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ARTICLE

An 11p;17p telomeric translocation in two families associated with recurrent miscarriages and Miller-Dieker syndrome

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Translocations occur in a proportion of couples affected by recurrent miscarriages. We describe two such families in which the underlying cause was a cryptic subtelomeric 11p;17p translocation detected only after the birth of an affected child carrying an unbalanced form of the rearrangement. Unbalanced subtelomeric rearrangements are now recognised as a significant cause of mental impairment and we believe that these rearrangements may also be an important cause of recurrent miscarriages. In these two families the translocation is most likely to have arisen from a single ancestral event because all translocation carriers shared almost identical haplotypes around the breakpoints on both chromosomes.
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Introduction

We present two families with a common subtelomeric 11p;17p translocation detected following the live birth of a child with Miller-Dieker syndrome (MDS). Both families had previously presented with recurrent miscarriages but routine cytogenetic analysis failed to identify the subtle rearrangement. In around 2–5% of couples who have had two or more spontaneous abortions, one member of the couple is a translocation carrier,[1–3] the translocation being reciprocal in around two-thirds and Robertsonian in one-third of cases.

Recent reports have suggested that unbalanced submicroscopic telomeric rearrangements might represent a significant cause of idiopathic mental retardation (IMR) either with or without associated phenotypic abnormalities,[4–11] although this has not been found in all studies.[12] In around 50% of reported cases the translocation is familial, the balanced form being carried by a parent and possibly other family members. Subtelomeric translocations have also been identified in couples presenting with recurrent miscarriages and affected live born offspring with mental retardation and a variety of other anomalies[13–18] or with a clinically recognisable syndrome such as cri du chat,[19] Wolf-Hirschhorn syndrome[20] or MDS.[21–25] Subtelomeric rearrangements may also be common in such families[26] and we present here two additional families in which a cryptic rearrangement is associated with multiple pregnancy losses.

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Case reports

Family 1

MW was the first child born to healthy, unrelated parents. The parents had previously been investigated cytogenetically, with normal results, because of two spontaneous miscarriages at 8 weeks gestation. Pregnancy was uneventful up to 30 weeks gestation when polyhydramnios was noted. Prenatal cytogenetic analysis following amniocentesis showed an apparently normal female karyotype. At 32 weeks gestation ultrasound examination showed dilated lateral ventricles and probable agenesis of the corpus callo-

sum. The patient was born at 34 weeks gestation, weighing 1520 g, following emergency Caesarean section for intrauterine growth retardation (IUGR), spontaneous rupture of membranes and polyhydramnios. Postnatal examination showed frontal bossing, a prominent nasal bridge, thin upper lip, micrognathia, tapering fingers with camptodactyly, broad big toes and small toe nails. A head ultrasound scan showed agenesis of the corpus callosum and probable lissencephaly. The patient died aged 1 day. Postnatal directed high resolution cytogenetic analysis, following a clinical suggestion of Miller-Dieker syndrome, showed a subtle terminal deletion of the short arm of one chromosome 17 (ISCN 550 band level). FISH with a cosmid from the locus D17S379 confirmed a deletion of the MDS critical region. Although parental karyotypes had previously been reported as normal following referral for recurrent miscarriages (ISCN 550 band level), high resolution analysis detected an apparently balanced maternal translocation between the distal short arms of chromosomes 11 and 17. FISH with the telomeric probe 2209a2 (11p) and the Miller-Dieker critical region probe D17S379 confirmed this translocation (Figure 1), 46,XX,t(11;17)(p15.5;p13.3).ish t(11;17)(2209a2-, D17S379+; D17S379-, 2209a2+). In addition to the proband's mother three further carriers of this balanced translocation were identified in this family using the telomeric FISH probes (Figure 2). All were male including the maternal grandfather and uncle. The grandmother of MW was noted to have had approximately six first trimester miscarriages over 4 years before the birth of MW's mother and uncles. Since the birth of MW, her parents have had five further 5–10 week miscarriages, of which one was karyotyped and confirmed to carry the same malsegregation pattern seen in MW.

Family 2

LT was the first child born to healthy, unrelated parents. The parents had also been investigated cytogenetically with normal results because of four previous spontaneous miscarriages. Prenatal cytogenetic analysis following amniocentesis showed an apparently normal female karyotype. An initial ultrasound scan at 23 weeks gestation showed mild dilation of posterior horns of the cerebral ventricles,

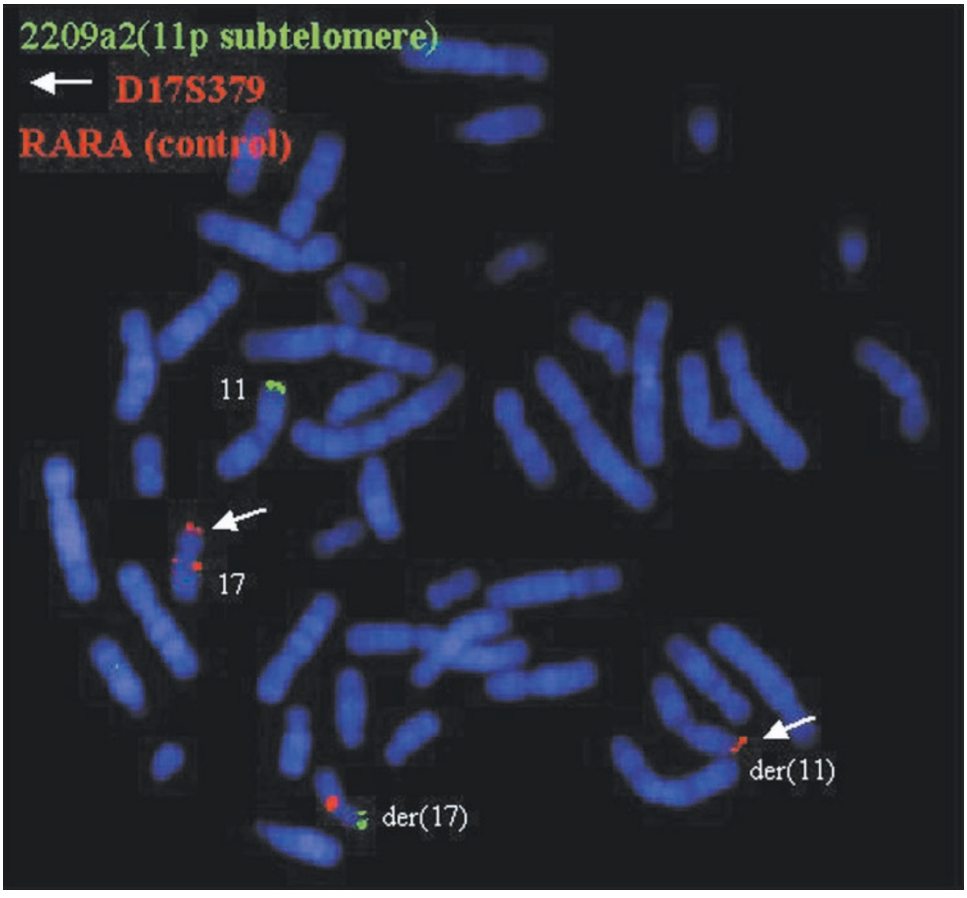

Figure 1 FISH result in the mother of MW with the 11p telomeric probe (2209a2) (green), the Miller-Dieker syndrome critical region probe (D17S379) (red, arrowed) and RARA (red, control).

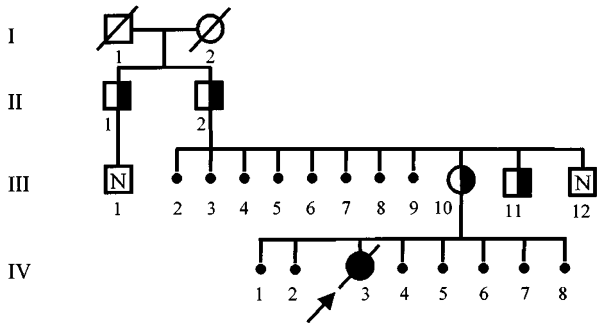

Figure 2 Pedigree of Family 1. The four translocation carriers produced four healthy live born children and 14 miscarriages, 13 of which were first trimester.

but by 33 weeks they were within normal limits. Further scans were performed every 2–3 weeks but showed a gradual decrease in growth velocity. LT was born at 36 weeks by ventouse vaginal delivery. She was noted to have symmetrical IUGR, hypoglycaemia and dysmorphic features – vertical forehead creases, long philtrum, micrognathia, microcephaly, bridged palmar creases and a high pitched cry. The patient was referred for re-investigation at 2 days of age with a suspected diagnosis of either MDS or Pallister Killian syndrome. However, despite a directed high resolution analysis no cytogenetically visible abnormality of 17p was identified (ISCN 500 band level). FISH with a cosmid from the locus D17S379 detected a submicroscopic deletion of the MDS critical region in the proband. Parental karyotypes had previously been reported to be normal following referral for recurrent miscarriages and repeat high resolution examination failed to identify an abnormality (both analyses at ISCN 500 band level). However, FISH analysis of parental blood samples using the same D17S379 cosmid showed that the deletion in LT was the result of unbalanced segregation of a maternal rearrangement involving 11p. FISH with the telomeric probes 2209a2 (11p) and 2111b1 (17p) confirmed the presence of an apparently balanced cryptic translocation between the distal short arms of chromosomes 11 and 17, 46,XX.ish t(11;17)(p15.5;p13.3) mat (2209a2-, 2111b1+; 2111b1-, 2209a2+). LT died at 14 months of age.

Materials and methods

Cytogenetic and subtelomere FISH analysis

Metaphase chromosomes prepared from Fluorodeoxyuridine (FdU)-synchronised phytohaemagglutinin-stimulated cultures[27] of peripheral blood lymphocytes were used for Giemsa-trypsin-Leishman's (GTL) banding as well as for fluorescence *in situ* hybridisation (FISH) analysis. FISH was performed according to the method described by Pinkel *et al*.[28,29] with some modifications. Dual-colour subtelomere FISH was undertaken using 11p and 11q subtelomere specific clones [6,30] labelled with digoxigenin and biotin respectively. Sites of hybridisation were subsequently detected using tetramethyl rhodamine isothiocyanate (TRITC) conjugated anti-digoxigenin (Boehringer) and fluorescein isothiocyanate (FITC) conjugated avidin (Vector). Slides were mounted in DAPI-Antifade (Vector) and viewed using a Zeiss Axiophot microscope. Images were captured using MacProbe (PSI) software.

Molecular genetic investigations

DNA was extracted from peripheral blood by a salt precipitation technique as described by Miller *et al*.[31] The translocation breakpoints on chromosomes 11p and 17p were determined by PCR amplification of microsatellite polymorphisms from genomic DNA extracted from MW, LT and their parents. Primer sequences were obtained from the Genome Database (GDB: www.gdb.org) and the relative order of loci inferred from the NCBI (www.ncbi.nlm.nih.gov) and Ensembl (www.ensembl.org) databases.

Results

The translocation breakpoints are shown in Table 1. It should be noted that for both regions the genomic sequence has not been completed and so the locus order has not been unequivocally established. At the level of microsatellite mapping the results are consistent with the breakpoints being identical in both families. On chromosome 17 the breakpoint for LT and MW is between D17S1584 and D17S1810 and on chromosome 11 between D11S1318 and D11S1758 (for the locus D11S4177, between D11S1318 and D11S1758, LT is trisomic but MW is not informative). Thus LT and MW are trisomic for approximately 2 Mb of distal chromosome 11p and, consistent with their MDS phenotypes, are monosomic for approximately 5 Mb of distal chromosome 17p.

Origin of the translocations

There are at least two simple explanations for the observation of apparently identical translocations in unrelated individuals. First that there is sequence similarity between the breakpoints and the translocation is a recurring one, analogous to the t(11q;22q) (q23;q11), but would go unrecognised in sporadic cases because of its cryptic nature. This hypothesis is supported by our observation that probe 2209a2 which hybridises to the telomeric region of 11p also hybridises weakly to the short arm telomeric region of chromosome 17 (Figure 3).[11] This implies that these subtelomeric regions share a degree of sequence similarity. It is possible that during early meiotic prophase, when the telomeres cluster at the nuclear periphery and homology searching and pairing initiates, this sequence similarity promotes a cross-over event resulting in the 11p;17p telomeric rearrangement. However, in each of the previously reported cases of MDS due to a familial cryptic translocation, the cross-over event involved a different chromosome arm: 3q, 8q, 9p, 10q, 19q and 20q.

Table 1 Breakpoints on chromosomes 11 and 17

Chromosome 11 breakpoint				*Chromosome 17 breakpoint*			
Mb from 11pter	*PCR locus*	*LT*	*MW*	*Mb from 17pter*	*PCR locus*	*LT*	*MW*
0.19	D11S2071	+++	+++	0.17	D17S849	DEL	DEL
0.39	D11S1363	+++	+++	0.53	D17S926	NI	DEL
0.45	D11S922	+++	+++	0.79	D17S1840	DEL	DEL
0.53	D11S4046	+++	+++	0.92	D17S1529	DEL	DEL
1.19	D11S1984	+++	NI	2.57	D17S1798	DEL	NI
–	TH	+++	+++	3.66	D17S829	DEL	DEL
2.10	D11S1318	+++	+++	4.04	D17S1828	NI	DEL
2.11	D11S4177	+++	NI	4.90	D17S1584	DEL	DEL
–	D11S1758	N	N	6.00	D17S1810	N	N
3.03	D11S2345	N	N	6.39	D17S513	N	N

+++=trisomic; N=normal (disomic); NI=non informative; ____=breakpoints.

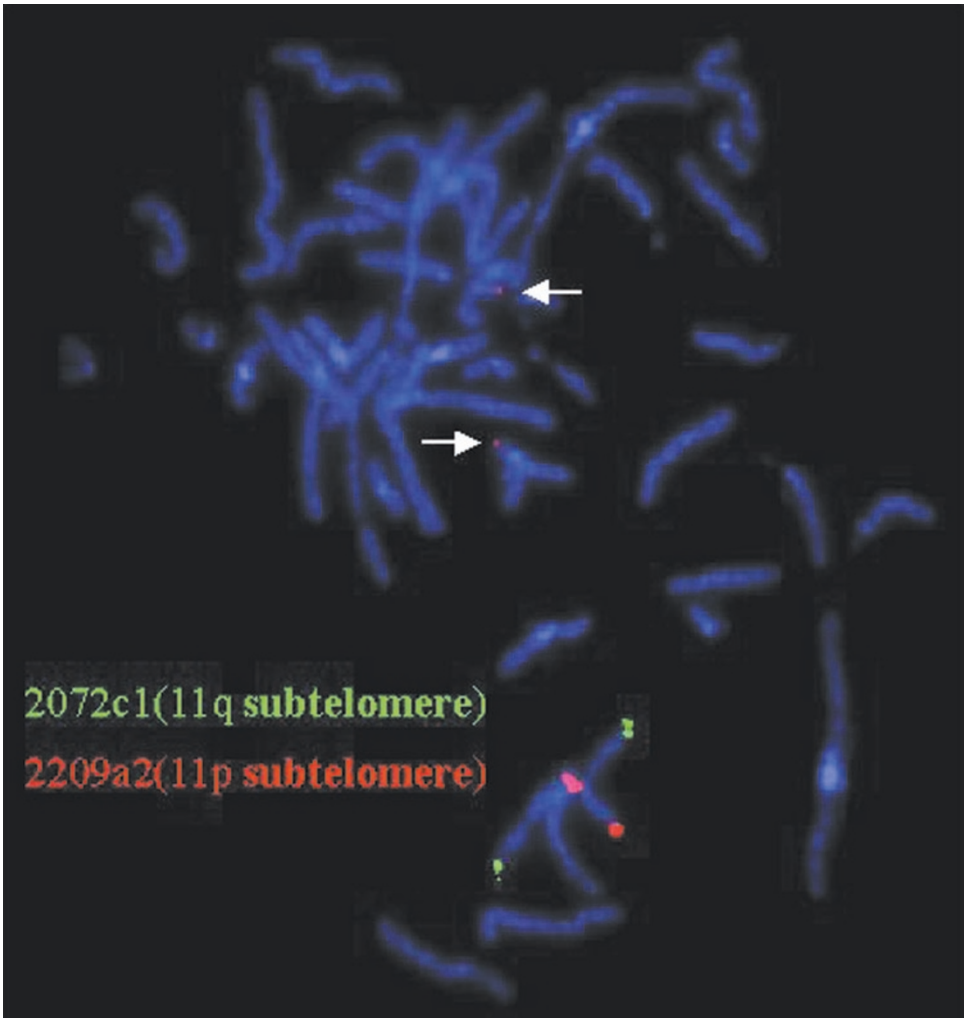

Figure 3 FISH in a control individual with the 11p telomeric probe (2209a2) (red) and the 11q telomeric probe (2072c1) (green). The arrows indicate the sites of cross hybridization of 2209a2 at the telomeric regions of the short arms of both chromosomes 17.

The second explanation is that the translocation is identical by descent and the patients are related. It is relatively easy to discriminate between these two hypotheses as the first involves two independent events and therefore the regions close to the breakpoints would be expected to have different haplotypes while the second involves a single event and the regions close to the breakpoints would be expected to have identical or very similar haplotypes.

In order to test these hypotheses we undertook haplotyping in the regions surrounding the breakpoints. For MW we had DNA from the maternal grandparents allowing us to determine the phase of alleles present in MW's mother. On chromosome 17, the mothers of LT and MW shared an allele at all nine loci tested between D17S1529 and D17S513, an interval of approximately 5.5 Mb and a genetic distance of 3.97 female cM (fcM) and 24.43 male cM (mcM) (see Table 2). For all seven informative loci a shared allele was transmitted to MW's mother from MW's grandfather, who carries the same translocation. On chromosome 11, the mothers of LT and MW shared at least one allele over an interval exceeding 18 Mb between the loci D11S2071 (the most distal marker tested) and D11S899, a genetic distance of 22.41 fcM and 33.24 mcM (Table 3). With the exception of one locus, D11S4124, a shared allele was demonstrated to be grandpaternal in origin in the mother of MW at all 25 informative loci. Thus all three translocation carriers tested share identical or virtually identical haplotypes for large chromosomal segments that encompass both translocation breakpoints. For chromosome 17 we have also demonstrated that multiple haplotypes exist for this region and could not detect the shared haplotype among any members of five randomly selected nuclear families tested with the same PCR primers (data not shown).

We have utilised sequence based maps of distal 11p and 17p to determine the normal levels of recombination during male and female meiosis. The estimated male genetic distances for the homozygous segments were 24.43 mcM for chromosome 17 and 33.24 mcM for chromosome 11. Given these values, a male recombination event should occur once in 4.1 meioses for chromosome 17 and once in 3.0 meioses for chromosome 11. The shorter female genetic distances of 3.97 fcM for chromosome 17 and 22.41 fcM for chromosome 11, indicate that a female recombination event would occur once in 25.2 meioses and once in 4.5 meioses respectively. Both breakpoints occur in regions with approximately average levels of female recombination but within male recombination hotspots. Thus, by virtue of their extensive sequence homology we consider that these two translocations are identical by descent. Although both families are from Southern England extending the pedigrees back for five generations in family 1 and three generation in family 2 did not identify a common ancestor.

Discussion

An 11p;17p telomeric translocation was found in the mothers of two liveborn probands with MDS. Both families had a history of unexplained recurrent miscarriages. The derived chromosome was visible by high resolution cytogenetic analysis in the proband of family 1 (ISCN 550 band level) but not in the proband of family 2 (ISCN 500 band level). Both maternal translocations were originally reported as normal. On re-examination the translocation

Table 2 Chromosome 17

Mb from 17pter	*PCR locus*	*Mother of LT*		*Mother of MW*		*Grandfather of MW*		*Grandmother of MW*		*Shared allele(s)*	*Origin*
–	D17S1866	169	171	165	167	167	171	155	165	none	–
0.17	D17S849	253	255	255	255	255	255	255	255	255	NI
0.53	D17S926	243	251	242	249	242	251	249	253	none	–
0.79	D17D1840	211	213	198	198	198	211	198	209	none	–
0.92	D17S1529	168	173	168	168	168	168	160	168	168	NI
2.57	D17S1798	251	251	251	253	251	251	251	253	251	GP
2.70	D17S1583	121	123	102	123	102	123	102	102	123	GP
3.66	D17S829	263	274	263	274	248	274	246	263	263, 274	GM, GP
4.04	D17S1828	210	212	210	216	210	218	208	216	210	GP
4.90	D17S1584	102	111	102	117	102	102	115	117	102	GP
→ 6.00	D17S1810	114	118	114	116	110	114	116	118	114	GP
6.12	D17S1584	93	93	93	93	91	93	93	93	93, 93	NI
6.39	D17S513	188	192	180	192	182	192	180	192	192	GP
6.43	D17S1832	188	188	180	188	180	180	184	188	188	GM
8.20	D17S1353	206	206	204	214	204	206	206	214	none	–
9.84	D17S786	124	124	122	122	120	122	120	122	none	–

□=shared allele(s); →=breakpoint; ⋮⋮=region of homology; NI=not informative; GM=grandmaternal; GP=grandpaternal.

Table 3 Chromosome 11 genotypes

Mb from 11pter	*PCR locus*	*Mother of LT*		*Mother of MW*		*Grandfather of MW*		*Grandmother of MW*		*Shared allele(s)*	*Origin*
0.19	D11S2071	**168**	**196**	**168**	**196**	192	**196**	**168**	187	168, 196	GM, GP
0.39	D11S1363	**245**	**249**	**245**	**249**	245	**249**	**245**	251	245, 251	GM, GP
0.45	D11S922	220	**243**	237	**243**	216	**243**	231	237	243	GP
0.53	D11S4046	**196**	**196**	182	**196**	**196**	**196**	182	194	196	GP
1.19	D11S1984	**180**	192	**180**	**180**	**180**	184	**180**	**180**	180	NI
–	TH	**310**	322	**310**	314	**310**	322	310	314	310	GP
2.10	D11S1318	**129**	**137**	**129**	**137**	121	**129**	127	**137**	129, 137	GP, GM
2.11	D11S4177	183	**209**	**209**	**209**	**209**	**209**	**209**	**209**	209	NI
–	D11S1758	242	**244**	**244**	246	242	**244**	246	248	244	GP
2.20	D11S4088	**210**	238	**210**	228	**210**	228	214	228	210	GP
3.03	D11S2345	**120**	124	**120**	130	**120**	**120**	124	130	120	GP
3.11	D11S4146	**197**	**199**	**197**	**199**	NT		NT		197, 199	GM, GP
4.45	HBB	**151**	**151**	149	**151**	**151**	**151**	149	151	151	GP
4.62	D11S1760	**80**	**80**	**80**	88	**80**	88	88	88	80	GP
4.79	D11S4124	170	**174**	166	**174**	166	172	170	**174**	174	GM
–	D11S4181	209	**217**	215	**217**	207	**217**	213	215	217	GP
5.32	D11S1338	**258**	262	256	**258**	**258**	262	256	262	258	GP
6.60	D11S1331	191	**197**	**197**	**197**	**197**	**197**	193	**197**	197	NI
7.41	D11S932	**153**	**153**	**153**	155	**153**	155	**153**	155	153	NI
–	D11S909	**117**	**119**	**117**	**119**	**117**	**119**	**119**	**119**	117, 119	GP, GM
8.56	D11S4149	212	**223**	**223**	**223**	**223**	225	221	**223**	223	NI
10.38	D11S1329	**262**	**264**	**262**	**264**	**262**	266	262	**264**	262, 264	GP, GM
10.86	D11S1346	264	**280**	278	**280**	278	**280**	271	278	280	GP
11.12	D11S4189	**259**	**259**	**259**	265	**259**	**259**	263	265	259	GP
11.43	D11S1349	273	**275**	**275**	**275**	264	**275**	260	**275**	275	NI
13.95	D11S569	**146**	**146**	**146**	162	**146**	150	150	152	146	GP
14.81	D11S4116	**210**	216	203	**210**	**210**	**210**	203	210	210	GP
14.12	D11S1794	**254**	**254**	**254**	**254**	**254**	260	**254**	273	254, 254	GM, GP
15.28	D11S4121	**108**	115	**108**	110	**108**	120	108	110	108	GP
16.35	D11S4099	165	**197**	**197**	201	193	**197**	171	201	197	GP
17.24	D11S4138	199	**203**	201	**203**	**203**	**203**	201	203	203	GP
18.94	D11S899	**91**	**91**	**91**	103	**91**	98	91	103	91	GP
26.50	D11S904	**200**	202	192	**200**	192	192	**200**	202	200	GM
35.46	D11S907	162	170	168	172	182	172	168	168	none	–

[ ]=shared allele(s); →=breakpoint; ⋯=region of homology; NI=not informative; NT=not tested; GM=grandmaternal; GP=grandpaternal.

was just visible in family 1 (ISCN 550 band level) but remained cryptic in family 2 (ISCN 500 band level). Therefore the rearrangement is very subtle and would only be detectable cytogenetically at an ISCN band level of 550 or above.

While it is likely that the sequence similarity between 11p and 17p described in hypothesis 1 contributed to the rearrangement formation, we consider that the translocation arose from a single ancestral event and then segregated in the two families presented here. There were identical haplotypes surrounding 5.5 Mb on the chromosome 17 breakpoint and 18 Mb on the chromosome 11 breakpoint. The single discrepant result at D11S4124 can be explained by microsatellite mutation which, while infrequent (estimates range from 10^{-2} to 10^{-4} per generation), is by no means unheard of. The ancestral allele would have been 166 or 170 and we assume the fact that the mothers of LT and MW share an allele present in MW's grandmother is coincidental. Broman and Weber[32] have reported that long homozygous segments, up to 77 cM in length, may be relatively common in the human genome. They include the example of 1.6 cM of sequence homology between D11S1794 and D11S4138 which is within the proximal part of our larger homozygous segment on chromosome 11p15.

After transmission of the derived chromosomes from the postulated common ancestor there must have been recombination proximal to the chromosome 11 breakpoint and on both sides of the chromosome 17 breakpoint. The male recombination hotspot on distal 17p is greater than that on distal 11p (4.46 mcM/Mb compared to 1.77 mcM/Mb) and

may account for the smaller stretch of shared alleles on chromosome 17. The microsatellite mutation and recombinations suggest that the derived chromosomes were transmitted through a number of generations, although the high rate of pregnancy loss argues against the relationship being very distant.

Irrespective of its original formation, the translocation is responsible for the poor obstetric history in both families presented here. In recent years there has been much emphasis on the search for cryptic telomeric rearrangements as underlying causes in cases of IMR. The possible role of these abnormalities in the etiology of recurrent miscarriages is now being considered. In a study detailing the clinical features associated with submicroscopic subtelomeric rearrangements de Vries *et al*.[33] observed only 2 of 24 probands with a family history of miscarriages. However, the study was not looking specifically for a link with miscarriages and included both sporadic and familial cases. Since the extent of chromosome loss or gain in the unbalanced products from a translocation will determine the clinical consequences, larger imbalances would be more likely to cause spontaneous foetal loss or give rise to severely affected live born children with multiple congenital abnormalities (MCA). Our two patients died aged 1 day and 14 months. The average age of the children studied by de Vries was 8.2 years (range 2 to 20) and a positive family history was common for mental retardation but rare for MCA. Therefore, although miscarriages were rare among the families of the translocation carriers selected, the study population is likely to underestimate the proportion of families with miscarriages. Fan and Zhang[34] tried to directly assess the significance of subtelomeric translocations in 80 patients referred with more than three miscarriages. They identified no structural abnormalities amongst their cohort of patients, but the power of the study was halved because the population comprised individuals rather than couples. In contrast, of five couples with five or more miscarriages Yakut *et al*[18] identified two with a balanced subtelomeric rearrangement.

Cryptic subtelomeric rearrangements were first identified in families with recurrent pregnancy losses through direct analysis of individual chromosome arms following a suggested clinical diagnosis.[19–26] With increased clinical awareness and advances in FISH technology, a growing number of cases are being identified through specific searches of the subtelomeric regions.[13–15,17] The balance of evidence suggests that cryptic translocations are responsible for recurrent miscarriages in a proportion of families and that the proportion is likely to rise in families with five or more miscarriages or where additional relatives are also affected.

In the families reported here, the occurrence of a live birth with a clinically recognisable microdeletion syndrome led to the diagnosis of a cryptic parental balanced translocation, but this is likely to be an uncommon means of ascertainment. Therefore a systematic screen of the subtelomeric regions should be undertaken in a cohort of families to determine the contribution of such rearrangements to reproductive loss.

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