of sSMC varied in all reviewed subgroups; between 15 (12) and 377,357 cases (2) and 0 (12,13) and 162 sSMC carriers (2), respectively. The included cases were studied in time frames between 0.5 (14) and 23 years (15).

Here and in Tables I-V as well as in Figs. 1 and 2 the results for the four aforementioned groups are listed.

Group 1: Prenatal cases. In routine prenatal diagnostics 688,030 cases provided by >240 laboratories, including our own detected in summary 514 sSMC (Table IA). The datasets, which were acquired in 20 different countries indicated a frequency of 0.075% of sSMC in unselected prenatal cases. As summarized in Tables VA and B the detection rate was the same, independent if chorion villi samples (CVS) or amniotic fluid cells (AFC) were studied (Fig. 1). According to study 42 the rate of *de novo* sSMC was 0.043%; studies 7 and 43-49 indicated an enhanced sSMC rate of 0.204% in ultrasound-abnormal cases, and in 4,625 ICSI cases (studies 50-62) 2 sSMC were detected (0.043%) (Table IB).

Group 2: Postnatal cases. In Table IIA we compiled ten newborn studies, to determine the sSMC frequency in the general living human population. Only studies on consecutively newborn children, without further selection criteria were included. In 12,694 postnatal subjects, 54 sSMC cases were described, corresponding to a rate of 0.044% of

Table II.

A, Consecutive newborn cases.^a

No. of study	Reference	Country study performed in	No. of centers involved	Studied cases	Cases with sSMC	
					Absolute	(%)
63	Lin <i>et al</i> , 1976 (14)	Canada	1	930	0	0.000
64	Xia <i>et al</i> , 1982 (81)	P.R. China	1	2,079	0	0.000
65	Maeda <i>et al</i> , 1991 (82)	Japan	1	14,835	4	0.027
66	Hook and Hamerton, 1977 (17)	Canada, UK, Denmark, USA	7	56,952	16	0.028
67	Buckton <i>et al</i> , 1985 (83)	UK	1	3,993	2	0.050
68	Nielsen and Rasmussen, 1975 (23)	Denmark	1	11,148	6	0.054
69	Bratkowska <i>et al</i> , 1985 (84)	Poland	1	3,665	2	0.055
70	Nielsen and Wohler, 1991 (22)	Denmark	1	23,762 ^b	18	0.076
71	Buchkov <i>et al</i> , 1974 (85)	Russia	1	2,500	2	0.080
72	Hansteen <i>et al</i> , 1982 (86)	Norway	1	1,830	4	0.219
Sum			16	121,694	54	0.044

^aTen studies on consecutive newborn cases detecting in summary 54 sSMC in 121,694 cases. ^bWithout the cases previously mentioned in Nielsen and Rasmussen, 1975.

B, Normal adult cases.^c

No. of study	Reference	Country study performed in	No. of centers involved	Studied cases	Cases with sSMC	
					Absolute	(%)
73	Tawn and Earl, 1992 (87)	UK	1	1,405	1	0.071

^cOnly one small study was available on sSMC frequency in normal adult humans.

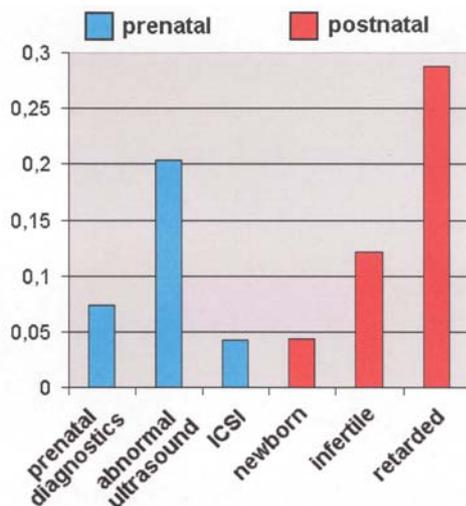


Figure 1. The frequency of sSMC. Data for prenatal (blue columns) and postnatal studies (red columns) according to Tables I-IV is summarized. The sSMC frequencies in prenatal diagnostics (Table IA), in ultrasound abnormal and in ICSI-induced pregnancies (Table IB) are shown in comparison to sSMC frequencies in newborn, infertile and developmentally retarded subjects.

sSMC carriers in the general population. In study 73, 1,405 normal probands were cytogenetically analyzed and 1 sSMC carrier was identified (Table IIB).

Group 3: Developmentally retarded patients. Twenty-six studies provided 69,332 developmentally retarded patients and 200 sSMC carriers were identified, i.e. a rate of 0.288% (Table III).

Group 4: Patients with fertility problems. Forty-one cytogenetic studies on a total of 30,510 patients with different fertility problems were available. In general a rate of 0.125% sSMC carriers was detected. When analyzing the dataset of Table IV in a gender-specific manner, the picture changes; i.e. 36/21,841 (0.165%) male and 2/9,165 (0.022%) woman sSMC carriers were found.

In Table VA-C also the rate of *de novo* and familial sSMC was determined based on the studies listed in Tables IA and IIA. According to the studies in which pertinent data was available, *de novo* sSMC constituted ~70% and familial, ~30% of the cases (Fig. 2).

Discussion

The frequency of sSMC carriers was given in the literature normally by citing 1-3 more or less randomly selected population studies, most frequently those of Hook and Hamerton (16), Hook and Cross (17), Sachs (18), Warburton (2), Brondum-Nielsen and Mikkelsen (19) and Hsu *et al* (20). Thus, the sSMC frequency was normally presented as between 0.028% and 0.150%.

No. of study	Reference	Studied cases	Cases with sSMC	
			Absolute	(%)
74	Cora <i>et al</i> , 2000 (88)	120	0	0.000
75	Kodama, 1982 (89)	197	0	0.000
76	Srsen <i>et al</i> , 1989 (90)	324	0	0.000
77	Al Husain and Zaki, 1999 (91)	337	0	0.000
78	Higurashi <i>et al</i> , 1985 (92)	455	0	0.000
79	Fryns <i>et al</i> , 1982 (93)	32,930	62	0.118
80	Rasmussen <i>et al</i> , 1982 (94)	1,905	3	0.158
81	Hernández <i>et al</i> , 1990 (95)	1,586	3	0.189
82	Moreno-Garcia <i>et al</i> , 2005 (96)	972	2	0.206
83	Wuu <i>et al</i> , 1984 (97)	470	1	0.213
84	Phelan <i>et al</i> , 1996 (98)	4,485	11	0.245
85	Price <i>et al</i> , 1976 (99)	611	2	0.327
67	Buckton <i>et al</i> , 1985 (83)	3,673	12	0.327
86	Bourgeois and Benezech, 1977 (100)	600	2	0.333
21	Woo <i>et al</i> , 2003 (47)	1,443	5	0.347
36	Kaluzewski <i>et al</i> , 2001 (56)	902	4	0.443
87	Hou and Wang, 1998 (101)	11,893	54	0.454
88	Hong <i>et al</i> , 1999 (102)	604	3	0.500
89	Kim <i>et al</i> , 1999 (103)	4,117	24	0.583
90	Singh <i>et al</i> , 1974 (104)	504	3	0.595
91	Kirkilionis and Sergovich, 1987 (105)	495	3	0.606
92	Toyota <i>et al</i> , 2001 (106)	161	1	0.621
93	Mulcahy and Jenkyn, 1972 (107)	154	1	0.649
94	Battaglia <i>et al</i> , 1999 (108)	120	1	0.833
95	Felix <i>et al</i> , 1998 (109)	202	2	0.990
96	Borgaonkar <i>et al</i> , 1971 (110)	72	1	1.389
Sum		69,332	200	0.288

^aTwenty-six studies provided data for sSMC frequency in developmentally retarded patients.

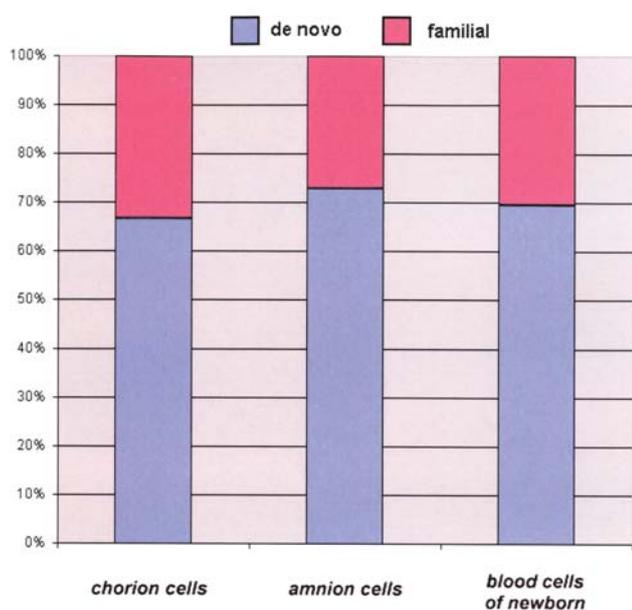


Figure 2. Frequency of *de novo* and familial sSMC. Comparison of the frequency of *de novo* and familial sSMC in CVS, AFC and blood of newborn cases based on the data provided in Table V. The rate is more or less always 2:1.

These variations are mainly caused by the studied population size and the bias a single study can be subjected to. The prenatal, as well as the postnatal studies included data worldwide, as detailed in Tables I and II in the category 'Country study performed in'. As countries such as Australia, Canada and the USA included people from Asian, African, Australian and European descent, all ethnic groups were represented in the studies. Moreover, different European, Egyptian, Japanese, Korean and Chinese studies were also included. For example, five studies performed in Germany, a country with a relatively homogeneous population showed variations between 0.042% and 0.225% of the detection rate for prenatal sSMC (Table I, studies 10, 16, 19, 29 and 39). An ethnic effect in sSMC frequency was not detected, at least not on the available sample size.

Here we attempted to ascertain the frequency of sSMC carriers in the general population and in some subpopulations.

As some well-recognized and frequently cited previous studies (2,21) summarized data from different laboratories in similar ways to us, the present attempt to compile patient data from 132 studies in 4 different main groups: prenatal, postnatal, developmentally and/or mentally retarded and infertile seemed to be legitimate and straight forward. However, during this review we encountered problems

Table IV. Patients with fertility problems.^a

No. of study	Reference	Number of studied cases			Cases with sSMC		
		Male	Female	Total	Male	Female	Total (%)
97	Martin <i>et al</i> , 1986 (12)	0	15	15	n.a.	0	0.000
51	Van Golde <i>et al</i> , 1999 (69)	23	n.a.	23	0	n.a.	0.000
98	Baschat <i>et al</i> , 1996 (111)	32	n.a.	32	0	n.a.	0.000
99	Kleiman <i>et al</i> , 1999 (112)	72	n.a.	72	0	n.a.	0.000
100	Penna Videau <i>et al</i> , 2001 (113)	84	n.a.	84	0	n.a.	0.000
101	Quilter <i>et al</i> , 2003 (114)	103	n.a.	103	0	n.a.	0.000
102	Stuppia <i>et al</i> , 1998 (115)	126	n.a.	126	0	n.a.	0.000
103	Raziel <i>et al</i> , 2002 (116)	65	65	130	0	0	0.000
104	Westlander <i>et al</i> , 1999 (117)	137	n.a.	137	0	n.a.	0.000
105	Lange <i>et al</i> , 1993 (118)	72	72	144	0	0	0.000
106	Schreurs <i>et al</i> , 2000 (119)	-	163	163	n.a.	0	0.000
107	Pauer <i>et al</i> , 1997 (120)	128	122	250	0	0	0.000
77	Al Husain and Zaki, 1999 (91)	128	129	257	0	0	0.000
108	Farzanfar and Azimi, 2005 (121)	257	n.a.	257	0	n.a.	0.000
109	Crüger <i>et al</i> , 2003 (122)	392	n.a.	392	0	n.a.	0.000
110	Micic <i>et al</i> , 1984 (123)	820	n.a.	820	0	n.a.	0.000
111	Matsuda <i>et al</i> , 1989 (124)	554	n.a.	554	0	n.a.	0.000
52	Causio <i>et al</i> , 1999 (70)	301	301	602	0	0	0.000
112	Haidl <i>et al</i> , 2000 (125)	305	305	610	0	0	0.000
2	Fortuny <i>et al</i> , 1988 (33)	445	445	890	0	0	0.000
113	Yoshida <i>et al</i> , 1997 (126)	1,007	n.a.	1,007	0	n.a.	0.000
114	Makino <i>et al</i> , 1990 (127)	639	639	1,278	0	0	0.000
115	Celep <i>et al</i> , 2006 (128)	645	645	1,290	0	0	0.000
116	Palka <i>et al</i> , 1978 (129)	2,078	n.a.	2,078	2	n.a.	0.048
117	Peschka <i>et al</i> , 1999 (130)	781	781	1,562	1	0	0.064
118	Radojic Badovinac <i>et al</i> , 2000 (131)	676	624	1,300	2	0	0.077
119	Gekas <i>et al</i> , 2001 (132)	2,196	1,012	3,208	3	0	0.094
120	Hens <i>et al</i> , 1988 (133)	500	500	1,000	1	0	0.100
121	Meschede <i>et al</i> , 1998 (134)	432	436	868	1	0	0.115
122	Scholtes <i>et al</i> , 1998 (135)	1,116	1,164	2,280	3	0	0.132
123	Morel <i>et al</i> , 2004 (136)	335	370	705	1	0	0.142
124	Van Assche <i>et al</i> , 1996 (137)	694	n.a.	694	1	n.a.	0.144
54	Testart <i>et al</i> , 1996 (72)	261	261	522	1	0	0.192
125	Tuerlings <i>et al</i> , 1998 (138)	1,792	n.a.	1,792	4	n.a.	0.223
126	Chandley <i>et al</i> , 1975 (139)	1,599	966	2,565	4	2	0.234
127	Pandiyan and Jequier, 1996 (140)	1,210	n.a.	1,210	3	n.a.	0.248
128	Mau <i>et al</i> , 1997 (141)	150	150	300	1	n.a.	0.333
129	Bourrouillou <i>et al</i> , 1985 (142)	952	n.a.	952	4	n.a.	0.420
130	Dohle <i>et al</i> , 2002 (143)	150	n.a.	150	1	n.a.	0.667
131	Retief <i>et al</i> , 1984 (144)	496	n.a.	496	2	n.a.	0.403
132	Nagvenkar <i>et al</i> , 2005 (145)	88	n.a.	88	1	0	1.136
Sum		21,841	9,165	30,510	36	2	0.125
					=0.165%	=0.022%	

^asSMC frequency in patients with fertility problems was found to be on average 0.125%, but 0.165% for male and 0.022% for female subjects. n.a., not available for all sSMC cases reported.

concerning the comparability of the research papers included. Especially for the prenatally analyzed cases (Table I) some of the reported details were different, e.g. study 42 (2) only reported *de novo* and no familial sSMC cases. Others such as study 18 of Ferguson-Smith MA and Yates (21) did not discriminate between familial or *de novo* sSMC; the later study also provided no information whether AFC and CVS

were analyzed. Thus, to answer questions concerning frequencies in different tissue types and the parental origin of sSMC, the data summarized in Tables IA and IIA was extracted, and the relevant studies were included in Table VA-C.

Another problem was that datasets of some of the studies included in Tables I-IV were repeatedly published in an



SPANDIDOS PUBLICATIONS Excerpt of Tables I and II concerning the frequency and the parental origin of sSMC in CVS, AFC and blood of cases.

A, Parental origin of sSMC in CVS.

No. of study	Reference	Studied cases	Cases with sSMC	Parental origin of sSMC	
				<i>De novo</i>	Familial
1	Jotterand-Bellomo <i>et al</i> , 1988 (32)	551	0	0	0
7	Eydoux <i>et al</i> , 1989 (13)	4 ^a	0	0	0
10	Stengel-Rutkowski and Nummermann, 1991 (40)	7,124	3	n.a.	n.a.
14	Vejerslev and Mikkelsen, 1989 (42)	7,800	4	n.a.	n.a.
15	Association of Clinical Cytogeneticists, 1994 (43)	7,415	4	n.a.	n.a.
23	Grati <i>et al</i> , 2006 (49)	15,109	11	n.a.	n.a.
26	Ledbetter <i>et al</i> , 1992 (52)	11,436	9	n.a.	n.a.
28	Blennow <i>et al</i> , 1994 (28)	4,159 ^a	1	1	0
30	Brondum-Nielsen and Mikkelsen M, 1995 (19)	1,644 ^a	1	1	0
33	Karaman <i>et al</i> , 2006 (27)	904 ^b	0	0	0
34	Sachs <i>et al</i> , 1987 (18)	1 ^a	0	0	1
37	Lippman <i>et al</i> , 1992 (57)	1,019 ^a	4	2	2
39	Authors' laboratory 2000-2005	140 ^a	0	0	0
41	van den Berg <i>et al</i> , 2000 (60)	1,838	7 ^c	4	1
Sum		59,144	44 =0.074%	8/9,705 =0.082%	4/9,705 =0.041%

^aAFC cases were excluded; ^bAFC cases and fetal blood cases were excluded; ^ctwo cases with unknown parental origin. n.a., not available for all sSMC cases reported.

B, Parental origin of sSMC in AFC.

No. of study	Reference	Studied cases	Cases with sSMC	Parental origin of sSMC	
				<i>De novo</i>	Familial
2	Fortuny <i>et al</i> , 1988 (33)	600	0	0	0
3	Crandall <i>et al</i> , 1980 (34)	2,500	0	0	0
4	Hsieh <i>et al</i> , 1992 (35)	2,975	0	0	0
5	Boue <i>et al</i> , 1982 (36)	5,315	0	0	0
6	Yaegashi <i>et al</i> , 1998 (37)	5,484	0	0	0
7	Eydoux <i>et al</i> , 1989 (13)	6,515	0	0	0
11	Tabor and Philip, 1987 (40)	2,264	1	0	1
13	Squire <i>et al</i> , 1982 (41)	2,036	1	1	0
16	Stengel-Rutkowski <i>et al</i> , 1978 (44)	5,165	3	n.a.	n.a.
18	Ferguson-Smith and Yates, 1984 (21)	52,965	32	n.a.	n.a.
19	Djalali, 1990 (46)	20,370	7	n.a.	n.a.
20	Hsu <i>et al</i> , 1996 (20)	179,663	113	n.a.	n.a.
21	Woo <i>et al</i> , 2003 (47)	1,541	9	n.a.	n.a.
22	Daniel <i>et al</i> , 1982 (48)	3,000	2	1	1
24	Golbus <i>et al</i> , 1979 (50)	2,699	2	n.a.	n.a.
25	Al-Kouatly <i>et al</i> , 2002 (51)	8,642 ^d	7	6	1
27	Hook and Cross, 1987 (16)	78,567	62	n.a.	n.a.
28	Blennow <i>et al</i> , 1994 (28)	34,908 ^d	30	n.a.	n.a.
29	Bartsch <i>et al</i> , 2005 (53)	43,273	42	29	13
30	Brondum-Nielsen and Mikkelsen M, 1995 (19)	11,055 ^d	13	8	5
31	Li <i>et al</i> , 2000 (54)	15,781	18	n.a.	n.a.
32	Benn and Hsu, 1984 (55)	6,500	8	5	3
33	Karaman <i>et al</i> , 2006 (27)	11,898 ^e	14	12	2
34	Sachs <i>et al</i> , 1987 (18)	9,999 ^d	14	14	0
37	Lippman <i>et al</i> , 1992 (57)	968 ^d	1	1	0
38	Carrasco Juan <i>et al</i> , 1990 (58)	1,000	2	0	2

Table VB. Continued.

No. of study	Reference	Studied cases	Cases with sSMC	Parental origin of sSMC	
				<i>De novo</i>	Familial
39	own laboratory 2000-2005	2,531 ^d	6	4	2
40	Dahoun-Hadorn and Delozier-Blanchet, 1990 (59)	811	2	1	1
Sum		519,025	382 =0.074%	82/122,051 =0.067%	31/122,051 =0.025%

^dPrenatal CVS were excluded; ^eprenatal CVS and fetal blood cases were excluded. n.a., not available for all sSMC cases reported.

C, Parental origin of sSMC in the blood of newborn cases.

No. of study	Reference	Studied cases	Cases with sSMC	Parental origin of sSMC	
				<i>De novo</i>	Familial
63	Aboulghar <i>et al</i> , 2001 (80)	930	0	0	0
68	Nielsen and Rasmussen, 1975 (23)	11,148	6	4	2
72	Hansteen <i>et al</i> , 1982 (86)	1,830	4	3	1
Sum		13,908	10 =0.071%	7 =0.050%	3 =0.022%

'overlapping' way in several papers, e.g. some data available in Nielsen and Wohlert (22) was previously reported in Nielsen and Rasmussen (23). Thus, one had to be extremely careful not to include the same data twice, especially, as in some publications not very detailed and/or comprehensive references were given on previously published data. To the best of our knowledge we avoided the double use of identical, but repeatedly published data in all Tables.

The last and maybe most critical point concerning the comparability of the studies included in Tables I-IV is that when all these studies were performed, no uniform definition of an sSMC was available. Thus, apart from the study of Warburton (2), it was hard if not impossible to know if an sSMC included or excluded isochromosomes 9p, 12p, 18p or Pallister-Killian syndrome and Cat-eye- or der(22)-syndrome chromosomes. While isochromosomes 9p are not sSMC according to the definition of Liehr *et al* (1), the other ones are. However, this point was not clarified and was also never questioned in the previous studies included in Tables I-IV. The presence of sSMC was only one of many different cytogenetic aberrations listed in these studies, and for each author/author group there was no question as to what an sSMC was - it seemed clear to them and thus required no closer reflection.

According to the data from ten consecutive, completely unselected newborn studies (Table IIA), a rate of 0.044% of sSMC carriers was determined in the general living newborn population. With an estimated human world population of 6,560,000,000 people there are at present $\sim 2.7 \times 10^6$ living sSMC carriers.

As previously described (1), the sSMC rate is $\sim 7x$ higher in (develop)mentally retarded patients (Table III) than in the normal population. This is similar, as we will discuss later, for the prenatal cases with ultrasound abnormalities, not surprising due to the fact that patients with i(12p), i(18p), i(22), der(22) but also larger inv dup(15) are overrepresented in this clinical group.

Patients with fertility problems (Table IV) have a $\sim 2.9x$ enhanced risk for an sSMC compared to the general population. To note, the rate of sSMC carriers in males versus females was 7.5:1. This observation was biased by heterogeneous reasons which led to the likely inclusion into the group of 'infertility patients' which were studied cytogenetically. However, the rate of male versus female sSMC carriers is strikingly different; and if valid, the mechanisms why an sSMC leads predominantly to male fertility problems have not been elucidated as yet. However, there are hints that oligozoospermia is significantly correlated with sSMC presence in 7% of subjects, while in azoospermia patients, sSMC is present in <1% of the corresponding cases (24). These observations also fit the recently outlined fact that familial sSMC are predominantly inherited via the maternal line (25).

According to the data reviewed here, sSMC are to be expected in 0.075% of all analyzed prenatal cases. There was no difference in the sSMC rate in CVS compared to AFC (Table VA and B). Thus, it can be carefully concluded that there seems to be no significant loss of pregnancies in connection with sSMC between weeks 10-14 (analysis of CVS) compared to weeks 9-15 (analysis of AFC).

Information on the parental origin in 9,705 CVS, 12,2051 AFC and 13,908 newborn cases was available (Table V). The rate of familial versus *de novo* cases is 1:2 in all three groups (Fig. 2). Consequently the rate of familial sSMC cases of $\sim 30%$ is significantly lower compared to the previously suggested value of $\sim 40%$ (reviewed in ref. 1). This data is also supported by the study of Hume *et al* (26), which could not be included in Table V due to the lack of data; they report that out of 19 sSMC cases only 5 were inherited, i.e. 26.3%.

The most cited and extensive prenatal study of Warburton (2) accounting for an sSMC frequency of 0.043% of *de novo* cases was based on a pre-selected collection, as i) she



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and not distinguish *de novo* and familial sSMC, and ii) she did not include extra chromosomes of identified origin, i.e. all cases with isochromosomes 9p, isochromosomes 18p, Pallister-Killian-, Cat-eye- and der(22)-syndrome chromosomes. So in summary, she underestimated the sSMC frequency in prenatal diagnostics compared to the other studies due to her inclusion criteria. Thus, her study was listed in Table IB together with the other pre-selected cytogenetic studies in prenatal diagnostics: those with detectable ultrasound abnormalities and those after induction of the pregnancy by ICSI. There was a strong positive correlation of sSMC presence and ultrasound abnormalities (Table IB). With 0.204% this rate was ~2.7x higher than in the general prenatal population. As well known syndromes such as Pallister-Killian, Cat-Eye, i(18p) or der(22) were included here, which normally are connected with malformations, this observation was not unexpected. For ICSI-induced pregnancies only 2 sSMC carriers among 4,625 cytogenetically studied newborns were observed. With a rate of 0.043% this was 1.7x less frequent than in all of the prenationally studied cases of Table IA. However, with high probability this observation was caused by the 146-fold smaller sample size available for ICSI pregnancies compared to all others (Table IA). In the prenatal study of Karaman *et al* (27) (study 33 in Table IA), 4 of 20 reported sSMC cases were ICSI-induced pregnancies; 1 familial case and three *de novo* sSMC cases were described. Unfortunately, no data is provided in this study as to how many ICSI-induced pregnancies were studied at all. Thus, at present a more or less identical rate of sSMC presence in ICSI-induced compared to a normal newborn population is to be suggested.

The sSMC rate in newborns (Table IIA) of 0.044% was only almost half of the prenationally detected one (Table IA) and highlights that, in prenatal diagnostics, only a pre-selected human subpopulation was studied. Concluding, the rate of 0.075% of sSMC carriers in prenatal studies was biased by three main points which were already discussed by Blennow *et al* (28): a higher rate of cases with sSMC in prenamentals compared to newborn can be due to i) the bias caused by the maternal age effect in prenatal series, ii) the fact that prenatal diagnosis is sometimes performed due to known or suspected fetal pathology, and/or iii) severely affected fetuses may result in miscarriages and will therefore not be included among newborn cases. Recently, proof for all three suggested effects plus a further effect (4) have been observed. (Ad 1) A maternal age effect, which was suggested for all chromosomes (16) was demonstrated at least for sSMC derived from chromosome 15 (29). (Ad 2 and 3) Prenatal diagnosis is performed also in a subset of fetuses which have suspect results in ultrasound and may result in miscarriages. This is the biological relevant subset which will not be seen in the newborn population. According to Kumar *et al* (30) 4.4% of sSMC pregnancies end in a stillbirth or spontaneous abortion. Also from the data summarized in Fig. 2 it is unlikely that this third effect takes place in a noteworthy proportion during the third trimester of pregnancy. If this would be the case in a significant rate, the percentage of *de novo* versus familial sSMC cases should decrease from early to later pregnancy. As shown in Fig. 2 this is not the

case, with the rate remaining always more or less ~2 in cells of CVS, amniocytic fluid and newborn blood. (Ad 4) The last but numerically relevant 'bias' is the fact, that at present still 30-50% of pregnancies diagnosed with an sSMC fetus are terminated (2,30,31), even though only 30% of sSMC cases manifest clinical symptoms (1). This means that a certain percentage of potentially healthy children with sSMC are aborted.

In conclusion, no data is available on the 'real rate' of sSMC carriers in the prenatal human general population. What is available is the clinically relevant frequency of 0.075% for the prenationally studied human population. Through this study for the first time sound and reliable values for the frequency of sSMC in prenatal, infertility and dysmorphism diagnostics are available and can be related to the sSMC rate in the normal control population.

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