Parental origin and mechanisms of formation of cytogenetically recognisable de novo direct and inverted duplications

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Abstract

Cytogenetic, FISH, and molecular results of 20 cases with de novo tandem duplications of 18 different autosomal chromosome segments are reported. There were 12 cases with direct duplications, three cases with inverted duplications, and five in whom determination of direction was not possible. In seven cases a rearrangement between non-sister chromatids (N-SCR) was found, whereas in the remaining 13 cases sister chromatids (SCR) were involved. Paternal and maternal origin (7:7) was found almost equally in cases with SCR (3:4) and N-SCR (4:3). In the cases with proven inversion, there was maternal and paternal origin in one case each. Twenty three out of 43 cytogenetically determined breakpoints correlated with common or rare fragile sites. In five cases, including all those with proven inverse orientation, all breakpoints corresponded to common or rare fragile sites. In at least two cases, one with an interstitial duplication (dup(19)(q11q13)) and one with a terminal duplication (dup(8)(p10p23)), concomitant deletions (del(8)(p23p23.3) and del(19)(q13q13)) were found.

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Tandem duplications are direct or inverted duplications of genetic material, ordered one after the other. Cytogenetically recognisable tandem duplications are rare and comprise several megabases. The clinical phenotype of most cases is characterised by multiple congenital anomalies and developmental delay. Case reports of tandem duplications of almost all regions of human chromosomes have been reported[1] and most of them have occurred de novo. Routine investigations include conventional karyotyping and fluorescence in situ hybridisation (FISH) with whole chromosome libraries. So far, mechanisms of formation and parental origin have not been investigated in a larger series. Mismatched pairing of homologues and unequal crossover between non-sister chromatids or sister chromatids in direct duplications, as well as three break rearrangements including a U type rearrangement in inverted duplications, have been assumed.[2] To the best of our knowledge, apart from a group of 16 cases with mono- and dicentric 8p duplications exclusively formed in maternal meiosis,[3] few cases with de novo tandem duplications have been investigated by molecular methods, for example, dup(5)(q11.2q14)pat, inv dup(6)(q22q23)pat, inv dup(6)(q24.3q27)pat, inv dup(7)(q21.2q36), dup(19)(q13.2q13.4) mat, and dup(21)(q11.2q22.3) mat.[4–9] The dup(21)(q11.2q22.3)mat was mosaic with a normal cell line despite meiotic formation.[9] In the group of dup(8p) and in the case with inv dup(7)(q21.2q36) concomitant deletions were found.[3 7]

Here, we report FISH and molecular findings in 20 cases of cytogenetically recognisable de novo direct or inverted tandem duplications of 18 different autosomal chromosome segments.

Subjects and methods

All patients were clinically evaluated by experienced clinical geneticists and referred to the Institute for Medical Genetics, Zürich, either for cytogenetic/molecular investigations or for clinical evaluation over a period of more than 10 years. It was not possible to receive further material for specific investigations in all cases. One patient (case 19) has been published in 1993.[10] Part of this study was presented at the 11th Annual Meeting of the German Society of Human Genetics.[11] Clinical data of the patients as well as parental ages of all cases are available from the authors on request.

Lymphocyte chromosome examinations were performed according to standard procedures (400-600 bands per haplotype). In most cases Q and G banded karyotypes were evaluated. Metaphase FISH procedures were also performed according to the manufacturer's instructions with standard protocols for whole chromosome libraries (ONCOR© Inc, Gaithersburg) and in part with specific probes located at subtelomeric or telomeric loci.[12] The latter were obtained from ATTC© (Rockville, MD). Briefly, purified DNA from clones was labelled with either biotin-16-dUTP (Boehringer Mannheim©, Mannheim, Germany) or digoxigenin-11-dUTP (Boehringer Mannheim©, Mannheim, Germany) by nick translation. FITC avidin and rhodamine antidigoxigenin were used to detect these probes.

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Analysis was performed using a Zeiss Axioplan epifluorescence microscope. Images were recorded by Photometrics CCD KAF1400 camera (Photometrics, Tucson, Ariz) and controlled with Smart Capture imaging software (Vysis© Inc, Downers Grove, IL).

Genomic DNA was isolated from blood by salt extraction. For Southern blot analysis DNA was digested, electrophoresed in agarose, transferred to nylon membranes, and hybridised with ^{32}P labelled probes before autoradiography. PCR analysis was performed as described by Dutly *et al*.[13] Briefly, highly polymorphic (heterozygosity >0.7) and commercially available microsatellites (Research Genetics©) were run on a 6% polyacrylamide gel and visualised by silver staining. Between 200 and 500 ng of DNA were amplified in a volume of 25 µl. PCR amplification was performed on a Perkin-Elmer 9600 with 32 cycles of 30 seconds at 94°C, 45 seconds at 55-60°C for annealing, and one minute 20 seconds at 72°C for extension. The products were mixed in an equal volume of urea loading buffer and loaded onto the gel. Dosage results were interpreted by visually comparing the intensity of both alleles.

In each uninformative case a minimum of 10, but mostly more than 20 microsatellites were used. Altogether, examinations with more than 300 different microsatellites were performed. Three alleles in one or more markers were considered as representing meiotic formation and non-sister chromatid rearrangement (N-SCR). A maximum of two alleles in the patient (one maternal and one paternal) for all informative markers was interpreted to be a result of sister chromatid rearrangement (SCR), if the marker was heterozygous in the parent of origin and the other parent had a different allele. Maternal and paternal uniparental disomy were excluded by markers located upstream and downstream of the duplicated region (data not shown). Non-paternity was excluded by markers located on various other chromosomes (data not shown).

Table 1 Cytogenetic and molecular results

Case	Cytogenetic results	Orientation	Estimated deletion size (cM)	Parental origin	Formation by molecular results
1	46,XX,dup(2)(q35q37)	?	20	pat	SCR
2	46,XX,dup(2)(q35q37)	?	20	mat	SCR
3	46,XX,dup(4)(p13p16)	dir	50	pat	N-SCR
4	46,XX,dup(4)(q25q33)	dir	75	mat	N-SCR
5	46,XY,dup(5)(p14p15.3)/46,XY	dir	20	?	?
6	46,XX,dup(5)(q22q35)	dir	60	mat	N-SCR
7	46,XY,dup(6)(q21q22)	dir	8	mat	SCR
8	46,XY,dup(7)(q22q31)	dir	12	mat	SCR
9	46,XX,dup(8)(p11p23), del(8)(p23p23.3)	inv	35	mat	N-SCR
10	46,XY,dup(8)(q22q24.3)	inv	48	pat	SCR
11	46,XX,dup(9)(p13p24)	dir	17	pat	N-SCR
12	46,XX,dup(9)(p13p24)	dir	17	pat	SCR
13	46,XX,dup(10)(q22q26)	dir	37	?	SCR
14	46,XX,dup(11)(q13.2q13.4)	?	8	pat	N-SCR
15	46,XX,dup(12)(q24.1q24.3)	dir	39	?	SCR
16	46,XY,dup(14)(q21q21)	?	9	?	SCR
17	46,XY,dup(15)(q13q13)	?	9	?	SCR
18	46,XX,dup(17)(p11p13)	dir	20	pat	N-SCR
19	46,XX,dup(19)(q11q13), del(19)(q13q13), del(16)(q21q21)	dir	15	mat	SCR
20	46,XY,dup(22)(q11.1q13.1)	inv	35	?	SCR

Breakpoints corresponding to common or rare fragile sites are underlined. mat = maternal, N-SCR = non-sister chromatid rearrangement, pat = paternal, SCR = sister chromatid rearrangement.

Results

Clinical findings in all patients (13 females, seven males) included multiple congenital anomalies, non-specific patterns of dysmorphism, and developmental delay. Details are available on request from the corresponding author.

The cytogenetic and molecular results of our study are summarised in table 1. Additional chromosomal material was visible on each evaluated metaphase in 19 cases. In one patient (case 5) mosaicism was detected. In most cases the abnormal banding pattern indicated a duplication of genetic material originating from the same chromosome. This was confirmed by FISH with whole chromosome libraries. In all cases only the abnormal chromosome and its normal homologue were completely painted and no further chromosome was involved. Chromosome analysis of all parents showed normal results (46,XX or 46,XY) with no abnormal banding pattern shown by GTG banded metaphases and in part by FISH with whole chromosome libraries. Therefore, all duplications had occurred de novo and a balanced insertion/deletion in one parent could be excluded. Inversion in three cases had already been shown by conventional banding. In some smaller duplications it was not possible to discriminate unambiguously between a direct and inverted duplication. Therefore, inverted duplications might be more frequent. Interstitial telomeres were not detected in the 12 cases (1-3, 5, 6, 9-13, 15, and 18) with terminal duplications, suggesting subtelomeric breakpoints or even small concomitant telomeric deletions. However, it was possible to verify this assumption by microsatellite analysis in only one case (case 9). The concomitant deletion in case 19 was detected by loss of heterozygosity of the microsatellites applied. Terminal markers displayed only one allele. However, since the subtelomeric FISH probe was present and most of the distally located microsatellites were not informative, an interstitial deletion was assumed.

The results of molecular investigations are summarised in table 2. There were seven cases each of maternal and paternal origin. A minimum of five markers clearly indicating different intensity without any contradiction regarding parental origin was required to define parental origin in cases with SCR (fig 1). Owing to lack of the required number of informative markers, evaluation of parental origin was not possible in six cases.

Formation by N-SCR was inferred in seven cases (fig 2). Maternal origin was found four times and paternal origin three times. In the remaining 13 cases the distribution of the parental alleles indicated a SCR. A meiotic origin was found in seven cases (fig 2). In three cases with an inverted duplication, paternal SCR was found twice and maternal N-SCR once. In two cases, a concomitant deletion was detected, one was telomeric (del(8)

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(p23p23.3)) in a case with an inverted duplication (dup(8)(p10p23)), the other (del(19)(q13q13)) was interstitial with a direct duplication (dup(19)(q11q13)). In 10 cases one breakpoint and in five additional cases both breakpoints of the duplications were localised to the same cytogenetic band as common or rare fragile sites.

Table 2 Results of investigated markers mapping to the duplicated regions

46,XX,dup(2)(q35q37)pat		*46,XX,dup(2)(q35q37)mat*		*46,XX,dup(4)(p13p16)pat*	
Marker	*M/P/F*	*Marker*	*M/P/F*	*Marker*	*M/P/F*
D2S371	ab/b/bc	D2S1391	cd/b*d*/ab	D4S43	ab/ab/b
D2S355	ac/a*d*/bd	D2S118	bc/a*b*/ab	D4S127	b/ab/ab
D2S2192	a/a*c*/bc	D2S2189	bc/ab/ac	D4S3023	b/*a*b*c*/ac
D2S325	ab/ab/ab	D2S355	ab/b/ab	D4S1599	b/ab/ac
D2S2274	b/ab/ab	D2S325	ab/bc/ac	D4S403	ab/ab/b
D2S2242	ac/bc/ab	D2S2208	ac/*a*b/bc	D4S1546	cd/*ab*d/ab
D2S2178	ac/bc/b	D2S2274	a/*a*b/bc	D4S391	ac/ab/bd
D2S1385	ac/a*c*/bc	D2S2208	ac/ab/bc	D4S2950	ab/ab/b
D2S1369	ab/a/ab	D2S2178	ab/bc/c	D4S1627	ac/ab/bd
D2S157	bd/bc/ac	D2S1385	ab/bc/bc		
D2S1380	a/a/a	D2S1369	ab/ab/ab		
D2S1345	ad/*c*d/bc	D2S157	ab/ab/ab		
D2S164	ac/a*c*/bc	D2S371	bc/*b*c/ac		
D2S163	ab/ab/ab	D2S1380	ad/ac/bc		
PAX3	a/a/a	D2S143	ac/bc/bc		
D2S1363	b/*a*b/ab	D2S163	ab/ab/ab		
D2S159	ab/b/ab	D2S120	ab/ab/bc		
D2S172	ab/ab/ab	D2S126	cd/a*d*/ab		
D2S206	ab/b*c*/cd	D2S159	bc/ab/ab		
D2S345	ab/a*b*/b	D2S172	abb/ab/ab		
D2S125	a/a*b*/b	D2S206	ab/ac/ac		
		D2S345	b/a*b*/a		

46,XX,dup(4)(q25q33)mat		*46,XX,dup(5)(q22q35)mat*		*46,XY,dup(6)(q21q22)mat*	
Marker	*M/P/F*	*Marker*	*M/P/F*	*Marker*	*M/P/F*
D4S427	b/ab/ac	D5S346	bc/a*c*/ab	D6S430	bc/a*c*/ab
D4S194	ad/*acd*/bc	D5S2098	bc/a*bc*/ac	D6S251	ab/ac/cd
D4S1615	bd/a*bd*/ac	D5S2057	a/ab/b	D6S275	ac/ac/bc
D4S175	ac/ac/ab	D5S816	ac/ac/bc	D6S300	ab/ac/bc
D4S2939	b/ab/ab	D5S393	ab/ab/a	D6S252	ab/b/b
D4S413	ab/*ab*d/cd	D5S673	ab/ab/bc	D6S434	bc/*b*c/ac
D4S1566	ab/*abc*/ac	D5S820	bc/a*bc*/ac	D6S268	cd/b*d*/ab
D4S1552	ac/bc/ab	D5S423	bd/a*bd*/ac	D6S302	c/a*c*/ab
		D5S400	a/a/ab	D6S261	bc/b*c*/ab
		D5S1456	ab/ab/bc	D6S407	ab/*b*c/ac
		D5S429	ac/*a*bc/ab	D6S262	ab/ac/c
		D5S498	ab/ab/b	D6S270	ac/ac/bc
		D5S2006	ab/b/b	D6S292	ac/bc/bd

46,XY,dup(5)(p14p15.3)/46,XY	
Marker	*M/P/F*
D5S406	a/ab/b
D5S208	ad/ab/bc
D5S807	ab/b/b
D5S1963	a/ab/b
D5S1473	b/ab/a
D5S502	ac/a/a
D5S663	ab/bc/c

46,XY,dup(7)(q22q31)mat		*46,XX,dup(8)(p10p23)mat, del(8)(p23p23.3)mat*			*46,XX,dup(8)(q22q24.3)pat*	
Marker	*M/P/F*	*Marker*	*M/P/F*	*Deletion*	*Marker*	*M/P/F*
D7S527	cd/a*c*/ab	D8S264	a/c/bc	mat del	D8S273	ab/ab/ac
D7S515	ab/b/bc	D8S262	bc/a/ab	mat del	D8S559	ab/a*b*/b
D7S496	bc/b*c*/ab	D8S1824	ac/a/ab	?	D8S521	ab/ab/a
D7S501	ab/ab/b	D8S1806	a/b/b	mat del	D8S556	b/ab/a
D7S523	ab/ab/ab	D8S201	b/b/ab	?	D8S281	ac/a*b*/b
D7S486	b/*b*c/ac	D8S307	ab/b/bc	?	D8S522	bc/ab/ab
D7S522	a/a/ab	D8S439	ab/b/bc	?	D8S514	a/a*b*/b
CF-108	b/b/ab	D8S552	a/*a*b/b		D8S266	bc/*a*b/ab
CF-332	c/b*c*/ab	D8S133	bc/bc/ac		D8S508	b/b/b
CF-333	ab/ab/b	D8S261	ab/ab/b		D8S263	a/ac/bc
D7S495	bc/b/ac	D8S1145	a/ab/b		D8S557	a/a/a
D7S483	bc/a*b*/ab	LPL	ab/*abc*/bc		D8S529	ac/bc/b
		NEFL	bc/bc/ac		D8S256	b/ab/ab
		D8S505	ab/ab/bc		D8S1746	bc/ac/ac
		D8S322	bc/*b*c/ac		D8S1710	ab/a*c*/c
		D8S136	ab/ab/bc		D8S537	ac/a*c*/bc
		D8S137	b/b/ab		D8S272	ab/b*c*/bc
		D8S87	ab/ab/ab		D8S274	ab/ab/ab
		ANK1	a/a/a			
		PLAT	ac/ac/ab			

Results of analysis of microsatellite markers of the duplicated regions from all investigated cases. Markers fully or in part informative are underlined and in italics. Markers are ordered from p to q telomere. M = mother, P = patient, F = father.

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Table 2 Results of investigated markers mapping to the duplicated regions (continued)

46,XY,dup(9)(p13p24)pat		*46,XX,dup(9)(p13p24)pat*		*46,XX,dup(10)(q22q26)*	
Marker	*M/P/F*	*Marker*	*M/P/F*	*Marker*	*M/P/F*
D9S1779	ab/a$\underline{c}$/c	D9S178	ab/ab/a	D10S195	a/a/ab
D9S132	b/a$\underline{b}$/ab	D9S132	b/b/ab	D10S2327	b/b/ab
D9S144	b/b/ab	D9S144	c/ac/ab	D10S1686	ac/ac/bc
D9S256	ac/$\underline{a}$c/ab	D9S256	a/a/a	D10S215	a/a/a
D9S269	a/a/ab	D9S269	ac/$\underline{b}$c/ab	D10S677	ab/bc/cd
D9S156	b/b$\underline{c}$/ac	D9S285	bc/$\underline{b}$c/ab	D10S534	ab/ab/ab
D9S157	ac/c$\underline{d}$/bd	D9S156	ab/a$\underline{b}$/b	D10S562	ab/a/ab
D9S171	ab/a$\underline{b}$/bc	D9S157	ab/a$\underline{b}$/bc	D10S1237	a/a/a
D9S161	b/$\underline{a}$b$\underline{c}$/ac	D9S162	ab/ab/bc	D10S209	ab/a/a
D9S1868	a/a$\underline{b}$/ab	D9S171	b/b/ab	D10S1230	ac/bc/bc
D9S304	ab/a/ab	D9S161	ab/a$\underline{c}$/cd	D10S1213	ab/bc/bc
		D9S1868	ac/bc/b	D10S590	a/a/a
		D9S104	ac/a$\underline{b}$/b	D10S212	a/a/ab
		D9S304	bc/b$\underline{d}$/ad		
		D9S200	b/$\underline{a}$b/ab		

46,XX,dup(11)(q13.2q13.4)pat		*46,XX,dup(12)(q22q24.3)*		*46,XX,dup(14)(q21q21)*	
Marker	*M/P/F*	*Marker*	*M/P/F*	*Marker*	*M/P/F*
D11S4191	ac/a$\underline{bc}$/bc	D12S1722	ab/b/b	D14S80	bc/bc/ab
D11S534	ab/a$\underline{c}$/ac	D12S43	b/b/ba	D14S70	bc/bc/ab
D11S916	b/bc/ac	D12S81	ab/ab/ab	D14S75	ac/ac/bc
D11S527	bc/ac/ab	D12S1660	c/ac/ab	D14S288	b/ab/ac
D11S2002	ab/$\underline{a}$b$\underline{c}$/ac	D12S1588	ab/ac/bc	D14S989	b/ab/ab
D11S901	ac/a$\underline{c}$/ab	IGFI	ab/b/b	D14S1057	ab/ab/ab
D11S876	bc/bc/ac	D12S84	b/ab/a	D14S276	ab/ab/ab
		D12S79	a/a/a	D14S1064	b/ab/ab
		D12S342	ab/ab/ab	D14S274	ac/bc/bc
		D12S1675	ab/b/b	D14S750	b/ab/ac
		D12S1679	ac/ab/b	D14S290	ab/ab/bc
		D12S834	a/ab/ab	D14S63	ab/ab/bc
		D12S1714	b/ab/ab	D14S77	bc/ab/a
		D12S1723	b/b/ab	D14S42	cd/ac/ab
		D12S343	a/a/ab	D14S67	ac/ac/bc
		D12S1638	a/ab/b	D14S68	ab/bc/bc
		D12S357	a/a/a	D14S51	ac/ab/ab
				D14S62	ab/ac/c

46,XY,dup(15)(q13q13)		*46,XX,dup(19)(q11q13)mat, del(19)(q13q13),del(16)(q21q21)mat*			*46,XY,dup(22)(q11.1q13.1)*	
Marker	*M/P/F*	*Marker*	*M/P/F*	*Deletion*	*Marker*	*M/P/F*
D15S113	a/a/a	-LIPE	ab/ab/bc		D22S264	ab/bc/bc
D15S122	ab/ac/c	D19S191	bc/bc/ab		D22S343	a/ac/bc
GABR3	ab/bc/c	D19S223	a/ab/b		D22S427	b/bc/ac
D15S217	ac/ab/bc	D19S900	ab/ab/bc		D22S941	bc/b/ab
D15S541	ab/b/ab	D19S913	ac/ab/ab		D22S944	ab/ab/ab
D15S542	bd/cd/ac	D19S219	a/ab/bc		D22S351	ab/bc/bc
D15S97	ab/ab/ac	MD	ac/ac/bc		D22S1638	bc/ac/ac
D15S11	ab/ac/c	APOC2	bc/ab/ab		D22S311	ad/ab/bc
		D19S412	bc/bc/ab		D22S306	ab/ab/bc
		D19S112	ab/ab/bc		D22S303	b/ab/a
46,XX,dup(17)(p10p13)pat		D19S866	ab/a/ac	?	D22S258	a/a/a
Marker	*M/P/F*	D19S574	ac/b/b	mat del	D22S315	ab/ab/bc
D17S1866	ac/a/ab	D19S246	bc/a/ac	mat del	D22S277	a/ac/bc
D17S926	ab/bc/cd	D19S206	b/a/ac	mat del	D22S274	ac/bc/b
D17S938	ac/a/ab	D19S888	b/c/ac	mat del		
D17S520	ab/a$\underline{b}$/bc	D19S180	ac/b/ab	mat del		
D17S1875	ab/b$\underline{c}$/cd	D19S572	ac/b/b	mat del		
D17S969	ab/a/a	D19S254	ac/b/ab	mat del		
D17S921	ab/ab/ab	D19S880	ab/b/ab	?		
D17S261	a/ab/ab	D19S877	a/a/a	?		
CHRNB1	bc/c/ac	D19S720	a/a/a	?		
D17S122	b/$\underline{a}$b$\underline{c}$/ac	D19S210	ab/a/ab	?		

Discussion

We report on cytogenetic, FISH, and molecular findings in 20 cases with de novo direct or inverted tandem duplications of 18 different autosomal chromosome segments. "Tandem duplication" is a cytogenetic term and refers to the direct or inverted order of cytogenetically recognisable genetic material one after the other.[2] Mechanisms of formation are not known. In theory, the duplication might be direct or inverted, either within the same chromatid (A, B) or between non-sister chromatids (C, D) during replication or by unequal crossover (fig 3).[2] The rearrangement between non-sister chromatids can also be regarded as an insertion within the same chromosome arm. Definitive discrimination is not possible by standard techniques. Molecular methods like Southern blotting or microsatellite analysis as well as FISH are only able to show the presence or absence of an allele or genetic material, but not the exact localisation of a specific allele. It is not possible to differentiate physical localisation of maternal and paternal alleles by these methods. Therefore, the results and relevance of our study and of previously reported cases are limited. For an exact evaluation it would be necessary to separate the aberrant chromosome

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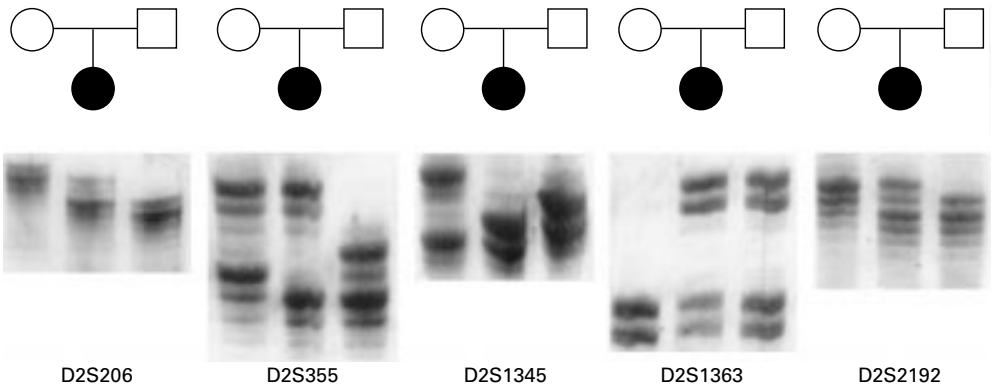


Figure 1 Molecular findings in case 1 (46,XX,dup(2)(q35q37)pat)) showing sister chromatid rearrangement by one maternal allele and one more intense paternal allele at each of the microsatellite markers investigated, D2S355, D2S2192, D2S1345, D2S1363, and D2S206.

from its homologue and afterwards to investigate markers located within the duplication. Only by this approach would it be possible to indicate the exact physical localisation of each allele either on the paternal or on the maternal homologue and the mechanism of formation dependent on the constellation of the two alleles.

Another problem of our study and of other published cases is the lack of data on the exact molecular breakpoints. Breakpoints determined either by conventional cytogenetic methods or by FISH with specific cosmids may differ by several megabases. What seems identical cytogenetically might be completely different at the molecular level. Therefore, the smaller the duplicated region, the less likely an informative result will be obtained.

Comparing the minimum 43 breakpoints in our cases with 150 common or rare fragile sites reported recently,[14] 23 breakpoints (53%) were localised to the same cytogenetic band as common or rare fragile sites (underlined in table 1). This is clearly more than the number of 13 breakpoints localised to the same cytogenetic band as common or rare fragile sites expected on a level of 500 bands. In two cases with direct duplications and in all three cases with obviously inverted duplications, both breakpoints mapped to common fragile sites.[14] As a consequence, an unequal crossover may be more likely. From these observations, it could be suggested that fragile sites are susceptible not only to breakage and subsequent deletions, but can finally result in direct or inverted duplications too.

Another way to explain deletions or duplications is a simple misalignment of homologous sequences. As recently shown in Charcot-Marie-Tooth type 1A neuropathy, duplications of approximately 1.5 Mb are the reciprocal products of homologous recombination events between misaligned flanking CMT1A-REP repeats.[15] The same mechanism is assumed in microdeletions such as Williams-Beuren syndrome or CATCH 22.[16]

Both SCR and N-SCR may also be found in mosaic cases. Three alleles always suggest meiotic formation and N-SCR. However, three alleles might also be present in cases formed premeiotically and going through meiosis without SCR or N-SCR. Two alleles are expected in cases formed either during meiosis by SCR or during mitosis by both N-SCR and SCR. In the latter situation a rearrangement between non-sister chromatids cannot be distinguished from a rearrangement between sister chromatids by microsatellite analysis, since there are always only two alleles. Mosaicism can only be detected by different cell lines on chromosome analysis. However, low level mosaicism or mosaicism restricted to the placenta or other uninvestigated tissues may be overlooked. Therefore, there might be more than the one mosaic case (case 5) in our sample. Since, in general, there are few reported cases with mosaicism of direct or inverted duplications,[1] we assumed meiotic origin in all of our cases without mosaicism in lymphocytes.

In our cases a 2:1 ratio of SCR versus N-SCR and an almost equal distribution of paternal and maternal origin, both in cases with N-SCR and in the informative cases with SCR, was found.

The mode of formation seems to be more complex in some cases. Almost all cases with an inverted duplication (8)(p10p23) are formed

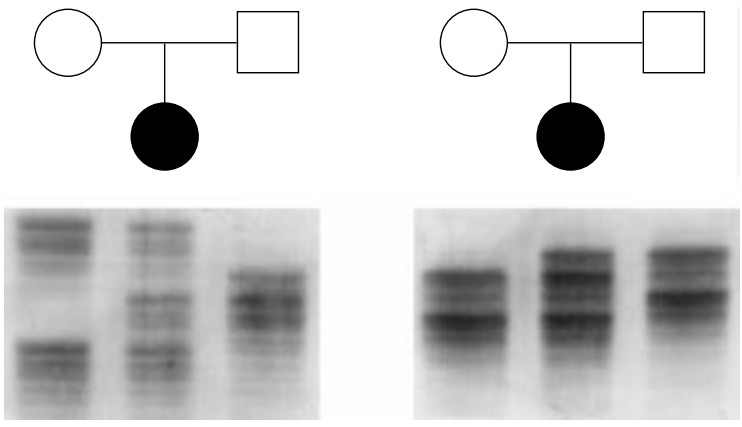


Figure 2 Molecular findings in case 4 (46,XX,dup(4)(q25q33)mat) showing non-sister chromatid rearrangement during maternal meiosis by two maternal alleles and one paternal allele at each of the investigated microsatellite markers D4S194 and D4S1615.

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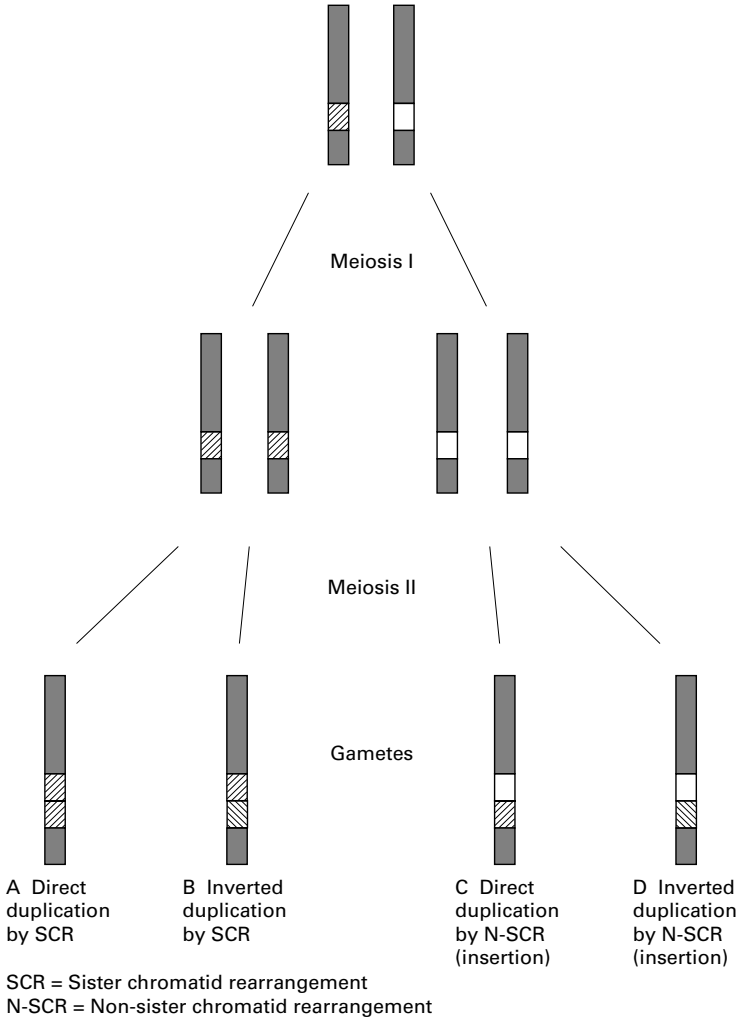


Figure 3 Theoretical possibilities of formation of direct or inverted tandem duplications.

during maternal meiosis and are often associated with a telomeric deletion (8)(p23p23.3).[3] These observations were confirmed in case 9 of our study. A similar result of a duplication associated with a deletion was obtained in a case with inv dup(7)(q21.2q36).[7] Abnormal pairing during maternal meiosis followed by an unequal crossover owing to misalignment of homologous sequences has been assumed. Moreover, in our study we found a concomitant deletion (del(19)(q13q13)) in one case with an interstitial duplication (dup(19)(q11q13)). The exact mechanism in this case remains unknown. However, based on this observation the phenotype of pure duplications should be interpreted with caution as other cases may also be associated with undetected small deletions.

In conclusion, our study shows that N-SCR and SCR occur in maternal and paternal meiosis with equal frequency, that N-SCR is less frequent than SCR, and that there is a weak association with common or rare fragile sites.

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