ORIGINAL ARTICLE

Subtelomere FISH analysis of 11 688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities

J B Ravnan, J H Tepperberg, P Papenhausen, A N Lamb, J Hedrick, D Eash, D H Ledbetter, C L Martin

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See end of article for authors' affiliations

Correspondence to: Dr C L Martin, Emory University, Department of Human Genetics, 615 Michael Street, Suite 301, Atlanta, GA 30322, USA; clmartin@genetics.emory.edu

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Background: Subtelomere fluorescence in situ hybridisation (FISH) analysis has increasingly been used as an adjunct to routine cytogenetic testing in order to detect small rearrangements. Previous reports have estimated an overall abnormality rate of 6%, with a range of 2–29% because of different inclusion criteria.
Methods: This study presents data compiled from 11 688 cases referred for subtelomere FISH testing in three clinical cytogenetic laboratories.
Results: In this study population, the detection rate for clinically significant subtelomere abnormalities was approximately 2.5%, with an additional 0.5% detection of presumed familial variants. Approximately half of the clinically significant abnormalities identified were terminal deletions, the majority of which were de novo. Most of the remaining cases were unbalanced translocations between two chromosomes or two arms of the same chromosome. Approximately 60% of the unbalanced translocations were inherited from a parent carrying a balanced form of the rearrangement. Other abnormalities identified included tandem duplications, apparently balanced translocations, partial deletions, and insertions. Interestingly, 9 cases (0.08%) were found to have interstitial deletions of non-telomeric control loci, either *BCR* on 22q or *PML* on 15q. The most common clinically significant imbalances found were deletions of 1p, 22q, 4p, 9q, 8p, 2q and 20p. The most common familial variants were a deletion or duplication of 10q, deletion of 4q, deletion of Yq, and duplication of X/Yp onto Xq.
Conclusions: This study of subtelomere rearrangements is a 20 fold increase in number over the previously reported largest study and represents an unbiased analysis of subtelomere rearrangements in a large, unselected patient population.

Over the past several years, the use of genome wide subtelomere screening has increasingly been used as an adjunct to routine cytogenetic testing and has already been incorporated into recommendations for the evaluation of individuals with unexplained mental retardation/developmental delay.[1 2] To assess the usefulness of this testing, Biesecker[3] reviewed 14 previously reported studies (comprising 1718 subjects) that performed subtelomere analysis. These studies showed an overall abnormality rate of 6%, with a range of 2–29% in individual studies. The large variance in frequency of abnormalities observed is most likely due to the different criteria used for inclusion and the sample size in each of the studies. In the largest single study of subtelomere abnormalities to date in patients with a normal karyotype, Knight *et al*[4] examined 466 individuals and reported a frequency of 7.4% (95% confidence interval (CI) 4.4 to 10.4%) in individuals with moderate to severe mental retardation and 0.5% (95% CI 0 to 1.6%) in individuals with mild mental retardation.

Interpreting the results from subtelomere testing can be complicated by the fact that in addition to those subtelomere rearrangements that are the most likely cause of the phenotype, there are also deletions or duplications of the subtelomere regions that appear to be benign familial variants, where an affected proband has an imbalance that is subsequently identified in one of the phenotypically normal parents. Many examples of such cases have previously been reported in the literature, including deletions of 2q, 4q, 10q, 14q, and 17p, and duplications of 4q, 6p, and 10q.[5–10] These findings underline the importance of follow up parental analysis when a subtelomere abnormality is identified in an affected proband to determine the clinical significance of the results.

In this study, subtelomere screening was carried out on 11 688 individuals who were referred to a diagnostic cytogenetics laboratory for fluorescence in situ hybridisation (FISH) testing as part of a clinical evaluation. Any unbalanced subtelomere rearrangements that were identified were categorised as causative of the phenotype or benign familial variants whenever parental analysis was performed and this interpretation could be made. This study of subtelomere rearrangements is a 20 fold increase in number over the previously reported largest study, and represents an analysis of the frequency and pattern of subtelomere rearrangements, both pathogenic and variants, in a large, unselected patient population.

METHODS

Cases included in this retrospective study were received by the clinical cytogenetics laboratory at either Genzyme Genetics (Sante Fe, NM, USA), Laboratory Corporation of America (Research Triangle Park, NC, USA) or the University of Chicago (Chicago, IL, USA) for subtelomere FISH screening.

Abbreviations: CNP, copy number polymorphism; FISH, fluorescence in situ hybridisation

Institutional review board procedures were followed by each institution. Samples were received from a variety of healthcare providers, including but not limited to geneticists, paediatricians, and paediatric neurologists. The majority of subjects were referred due to developmental delay or mental retardation with or without dysmorphic features; however, a wide range of indications was noted, such as behavioural disorder, autism, or growth delay. Chromosome analysis was recommended before subtelomere FISH studies were performed. Banding resolution of studies performed in these three laboratories was routinely 550 bands and above. Cases with cytogenetically visible abnormalities were excluded from this study. The indication for referral for cases in which a subtelomere abnormality was identified is listed in table 1.

Peripheral blood samples were cultured and harvested according to standard protocols. Genome wide subtelomere FISH analyses were carried out using either the ToTelVysion assay (Vysis/Abbott, Inc., Downers Grove, IL, USA) or the Chromoprobe Multiprobe-T System (Cytocell Technologies, Ltd., Cambridge, UK), both of which consist of 41 telomere probes.[11] The Cytocell Chromoprobe system was used for only a few specimens in one laboratory at the beginning of the study. Thereafter, all analyses were carried out using the Vysis ToTelVysion probe panel. The majority of the probes in the two systems target the same loci; additional information can be found on the manufacturers' websites (www.vysis.com and www.cytocell.com). The manufacturers' instructions were followed and 5–10 metaphase cells were analysed for each hybridisation area.

Hybridisation signals were evaluated for hybridisation to the correct subtelomere region as well as signal intensity, judged by direct microscopic visualisation. If the signal intensity was consistently unequal between homologues, the signal was defined as diminished or duplicated, depending on the pattern observed in interphase cells. A diminished signal represents a partial deletion where the breakpoint is contained within the clone and only involves a portion of the subtelomere probe target sequences. Hybridisation signals observed on non-target chromosomes were characterised as cross hybridisation if the signal was observed on a pair of homologues with similar intensity that was reduced relative to the signal on the target chromosome.

All abnormalities identified were confirmed with either repeat FISH analysis using a single subtelomere probe or targeted G banded chromosome confirmation. Parental FISH analyses of probands with subtelomere rearrangements were performed when possible to determine whether the abnormality was inherited or de novo. For parental analysis, targeted FISH was performed, using only the probes that showed abnormal hybridisation patterns in the proband. Rearrangements were characterised as familial variants when an unbalanced subtelomere rearrangement was inherited from a parent with the same imbalance and a normal phenotype, as reported by the referring physician. When parental analysis was not available, a rearrangement was designated as a possible variant if the same imbalance was observed and characterised as a familial variant in at least one other case.

RESULTS

Genome wide subtelomere FISH analysis was used in this study to analyse 11 688 peripheral blood specimens. As listed in table 1, 357 abnormalities were identified in 355 individual probands, an overall abnormality rate of 3.0%. Two probands were found to each have two presumably unrelated subtelomere rearrangements, one of which was clinically significant and the other a familial variant. The male:female sex ratio was virtually even at 169:170. The average age at diagnosis was 7 years (range 1 day to 70 years).

The most common subtelomere rearrangements observed were deletions and unbalanced translocations. As summarised in table 2, terminal deletions were identified in 175 cases (49.3% of all rearrangements found); an additional 19 cases (5.4%) showed a partial deletion. Also shown in table 2, 145 cases (40.8%) had unbalanced derivative chromosomes that were divided into three categories: (*a*) a derivative chromosome with both a duplication and a deletion of subtelomere regions (108 cases, 30.4%); (*b*) a derivative chromosome with only a duplication of subtelomere sequences translocated distal to an intact subtelomere region of another chromosome (20 cases, 5.6%); or (*c*) a derivative chromosome with a duplication onto the short arm of an acrocentric chromosome (17 cases, 4.8%). Representative images from each of these types of subtelomeric rearrangements are shown (fig 1). Other subtelomeric rearrangements were observed at lower frequencies; four (1.1%) tandem duplications, four (1.1%) apparently balanced reciprocal translocations, and one (0.3%) apparently balanced insertion were found.

Interestingly, nine interstitial deletions of non-telomeric regions were identified in this study by noting the absence of a signal for one of the control probes used in the subtelomere probe panel. Eight interstitial deletions involved the 22q11.2 *BCR* probe, which is used as a control probe in the mixture containing the 22q subtelomere probe. A FISH probe for the DiGeorge/velocardiofacial syndrome region at 22q11.2 was subsequently tested and was normal in all eight cases. In addition, one case showed an interstitial deletion of sequences targeted by the 15q24 *PML* probe used as a control probe in the mixture containing the 15q subtelomere probe. These cases may represent new microdeletion syndromes and comparisons of the phenotype of these individuals and further characterisation of their deletions is in progress.

The overall frequency of rearrangements found for each subtelomere region is shown in fig 2, classified by whether the rearrangement led to a duplication or deletion of the chromosome end, or whether the end remained intact but had additional material from another chromosome end added distally.

Parental FISH studies were recommended for all patients in whom a subtelomere rearrangement was found. Targeted studies using the probes showing an abnormality in the proband were performed on 136 sets of parents. Table 2 summarises the inheritance pattern observed for each type of abnormality. The majority of the terminal deletions (48 of 60; 80%) were found to be de novo. Twelve (20%) were inherited from a parent who carried the same deletion; 10 of these parents were reported by the referring physician to be phenotypically normal and two were reported to have abnormalities suggestive of a genetic imbalance. Parental follow up studies were available for 10 partial deletions, of which one (10%) was found to be de novo, with the remaining nine (90%) inherited from a parent with the same partial deletion. All of these parents were reported to be phenotypically normal. Only two of the interstitial deletions had complete parental follow up studies; both (100%) were found to be de novo.

Of the four cases with tandem duplications, parental studies were available for one; this dup(8)(pter) was found to be inherited from a phenotypically normal mother with the same duplication.

Unbalanced translocations involving more than one subtelomere region may result from meiotic segregation of a balanced rearrangement carried by a parent. Parental studies showed that of the 63 rearrangements involving more than one chromosome end, 29 (46%) were inherited from a parent carrying a balanced form of the rearrangement. Of the different types of rearrangements included in this group, inheritance from a balanced parent was less frequent for the

Table 1 Subtelomere rearrangements listed by abnormality type

Case	Abnormality	Del	Dup	Recip	Inheritance	Var	Clinical indication	Age‡	Sex
Deletion: terminal									
1	del(1)(pter)	1p			De novo		DD, MR	1 1/12	F
2	del(1)(pter)	1p			De novo		Gastro-oesophageal reflux, apnea, high pitched cry, hypotonia	1 9/12	F
3	del(1)(pter)	1p			De novo		MCA, agenesis of the corpus callosum, Ebstein's anomaly, hemivertebrae	1/12	M
4	del(1)(pter)	1p			De novo		DD, MR, SGA, seizures	6/12	F
5	del(1)(pter)	1p			De novo		DD, seizures, microcephaly	1 8/12	M
6	del(1)(pter)	1p			De novo		Features of PWS, speech delay	10 10/12	F
7	del(1)(pter)	1p			De novo		Features of PWS, seizures, speech delay	5 2/12	F
8	del(1)(pter)	1p			De novo		MR, seizures, myocardiopathy	3 11/12	F
9	del(1)(pter)	1p			De novo		MR, obesity	15 2/12	M
10	del(1)(pter)	1p			De novo		MCA, seizures, hypotonia	2	F
11	del(1)(pter)	1p							
12	del(1)(pter)	1p					FTT, DD, short stature	2 7/12	F
13	del(1)(pter)	1p					DD	4 2/12	M
14	del(1)(pter)	1p					DD	7 4/12	F
15	del(1)(pter)	1p					DD, MR	24 2/12	F
16	del(1)(pter)	1p					FTT, DD, DF, MR	14 2/12	M
17	del(1)(pter)	1p					DD, speech delay	9	F
18	del(1)(pter)	1p					MR, speech delay, DF	12 11/12	F
19	del(1)(pter)	1p					Features of DGS/VCFS	1 7/12	F
20	del(1)(pter)	1p							F
21	del(1)(pter)	1p					Features of Rett syndrome	10	F
22	del(1)(pter)	1p						7 5/12	F
23	del(1)(pter)	1p							
24	del(1)(pter)	1p							
25	del(1)(pter)	1p							
26	del(1)(pter)	1p							
27	del(1)(pter)	1p					DD, facial telangiectasia, left hemiparesis	8 4/12	F
28	del(1)(pter)	1p					Sensoral neural hearing loss	4/12	F
29	del(1)(qter)	1q			De novo		MCA, DF	4/12	M
30	del(2)(pter)	2p					DD, DF	3 9/12	M
31	del(2)(pter)	2p					Features of AS	2 5/12	F
32	del(2)(qter)	2q			De novo		DD, DF, hip dislocation	1 6/12	M
33	del(2)(qter)	2q					Encephalopathy	6 11/12	M
34	del(2)(qter)	2q						14 10/12	M
35	del(2)(qter)	2q					DD, MR, macrocephaly, autism	6 5/12	F
36	del(2)(qter)	2q					DD	20	F
37	del(2)(qter)	2q						11/12	F
38	del(2)(qter)	2q					Features of DGS/VCFS	2 11/12	M
39	del(3)(pter)	3p			Father same	Yes	Features of fragile X syndrome	2 1/12	M
40	del(3)(pter)	3p					DD, DF, birth defect	12 3/12	M
41	del(3)(pter)	3p						11 11/12	F
42	del(3)(pter)	3p						2 5/12	M
43	del(3)(pter)	3p						2 11/12	M
44	del(3)(qter)	3q			De novo		DD, MR	9 5/12	F
45	del(3)(qter)	3q			De novo		DD	4 11/12	F
46	del(3)(qter)	3q			De novo		DD, CLP	2 1/12	F
47	del(3)(qter)	3q			De novo		Features of AS	4 3/12	M
48	del(3)(qter)	3q					MCA	11/12	F
49	del(3)(qter)	3q							M
50	del(3)(qter)	3q					MR	12 5/12	M
51	del(3)(qter)	3q					DD	1 2/12	F
52	del(4)(pter)	4p			De novo		MR, DD, ASD, severe seizures, growth delay, features of WHS	5	F
53	del(4)(pter)	4p					Nervous system anomaly	20 8/12	M
54	del(4)(pter)	4p					MCA, DD, MR	7 8/12	M
55	del(4)(pter)	4p					FTT, DD, MR	2 10/12	F
56	del(4)(pter)	4p					DD, microcephaly, lack of coordination, abnormal involuntary movements	7 10/12	F
57	del(4)(pter)	4p					MR, DF, microcephaly, absent speech, epilepsy	18 11/12	F
58	del(4)(pter)	4p					Microcephaly, seizures, speech delay, DD	8 8/12	M
59	del(4)(pter)	4p						4/12	F
60	del(4)(qter)	4q			Father same	Yes	DD, MR, obese, upper palbebral fissures, 5th finger clinodactyly, chrorea movements	9 5/12	F
61	del(4)(qter)	4q			Father same	Yes	DD, MR	2	M
62	del(4)(qter)	4q			Mother same*	No	MR, DD, not walking, some speech	1 7/12	F
63	del(4)(qter)	4q			De novo		Growth delay, hypotonia, kidney hypoplasia	1	F
64	del(4)(qter)	4q						3 10/12	F
65	del(4)(qter)	4q					DD, MR	1 7/12	F

Table 1 Continued

Case	Abnormality	Del	Dup	Recip	Inheritance	Var	Clinical indication	Age‡	Sex
66	del(4)(qter)	4q					DD	22 11/12	M
67	del(4)(qter)	4q						14 6/12	F
68	del(4)(qter),der(8)t(4;8)(pter;pter)†	4q					MCA	41 10/12	F
69	del(5)(pter)	5p						4	M
70	del(6)(pter)	6p						1 6/12	F
71	del(6)(qter)	6q			De novo		DD, behavioural problems, bilateral epicanthal folds, prominent philtrum	2	F
72	del(6)(qter)	6q			De novo		DD	8 1/12	M
73	del(6)(qter)	6q					DD, inverted nipples, colpocephaly, family history of DD, behavioural disorder	9 8/12	F
74	del(6)(qter)	6q					DF, CHD, features of WS	1	M
75	del(7)(pter)	7p			De novo		Neonatal diabetes, growth retardation, microcephaly	1/12	F
76	del(7)(pter)	7p					DD, microcephaly	5 5/12	F
77	del(7)(qter)	7q					Features of WS	1 4/12	F
78	del(8)(pter)	8p			De novo		Severe MR, absent speech, DF, barely ambulatory	13 4/12	F
79	del(8)(pter)	8p					Short stature, DD	8/12	M
80	del(8)(pter)	8p					MR, hyperpigmentation	18 3/12	F
81	del(8)(pter)	8p						3 1/12	F
82	del(9)(pter)	9p			De novo		Behavioural disorder	8	F
83	del(9)(pter)	9p			De novo		DD, MR	2 1/12	M
84	del(9)(pter)	9p			De novo		Autism, hypotonia, speech delay	2 4/12	F
85	del(9)(pter)	9p			De novo		Features of DGS/VCFS, features of fragile X syndrome	1 1/12	F
86	del(9)(pter)	9p						5 11/12	F
87	del(9)(qter)	9q			De novo		DD, MR	2 8/12	M
88	del(9)(qter)	9q			De novo		Microcephaly, corneal anomalies	3 10/12	M
89	del(9)(qter)	9q			De novo		MCA, DD, MR	6 6/12	M
90	del(9)(qter)	9q			De novo		Features of SMS	3 9/12	F
91	del(9)(qter)	9q			De novo		DF, mild DD	4	M
92	del(9)(qter)	9q			De novo		MR, MCA, DF, hypotonia	6 4/12	M
93	del(9)(qter)	9q					DD, MR, mild DF, anteverted nasal tip, ASD, low tone, gastro-oesophageal reflux	1 11/12	F
94	del(9)(qter)	9q					Features of PWS	11/12	M
95	del(9)(qter)	9q					DD, MR, seizures, absent speech	4 7/12	F
96	del(9)(qter)	9q					DD, features of SMS	2 2/12	F
97	del(9)(qter)	9q					Features of Down syndrome	5 5/12	F
98	del(10)(pter)	10p			De novo		FTT, DD, hypotonia, speech delay, growth delay, prominent forehead, hydronephrosis	2 1/12	F
99	del(10)(pter)	10p					MCA	10 8/12	F
100	del(10)(pter)	10p					DD, ADHD, seizure disorder	7 7/12	F
101	del(10)(qter)	10q			Father same	Yes	Short stature, hearing loss	4 5/12	F
102	del(10)(qter)	10q			De novo		DD	2	F
103	del(10)(qter)	10q			De novo		DD, CHD, features of DGS/VCFS		F
104	del(10)(qter)	10q					DD, precocious puberty	12 3/12	M
105	del(10)(qter)	10q					Features of DGS/VCFS, features of WS, autism	5	M
106	del(10)(qter)	10q							
107	del(10)(qter)	10q							
108	del(10)(qter)	10q						30	M
109	del(10)(qter)	10q						1/12	M
110	del(11)(qter)	11q			Mother same*	No	DD, seizures, thrombocytopenia	9 2/12	F
111	del(11)(qter)	11q							
112	del(11)(qter)	11q					Features of fragile X syndrome, features of DGS/VCFS	13	M
113	del(11)(qter)	11q					Severe MR	18 1/12	M
114	del(12)(pter)	12p					DD, features of DGS/VCFS	8	F
115	del(13)(qter)	13q			De novo		Microcephaly, DD, DF, hypospadius, mild MR	7 6/12	M
116	del(13)(qter)	13q					DD, MR	1 1/12	F
117	del(13)(qter)	13q					DD	13	M
118	del(14)(qter)	14q					Lissencephaly, features of MDS, features of SMS	14 6/12	F
119	del(14)(qter)	14q					Unspecified disorder of metabolism	8/12	M
120	del(14)(qter)	14q					Microcephaly, speech or language disorder, DD	1 5/12	M
121	del(15)(qter)	15q					Feeding problems	3 1/12	F
122	dim(10)(qter),del(16)(pter)†	16p			De novo		DD, DF	4 5/12	M
123	del(16)(pter)	16p			De novo		DD, DF	20 1/12	M
124	del(16)(pter)	16p			De novo		Motor delay	2 3/12	M
125	del(16)(pter)	16p			De novo		DD	1 2/12	F

Table 1 Continued

Case	Abnormality	Del	Dup	Recip	Inheritance	Var	Clinical indication	Age‡	Sex
126	del(16)(pter)	16p						1/12	M
127	del(17)(pter)	17p			Mother same	Yes	Features of DGS/VCFS	10 7/12	M
128	del(17)(pter)	17p					DD	1 4/12	F
129	del(17)(pter)	17p					Features of WS	5/12	F
130	del(18)(qter)	18q			De novo		MCA	2 9/12	F
131	del(18)(qter)	18q						16 1/12	M
132	del(18)(qter)	18q					MCA	5/12	M
133	del(20)(pter)	20p			Father same	Yes	Features of SMS	2 4/12	M
134	del(20)(pter)	20p			De novo		DD	1 11/12	M
135	del(20)(pter)	20p			De novo		DD, DF, growth delay	3 9/12	F
136	del(20)(pter)	20p			De novo		Growth delay, hypotonia, kidney hypoplasia	1	M
137	del(20)(pter)	20p					DD	1	M
138	del(20)(pter)	20p						5 8/12	F
139	del(20)(pter)	20p						4 11/12	M
140	del(20)(pter)	20p						30 8/12	M
141	del(20)(pter)	20p						1 10/12	M
142	del(20)(qter)	20q			De novo		MCA	11 1/12	M
143	del(20)(qter)	20q			De novo		DD, MR	7 8/12	M
144	del(21)(qter)	21q			Mother same	Yes	DD, MR, hypotonia, hip dysplasia, plagiocephaly	7/12	M
145	del(21)(qter)	21q			Mother same	Yes	MR, ataxia, abnormal cerebellum	1	M
146	del(21)(qter)	21q					DF, CLP	2/12	M
147	del(22)(qter)	22q			De novo		DD	16	M
148	del(22)(qter)	22q			De novo		DD, speech delay	4	F
149	del(22)(qter)	22q					DD	7 4/12	F
150	del(22)(qter)	22q					MR, DD, ataxic gait	6 6/12	F
151	del(22)(qter)	22q					DF, CLP, haemangioma of eye	2 5/12	F
152	del(22)(qter)	22q					DD, MR	12 3/12	F
153	del(22)(qter)	22q					DD, DF, MR	3 5/12	F
154	del(22)(qter)	22q					DD, overgrowth	1 3/12	F
155	del(22)(qter)	22q					Cerebral palsy	13 7/12	F
156	del(22)(qter)	22q					DD, MR	4 3/12	F
157	del(22)(qter)	22q					DD, DF, hypotonia	2 10/12	M
158	del(22)(qter)	22q					DD	7	F
159	del(22)(qter)	22q					DD, speech delay, DF	4	F
160	del(22)(qter)	22q					DD, DF	5	F
161	del(22)(qter)	22q						1	F
162	del(X)(pter)	Xp						45 2/12	F
163	del(X)(qter)	Xq						39 5/12	F
164	del(Y)(pter)	Yp					DD, obesity, hypotonia, polydipsia	10 11/12	M
165	del(Y)(pter)	Yp					Features of fragile X syndrome	3 11/12	M
166	del(Y)(qter)	Yq			Father same	Yes	DD	8 6/12	M
167	del(Y)(qter)	Yq			Father same	Yes			M
168	del(Y)(qter)	Yq				?	DD, MR	7 8/12	M
169	del(Y)(qter)	Yq				?	Autism spectrum disorder	6 1/12	M
170	del(Y)(qter)	Yq				?	DD, MR	1 6/12	M
171	del(Y)(qter)	Yq				?	DD, DF	11	M
172	del(Y)(qter)	Yq				?	DD, ADHD	6 6/12	M
173	del(Y)(qter)	Yq				?		1 8/12	M
174	del(Y)(qter)	Yq				?	Features of DGS/VCFS	8 10/12	M
175	del(Y)(qter)	Yq			Brother same	?	DD	5	M
Deletion: partial									
176	dim(4)(pter)	4p			Mother same	Yes	MR, DD, CLP, coloboma of the iris, hypospadias, hypertelorism, short stature	16 7/12	M
177	dim(10)(qter)	10q			Father same	Yes	DD	8 8/12	F
178	dim(10)(qter)	10q			Father same	Yes	DD, hydrocephaly, seizure disorder	4/12	M
179	dim(10)(qter)	10q			Mother same	Yes	MCA, polymicrogyria	2 10/12	M
180	dim(10)(qter),del(16)(pter)†	10q			Mother same	Yes	DD, DF	4 5/12	M
181	dim(10)(qter)	10q			Mother same	Yes			
182	dim(10)(qter)	10q			Mother same	Yes	DD	3 5/12	M
183	dim(10)(qter)	10q			De novo	?	DD, DF, microcephaly	1 10/12	F
184	dim(10)(qter)	10q				?	MCA	4 4/12	M
185	dim(10)(qter)	10q				?	DD	2 8/12	M
186	dim(10)(qter)	10q				?	Autism	4 11/12	M
187	dim(10)(qter)	10q				?	Severe behavioural disorder, MR	2 4/12	M
188	dim(10)(qter)	10q				?	Features of fragile X syndrome	10 1/12	M
189	dim(10)(qter)	10q				?			
190	dim(10)(qter)	10q				?		1 1/12	M
191	dim(14)(qter)	14q			Mother same	Yes		3 5/12	M
192	dim(14)(qter)	14q				?	Hypotonia, hypertrophy, cardiomyopathy, small fontanelle	2/12	M
193	dim(14)(qter)	14q				?		7/12	M
194	dim(Y)(pter)	Yp			Father same	Yes	MCA	1 1/12	M
Deletion: interstitial									
195	del(15)(q22q22)	15q-PML			De novo		DD, features of AS/PWS, features of fragile X syndrome	3 4/12	F

Table 1 Continued

Case	Abnormality	Del	Dup	Recip	Inheritance	Var	Clinical indication	Age‡	Sex
196	del(22)(q11.2q11.2)	22q-BCR			De novo		Hearing loss	2 10/12	F
197	del(22)(q11.2q11.2)	22q-BCR					DD	6 7/12	M
198	del(22)(q11.2q11.2)	22q-BCR					DD, microcephaly	1	M
199	del(22)(q11.2q11.2)	22q-BCR					DD, cerebral palsy, growth delay	1	M
200	del(22)(q11.2q11.2)	22q-BCR					Hypotonia, microcephaly	1 7/12	M
201	del(22)(q11.2q11.2)	22q-BCR					Features of Turner syndrome, features of DGS/VCFS	1 10/12	F
202	del(22)(q11.2q11.2)	22q-BCR					Congenital musculoskeletal anomalies	7 5/12	M
203	del(22)(q11.2q11.2) Duplication	22q-BCR						11 7/12	M
204	dup(4)(qter)		4q				DD, brain disorder	3 7/12	F
205	dup(8)(pter)		8p		Mother same	Yes	DD, DF	4 1/12	M
206	?dup(10)(qter)		10q			?	Features of SMS	1 5/12	M
207	mos dup(10)(qter)		10q			?	DD	3 1/12	M
Derivative: duplication only									
208	der(1)t(1;17)(pter;pter)		17p	1p			DD	12 10/12	F
209	der(3)t(3;14)(pter;qter)		14q	3p	Mother same	Yes	DF, anxiety	4 4/12	M
210	der(7)t(7;16)(pter;pter)		16p	7p	Father balanced		MCA, pachygyria	0	F
211	der(10)t(10;16)(qter;pter)		16p	10q			MCA, MR	6 2/12	F
212	der(10)t(10;16)(qter;pter)		16p	10q	Mother balanced		MR, MCA, family history of MCA and MR	7 5/12	F
213	der(15)t(7;15)(qter;qter)		7q	15q					
214	der(18)t(16;18)(qter;pter)		16q	18p	Mother same	Yes		2 1/12	M
215	der(18)t(16;18)(qter;pter)		16q	18p	Mother same	Yes	DD, MR	4 1/12	F
216	der(18)t(16;18)(qter;pter)		16q	18p		?		4 5/12	M
217	der(18)t(16;18)(qter;pter)		16q	18p		?	DD, MR	15 6/12	F
218	der(18)t(16;18)(qter;pter)		16q	18p		?	Arhinencephaly, hemiplagia, features of SMS, features of DGS/VCFS	8 8/12	F
219	der(18)t(17;18)(pter;pter)		17p	18p	Mother same	Yes	DD	9 2/12	F
220	der(19)t(19;19)(pter;qter)		19q	19p	De novo			2 5/12	F
221	der(19 or 20)t(X or Y;19 or 20)(qter;pter or qter)		Xq or Yq	F-group			DD	7/12	F
222	der(20)t(X or Y;20)(qter;pter)		Xq or Yq	20p	Mother same	Yes	Features of fragile X	5	M
223	der(X)t(X;X or Y)(qter;pter)		Xp or Yp	Xq	Father same	Yes	Autism	1 10/12	F
224	der(X)t(X;X or Y)(qter;pter)		Xp or Yp	Xq	Father same	Yes	Low muscle tone	4/12	F
225	der(X)t(X;X or Y)(qter;pter)		Xp or Yp	Xq	Mother same	Yes	DD	2 5/12	M
226	der(X)t(X;X or Y)(qter;pter)		Xp or Yp	Xq	Mother same	Yes	DD, behavioural problem, DF, spinal stenosis, obsessive compulsive disorder, nasal speech	17 1/12	M
227	der(X)t(X;X or Y)(qter;pter)		Xp or Yp	Xq		?	DD, CHD	11 9/12	F
Derivative: duplication on acrocentric short arm									
228	der(13)t(9;13)(qter;pter)		9q	13p	De novo			15 1/12	M
229	der(13)t(11;13)(qter;pter)		11q	13p				8/12	M
230	der(13)t(13;17)(pter;qter)		17q	13p	De novo		CHD, MCA, Dandy-Walker malformation	7 4/12	M
231	der(14)t(7;14)(qter;pter)		7q	14p			Family history of speech delay and autism	2 7/12	M
232	der(14)t(14;14)(pter;qter)		14q	14p	De novo		DD, MR	3 1/12	M
233	der(14)t(14;16)(pter;pter)		16p	14p	De novo		DD	3 1/12	M
234	der(15)t(15;16)(pter;qter)		16q	15p				13 10/12	M
235	der(15)t(15;19)(pter;qter)		19q	15p	Mother balanced		DF, speech delay, short stature	4	F
236	der(15)t(X;15)(qter;pter)		Xq	15p	De novo		Features of AS		F
237	der(15)t(X or Y;15)(qter;pter)		Xq or Yq	15p				4 4/12	F
238	der(21)t(17;21)(qter;pter)		17q	21p			CHD, lissencephaly, hypoplastic kidney	2 7/12	F
239	der(21)t(21;22)(pter;qter)		22q	21p			MR	7 10/12	F
240	der(22)t(1;22)(qter;pter)		1q	22p			Features of fragile X syndrome	2	F
241	der(22)t(4;22)(pter;pter)		4p	22p	Mother same	Yes	DD, behavioural abnormalities, non-dysmorphic	4 1/12	M
242	der(22)t(4;22)(pter;pter)		4p	22p	Mother same	Yes	DD	1 5/12	M
243	der(22)t(4;22)(pter;pter)		4p	22p		?	Features of fragile X syndrome, autism	28 8/12	M
244	der(22)t(22;22)(pter;qter)		22q	22p	De novo		MCA	12 8/12	M
Derivative: deletion and duplication									
245	der(1)t(1;1)(pter;qter)	1p	1q				DD, MR	16 1/12	F
246	der(1)t(1;4)(pter;pter)	1p	4p		De novo		MCA	4 3/12	M
247	der(1)t(1;10)(pter;pter)	1p	10p		De novo		DD, MR	13 1/12	F
248	der(1)t(1;10)(pter;pter)	1p	10p				MR	10 7/12	M
249	der(1)t(X or Y;1)(qter;pter)	1p	Xq or Yq						M
250	der(1)t(1;4)(qter;qter)	1q	4q					6/12	F
251	der(1)t(1;3)(qter;qter)	1q	3q				MCA	0	F
252	der(1)t(1;15)(qter;qter)	1q	15q						F
253	der(1)t(1;18)(qter;pter)	1q	18p						
254	der(1)t(1;22)(qter;qter)	1q	22q					70 5/12	F
255	der(2)t(1;2)(qter;qter)	2q	1q					15 11/12	F

Table 1 Continued

Case	Abnormality	Del	Dup	Recip	Inheritance	Var	Clinical indication	Age‡	Sex
256	der(2)t(2;12)(qter;qter)	2q	12q				MR	31 3/12	M
257	der(2)t(2;17)(qter;pter)	2q	17p		Father balanced		Features of cerebro-oculo-facial-skeletal syndrome	8 10/12	M
258	der(2)t(2;20)(qter;qter)	2q	20q		Father balanced		DD, MR	11 6/12	F
259	der(2)t(2;22)(qter;qter)	2q	22q		Mother balanced		DD, autism	2 6/12	M
260	der(4)t(3;4)(qter;pter)	4p	3q		Father balanced		FTT, DD	10/12	M
261	der(4)t(4;6)(pter;pter)	4p	6p						
262	der(4)t(4;6)(pter;pter)	4p	6p				Features of DGS/VCFS	8 10/12	F
263	der(4)t(4;8)(pter;pter)	4p	8p				DD	1 7/12	M
264	der(4)t(4;8)(pter;pter)	4p	8p				MCA	16 1/12	M
265	der(4)t(4;11)(pter;pter)	4p	11p				DD	5 9/12	M
266	der(4)t(4;5)(qter;pter)	4q	5p		Father balanced		DD, seizures	7 10/12	M
267	der(4)t(X;4)(qter;qter)	4q	Xq		De novo		DD, MR, seizure disorder	12 2/12	M
268	der(4)t(X or Y;4)(qter;qter)	4q	Xq or Yq				FTT	1 6/12	M
269	der(5)t(2;5)(pter;pter)	5p	2p		Mother balanced		DD, family history of DD	2 3/12	F
270	der(5)t(2;5)(pter;pter)	5p	2p				FTT, DD, hypotonia, macrocephaly	1 1/12	F
271	der(5)t(5;7)(pter;pter)	5p	7p		Mother balanced		DD, MR, short stature	11 7/12	F
272	der(5)t(5;9)(pter;pter)	5p	9p				DF, delayed bone age, mild MR	2 4/12	F
273	der(5)t(5;10)(pter;qter)	5p	10q				MR	1	F
274	der(5)t(5;14)(pter;qter)	5p	14q				DD, mild MR, short stature	5 4/12	F
275	der(5)t(5;20)(pter;pter)	5p	20p		Father balanced		DD, MR	9 8/12	F
276	der(6)t(6;10)(pter;qter)	6p	10q				MCA, DD, DF	12 9/12	F
277	der(6)t(6;12)(pter;qter)	6p	12q				DD, deafness	6/12	F
278	der(6)t(1;6)(qter;qter)	6q	1q		De novo		Autism	4 9/12	F
279	der(6)t(3;6)(qter;qter)	6q	3q					2 7/12	F
280	der(6)t(6;7)(qter;pter)	6q	7p				DD, MR	2 11/12	M
281	der(6)t(6;21)(qter;qter)	6q	21q		Mother balanced		MR, brain malformation	6 6/12	F
282	der(7)t(7;7)(pter;qter)	7p	7q				CLP, abnormal thumbs	1/12	F
283	der(7)t(7;16)(pter;pter)	7p	16p				MCA, CLP, DF	0	M
284	der(7)t(7;19)(pter;qter)	7p	19q		Mother balanced		MCA	1 11/12	F
285	der(7)t(2;7)(qter;qter)	7q	2q					0	M
286	der(7)t(5;7)(pter;qter)	7q	5p		Mother balanced		DF, MR	2 4/12	F
287	der(7)t(7;8)(qter;pter)	7q	8p		Mother balanced		MR, microcephaly, growth delay, CLP	10	M
288	der(7)t(7;9)(qter;qter)	7q	9q				DD, short stature	7 2/12	M
289	der(7)t(7;11)(qter;pter)	7q	11p		Mother balanced		MCA, DD, DF	10 5/12	M
290	der(7)t(7;22)(qter;qter)	7q	22q		De novo		DD, DF	2 9/12	M
291	der(8)t(2;8)(pter;pter)	8p	2p		Mother balanced		DD	4 1/12	M
292	del(4)(qter),der(8)t(4;8)(pter;pter)†	8p	4p				MCA	41 10/12	F
293	der(8)t(8;8)(pter;qter)	8p	8q				Autism	7	F
294	der(8)t(8;9)(pter;qter)	8p	9q		De novo		DD, DF, microcephaly	2	M
295	der(8)t(8;10)(pter;qter)	8p	10q				DD, constipation, oesophageal reflux, cerebral palsy	10 1/12	F
296	der(8)t(8;12)(pter;pter)	8p	12p					2	M
297	der(8)t(8;12)(pter;pter)	8p	12p				Severe MR, hypotonia, ASD, blindness	2 11/12	F
298	der(8)t(8;18)(pter;qter)	8p	18q					6 4/12	F
299	der(9)t(3;9)(pter;pter)	9p	3p		De novo		DD, speech delay	3 9/12	F
300	der(9)t(X;9)(qter;pter)	9p	Xq		De novo		DD, short stature, irregular auricular deformity	2 3/12	M
301	der(9)t(7;9)(pter;qter)	9q	7p				MR, absent speech, repetitive movements	11 3/12	M
302	der(9)t(9;16)(qter;pter)	9q	16p		Mother balanced		DD, MSAB, features of DGS/VCFS	5 1/12	F
303	der(9)t(9;17)(qter;pter)	9q	17p				DD, MR	10 1/12	F
304	der(10)t(7;10)(qter;pter)	10p	7q					3 11/12	M
305	der(10)t(10;16)(pter;pter)	10p	16p		De novo		DD, MR, short stature	10 7/12	F
306	der(10)t(1;10)(pter;qter)	10q	1p		Mother balanced		DF, microcephaly	3	M
307	der(10)t(4;10)(pter;qter)	10q	4p				DD	1 4/12	M
308	der(10)t(4;10)(qter;qter)	10q	4q				DD	1 8/12	F
309	der(10)t(8;10)(qter;qter)	10q	8q		Mother balanced		MR, MCA, IUGR	13	M
310	der(10)t(8;10)(qter;qter)	10q	8q						
311	der(10)t(10;10)(qter;pter)	10q	10p					0	F
312	der(10)t(10;17)(qter;qter)	10q	17q		Mother balanced		MCA	1/12	M
313	der(10)t(10;21)(qter;qter)	10q	21q				Congenital anomalies, unspecified	3 8/12	F

Table 1 Continued

Case	Abnormality	Del	Dup	Recip	Inheritance	Var	Clinical indication	Age‡	Sex
314	der(11)t(2;11)(qter;qter)	11q	2q		Mother balanced		MCA	1/12	M
315	der(11)t(11;12)(qter;qter)	11q	12q					9	M
316	der(11)t(11;12)(qter;qter)	11q	12q					27 10/12	F
317	der(12)t(12;17)(pter;pter)	12p	17p		Father balanced		DD	1 1/12	M
318	der(12)t(12;19)(pter;qter)	12p	19q		Father balanced		DD, FTT	1 4/12	
319	der(12)t(12;20)(pter;pter)	12p	20p					13 7/12	M
320	der(12)t(12;19)(qter;pter)	12q	19p		Father balanced		MCA	6/12	M
321	der(13)t(2;13)(qter;qter)	13q	2q				MCA, VSD, DF	2/12	F
322	der(13)t(3;13)(qter;qter)	13q	3q		De novo		MCA, DD, MR	12 2/12	F
323	der(13)t(3;13)(qter;qter)	13q	3q				DD	1 6/12	F
324	der(14)t(X or Y;14)(pter;qter)	14q	Xp or Yp				DF, Hypotonia	2 4/12	F
325	der(15)t(3;15)(qter;qter)	15q	3q				Syndrome of unknown aetiology		M
326	der(15)t(9;15)(qter;qter)	15q	9q				Club foot, CHD, DD, MR	1 7/12	F
327	der(15)t(X;15)(qter;qter)	15q	Xq		De novo		DF, growth retardation	2 6/12	F
328	der(16)t(16;16)(pter;qter)	16p	16q					3	M
329	der(16)t(16;22)(pter;qter)	16p	22q		De novo		DF, thalassaemia trait	3	M
330	der(17)t(9;17)(qter;pter)	17p	9q		Mother balanced		Redundant neck skin (cystic hygroma on ultrasound), CHD, hypotonia, features of DGS/VCFS	1/12	F
331	der(18)t(18;18)(pter;qter)	18p	18q				DD, cerebral palsy	8	M
332	der(18)t(2;18)(pter;qter)	18q	2p				DD	4 7/12	F
333	der(18)t(4;18)(qter;qter)	18q	4q		Mother balanced		Features of SMS	8	F
334	der(18)t(4;18)(qter;qter)	18q	4q				Features of SMS, features of DGS/VCFS, features of fragile X syndrome	12 1/12	F
335	der(18)t(4;18)(qter;qter)	18q	4q				MCA	10/12	F
336	der(18)t(10;18)(pter;qter)	18q	10p				MCA	3 6/12	M
337	der(18)t(14;18)(qter;qter)	18q	14q				Short stature, mood disorder, features of DGS/VCFS, features of SMS	17 4/12	F
338	der(20)t(5;20)(qter;pter)	20p	5q				Static encephalopathy	14 5/12	F
339	der(20)t(9;20)(pter;pter)	20p	9p		De novo		FTT, DD	2 10/12	M
340	der(21)t(5;21)(pter;qter)	21q	5p		Father balanced		DD	3 8/12	M
341	der(21)t(14;21)(qter;qter)	21q	14q						M
342	der(22)t(6;22)(pter;qter)	22q	6p		De novo		DD	4 8/12	F
343	der(22)t(12;22)(qter;qter)	22q	12q					2 5/12	F
344	der(22)t(16;22)(qter;qter)	22q	16q		Father balanced		MCA	2 4/12	F
345	der(X)t(X;14)(pter;qter)	Xp	14q				Features of fragile X syndrome	16	M
346	der(X)t(X;X or Y)(pter;qter)	Xp	Xq or Yq		Mother same*	No	Hypotonia	1	M
347	der(X)t(X;X or Y)(pter;qter)	Xp	Xq or Yq				DD, MR	14 10/12	M
348	der(X)t(X;X or Y)(pter;qter)	Xp	Xq or Yq				Short stature, ADHD, mild autism	6 5/12	M
349	der(X)t(X;3)(qter;pter)	Xq	3p				MCA, bilateral optic coloboma	1 5/12	F
350	del(X)(pter)/der(X)t(X;15)(pter;qter)	Xp	mos 15q				DD	1 7/12	F
351	idic(Y)(q11.2)	Yq	Yp					12 4/12	M
352	der(Y)t(Y;X or Y)(qter;pter)	Yq	Xp or Yp		De novo		DD	6 8/12	M
Insertion									
353	der(22)ins(22;X or Y)(q11.2;pter)		Xp or Yp				Fine motor delay, features of fragile X syndrome	2 2/12	M
Translocation									
354	t(1;5)(qter;qter)								
355	t(6;12)(qter;qter)						DF, DD		F
356	t(19;21)(pter;qter)						MR	3 2/12	M
357	t(5;6)(qter;qter)								

*Abnormalities inherited from a parent with the same rearrangement are not considered to be familial variants when the parent has been reported to have an abnormal phenotype; †two patients had two unrelated subtelomere rearrangements; they are each listed twice, once for each abnormality (numbers 68 and 180 represent the same individual; 122 and 292 represent the same individual); ‡ages are given as years and/or number of months out of 12. ?, possible variant. Del, deletion; Dup, duplication; Recip, recipient of translocation; ADHD, attention deficit hyperactivity disorder; AS, Angelman syndrome; ASD, atrial septal defect; CHD, congenital heart defect; CLP, cleft lip and/or palate; DD, developmental delay; DF, dysmorphic features; DGS/VCFS, DiGeorge/velocardiofacial syndrome; FTT, failure to thrive; IUGR, intrauterine growth retardation; MCA, multiple congenital anomalies; MDS, Miller-Dieker syndrome; MR, mental retardation; MSAB, multiple spontaneous abortions; PWS, Prader-Willi syndrome; SGA, small for gestational age; SMS, Smith-Magenis syndrome; Var, variant; VSD, ventral septal defect; WHS, Wolf-Hirschhorn syndrome; WS, Williams syndrome. The mode of inheritance is listed only when both parents were tested. Clinical indication, age, and sex of the patient are given when available.

derivatives containing a duplication only (two of 12; 17%) or a duplication onto the short arm of an acrocentric chromosome (one of 9; 11%) than for the derivatives containing both a duplication and a deletion (26 of 42; 62%).

The same unbalanced translocation observed in a proband was found in a parent in nine of 12 (75%) cases containing a duplication only and in two of 9 (22%) cases with a duplication onto the short arm of an acrocentric chromosome; all of these carrier parents were reported to be phenotypically normal. One case with an abnormal X chromosome containing a deletion of Xp and a duplication of Xq/Yq was found to be inherited from a mother with the same rearrangement; however, the mother was reported to have an abnormal phenotype.

Table 2 Summary of each type of subtelomere abnormality identified in this study and the inheritance patterns observed when parental studies were performed

					Parent same		Parent balanced	
Abnormality	**Total**	**Variant***	**Inheritance known**	**De novo**	**Father**	**Mother**	**Father**	**Mother**
Deletion								
Terminal	175	18	60	48	7	5	0	0
Partial	19	19	10	1	3	6	0	0
Interstitial	9	0	2	2	0	0	0	0
Duplication	4	3	1	0	0	1	0	0
Derivative								
Duplication and deletion	108	0	42	15	0	1	10	16
Duplication only	20	13	12	1	2	7	1	1
Duplication onto acrocentric p arm	17	3	9	6	0	2	0	1
Insertion†	1		0					
Translocation	4		0					
Total	357	56	136	73	12	22	11	18

*Variants include both known familial variants as well as possible variants; †no parental studies were available for the insertion or translocation patients.

Of 63 unbalanced translocations, 22 (35%) were found to be de novo. This includes 15 of 42 cases (36%) with both a duplication and deletion, 1 of 12 cases (8%) with a duplication only, and 6 of 9 cases (67%) with a duplication onto the short arm of an acrocentric chromosome. No parental follow up studies were available for the probands with apparently balanced insertions or translocations.

The discovery of so many unbalanced subtelomere rearrangements found in reportedly normal parents complicates the interpretation of the clinical significance of individual abnormal subtelomere findings. In an attempt to clarify which subtelomere regions may have imbalances that do not result in phenotypic abnormalities, the rearrangements found in this study were classified as probably clinically significant or not. A rearrangement was considered a familial variant if parental FISH studies were performed and a phenotypically normal parent was shown to carry the same unbalanced rearrangement as the affected proband. When parental analysis was not available, a rearrangement was classified as a possible variant if the same rearrangement was shown to be a familial variant in at least one other unrelated case. All other rearrangements were assumed to be clinically significant. Of 357 abnormalities found, 31 were considered familial variants and 25 possible variants, a total of 56 likely variants; 16% of the total number of abnormalities found. The remaining 84% (301) of the abnormalities found were assumed to be clinically significant. However, without performing family studies or fine mapping to determine the actual size of the deleted or duplicated regions for the 24 cases categorised as possible variants, it is not accurately known if these rearrangements are benign variants versus clinically significant. Therefore, the number of clinically significant abnormalities identified could be under-represented.

Table 3 shows the rearrangements classified as likely variants in this study. The most common variants observed involved rearrangements of the 10q subtelomere region. Fourteen cases had a partial deletion for the 10q subtelomere probe region, one case had a deletion of the entire 10q subtelomere probe region, and two had apparent tandem duplications of the 10q probe region.

Three unbalanced translocations were found to be familial variants in more than one family. Five cases were found with an extra Xp/Yp subtelomere signal on the long arm of the X chromosome adjacent to the Xq/Yq subtelomere probe signal (both maternal and paternal inheritance were observed), three cases had an additional copy of the 4p subtelomere probe region on the short arm of chromosome 22, and five cases had an extra hybridisation signal for the 16q subtelomere region present on 18p adjacent to the 18p subtelomere probe signal. Other variants observed in more than one family included deletions of 4q, 21q, and Yq, and partial deletions of 14q.

Of particular interest are the two patients who each had two unrelated subtelomere abnormalities. One proband was found to have a deletion of 16p as well as a partial deletion of 10q (122 and 180 in table 1). Parental studies showed that the 16p deletion was de novo, but the partial 10q deletion was inherited from the mother, who showed the same diminished 10q signal. The second proband was found to have a deletion of 4q and a derivative 8 from a translocation between 4p and 8p (68 and 292 in table 1). Parental studies were not available for this family, but subtelomere FISH analysis of a sibling showed the same derivative 8 as the proband, but no deletion of 4q. The two siblings were reported to have similar phenotypes.

DISCUSSION

This study of 11 688 individuals demonstrates that rearrangements of the subtelomere regions contribute significantly to idiopathic mental retardation. The individuals tested here were referred for clinical subtelomere testing by a broad spectrum of medical specialties, including paediatrics, neurology, and genetics. The clinically significant abnormality rate of 2.6% detected in this cohort is similar to that previously reported for routine cytogenetic and fragile X testing of probands with unexplained developmental disabilities.[4 12] Thus, subtelomere testing is a vital diagnostic tool for individuals with unexplained mental retardation or developmental delay. In addition, as 46% of the clinically significant subtelomere alterations identified in this study were inherited from a parent carrying the balanced form of the rearrangement, these findings have obvious implications for recurrence risk estimates and genetic counselling.

Previous studies examining the incidence of subtelomeric rearrangements have reported a frequency of 2–29%.[3 13] The largest study published to date, on individuals with normal routine chromosome analysis, showed a detection rate of 7.4% in individuals with moderate to severe mental retardation and 0.5% in individuals with mild mental retardation.[4] In the present study, the inclusion of all patients referred to a clinical cytogenetics laboratory for subtelomere testing, regardless of the severity of mental retardation or presence of dysmorphic features, may explain the lower frequency of abnormalities. Additionally, as subtelomere imbalances are identified in individuals with only developmental delay, mild mental retardation, and/or mild dysmorphic features, the

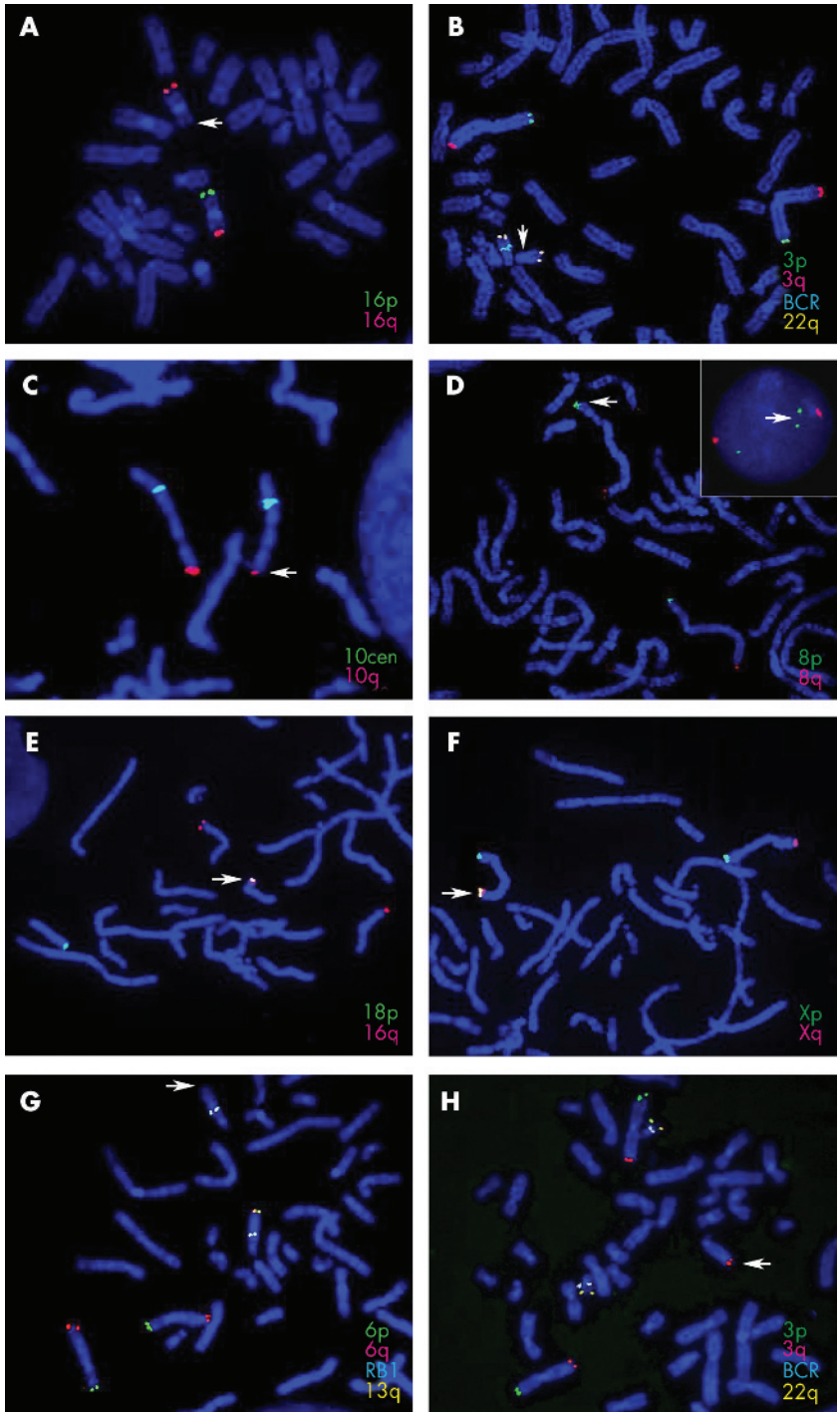

Figure 1 Representative FISH images of the various subtelomere rearrangements identified in this study. Arrows mark the abnormal chromosome. The probes used are listed for each image in the colour that corresponds to the colour of their hybridisation signal. (A) 16p subtelomere deletion; (B) deletion of the *BCR* control probe on chromosome 22q; (C) partial deletion of the 10q subtelomere; (D) duplication of the subtelomeric region of 8p shown on metaphase chromosomes and in an interphase nucleus (inset); (E) translocation of the 16q subtelomere probe to the short arm of chromosome 18, distal to the 18p subtelomere probe; (F) additional signal for Xp/Yp located on Xq adjacent to the X/Yq subtelomere probe signal; (G, H) unbalanced translocation between the subtelomeric regions of 3q and 13q resulting in monosomy for 13q (G) and trisomy for 3q (H).

population of individuals being tested could also be expanding, thus leading to an overall lower frequency. However, by using samples that were analysed as part of routine clinical care and not specifically solicited according to strict clinical criteria, this study better represents the incidence in the general delayed patient population.

A wide range of abnormalities, including deletions, duplications, and unbalanced translocations were observed in this study. One of the most important categories is the unbalanced translocations that involve a subtelomere region and the short arm of an acrocentric chromosome. These cases demonstrate that apparent acrocentric short arm polymorphisms by G banding analysis can actually represent clinically significant genomic imbalances, underlining the importance of using subtelomere FISH for individuals with an abnormal clinical phenotype and a normal karyotype.

Deletions were observed much more frequently than duplications in this study, as was found in previous reports.[4 14] Duplications are difficult to identify using metaphase FISH analysis. Therefore, the incidence of subtelomeric duplications may be higher, but is underrepresented by current testing methods. As more studies are performed using newer technologies, such as array based comparative genomic hybridisation or quantitative DNA based assays, which are better suited to detect duplications, the true frequency of these abnormalities will be revealed.

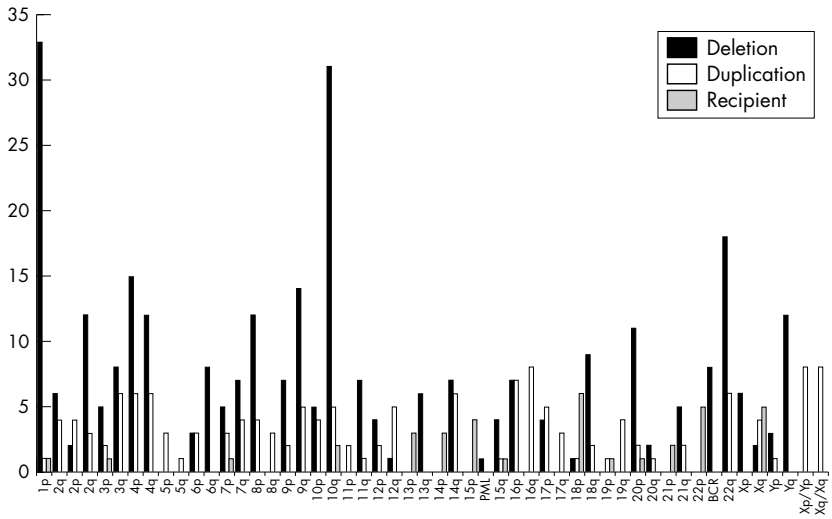

Figure 2 Number of subtelomeric rearrangements found involving each chromosome region. Solid bars, rearrangements in which the subtelomere region was monosomic; white bars, rearrangements in which the subtelomere region was trisomic; grey bars, rearrangements in which the subtelomere region targeted by the probe was intact but an additional subtelomere region was present distally. *PML* and *BCR* deletions represent interstitial deletions detected by the absence of the control probe signal for chromosomes 15 and 22, respectively.

In this study, 1p and 22q subtelomere deletions were the most frequent clinically significant imbalances observed. Both of these chromosomal regions are difficult to analyse using routine G banding analysis, which could contribute to this finding. The increased frequency of these deletions suggests there may be an underlying genomic mechanism contributing to their recurrence. To date, no common mechanism that accounts for these recurring deletions has been identified.

One of the difficulties in interpreting results from subtelomere testing is determining the clinical significance of the findings. Once an abnormality is identified, additional familial studies (preferably performed in the same laboratory that first identified the imbalance) are critical for accurately interpreting the results for the proband and for estimating recurrence risks for other family members. In this study, parental analysis was not available for many of the abnormalities identified, in spite of strong recommendations by the laboratories. Therefore, some of the deletions or derivative chromosomes with additional material from another subtelomeric region that were categorised as clinically significant may actually be familial variants.

Table 3 Subtelomeric rearrangements classified as variants

Abnormality	Familial variant*	Possible variant†	Case number(s)‡
Deletion: terminal			
del(3)(pter)	1		39
del(4)(qter)	2		60–61
del(10)(qter)	1		101
del(17)(pter)	1		127
del(20)(pter)	1		133
del(21)(qter)	2		144–145
del(Y)(qter)	2	8	166–175
Deletion: partial			
dim(4)(pter)	1		176
dim(10)(qter)	6	8	177–190
dim(14)(qter)	1	2	191–193
dim(Y)(pter)	1		194
Duplication			
dup(8)(pter)	1		205
dup(10)(qter)		2	206–207
Derivative: duplication only			
der(3)t(3;14)(pter;qter)	1		209
der(18)t(16;18)(qter;pter)	2	3	214–218
der(18)t(17;18)(pter;pter)	1		219
der(20)t(X or Y;20)(qter;pter)	1		222
der(X)t(X;X or Y)(qter;pter)	4	1	223–227
Derivative: duplication on acrocentric short arm			
der(22)t(4;22)(pter;pter)	2	1	241–243

*Abnormalities inherited from a phenotypically normal parent who carries the same rearrangement; †abnormalities that appear to be the same as familial variants but for which parental studies were not available; ‡the case number corresponds to the cases listed in table 1.

Partial deletions of the 10q subtelomere region were commonly observed in this study. Owing to the high frequency of this observation, a partial deletion of the 10q subtelomere clone is most likely a common polymorphism, much like the common polymorphism previously described for the 2q telomere region.[8] However, parental analysis is still recommended for these cases until more accurate genotype/phenotype correlations can be defined. As partial deletions or duplications of the 10q subtelomere clone were observed frequently in this study and have also been encountered in other studies,[7 9 15] one commercially available assay used in this study (ToTelVysion) was modified to avoid detection of this polymorphism.

The variants that have been reported in studies using subtelomere probes are interesting. Historically, most cytogenetic rearrangements that are identified in an affected proband and subsequently in an unaffected parent are deemed probably unrelated to the clinical findings in the proband. The same holds true for our current understanding of subtelomere rearrangements. In addition, with the advent of new technologies to examine copy number changes across the entire genome, several reports have now documented the existence of genome wide copy number polymorphisms (CNPs).[16–18] A comparison of the subtelomere clones with variants identified in this study against a database of previously reported CNPs (http://projects.tcag.ca/variation/) revealed that deletions of 4q,[9 17] 10q,[9 15] and 14q,[17] and duplications of 10q[9 15] have been previously observed. However, the possibility still exists that the difference in phenotypic expression between the parent and the affected child could be due to subtle differences in the rearrangement, modifier genes, genes present on the normal homologue, epigenetic effects or other, not yet described phenomena.

In order to gain accurate knowledge about the consequences of specific subtelomere rearrangements, additional mapping studies for each subtelomere region are necessary to define the size of a deletion or duplication that has a phenotypic effect compared with those that are tolerated without clinical effects. This mapping information, together

with the phenotypic observations, will allow the development of genotype/phenotype correlations to aid in the diagnosis, prognosis, and clinical management of individuals with subtelomere rearrangements. In the near future, through the use of DNA based methods for detecting genomic imbalances, such as array CGH, in conjunction with expanded clone coverage for the subtelomere regions, these determinations will be available as a more efficient and comprehensive diagnostic test.

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Authors' affiliations

J B Ravnan, A N Lamb, Genzyme Genetics, Santa Fe, New Mexico, USA
J H Tepperberg, P Papenhausen, Laboratory Corporation of America, Research Triangle Park, North Carolina, USA
D H Ledbetter, C L Martin, Emory University, Department of Human Genetics, Atlanta, GA, USA
J Hedrick, D Eash, C L Martin, University of Chicago, Department of Human Genetics, Chicago, IL, USA

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The first two authors contributed equally to this work.

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