15 Donnenfeld AE, Graham JM Jr, Packer RJ, Aquino R, Berg SZ, Emanuel BS. Microphthalmia and chorioretinal lesions in a girl with an Xp22.2-pter deletion and partial 3p trisomy: clinical observations relevant to Aicardi syndrome gene localization. *Am J Med Genet* 1990;**37**:182-6.
16 Gericke GS, Myburgh E, Bester R, van Rensberg EJ, Neethling E. Further delineation of the Xp22.2-pter syndrome of linear skin lesions, microphthalmia and anterior chamber eye anomalies. *Am J Hum Genet Suppl* 1991;**49**:A271.
17 Lindor NM, Michels VV, Hoppe DA, Driscoll DJ, Leavitt JA, Dewald GW. Xp22.3 microdeletion syndrome with microphthalmia, sclerocornea, linear skin defects, and congenital heart defects. *Am J Med Genet* 1992;**44**:61-5.
18 Kulharya AS, Roop H, Kukolich MK, Nachtman RG, Belmont JW, Garcia-Heras J. Mild phenotypic effects of a de novo deletion Xpter-Xp22.3 and duplication 3pter-3p23. *Am J Med Genet* 1995;**56**:16-21.
19 Wapenaar MC, Schiaffino MV, Bassi MT, Schaefer L, Chinault AC, Zoghbi HY, Ballabio A. A YAC-based binning strategy facilitating the rapid assembly of cosmid contigs: 1.6 Mb of overlapping cosmids in Xp22. *Hum Mol Genet* 1994;**3**:1155-61.
20 Wapenaar MC, Bassi MT, Schaefer L, Grillo A, Ferrero GB, Chinault AC, Ballabio A, Zoghbi HY. The genes for X-linked ocular albinism (OA1) and microphthalmia with linear skin defects (MLS). Cloning and characterization of the critical regions. *Hum Mol Genet* 1993;**2**:947-52.
21 Cox TC, Cox LL, Ballabio A. A very high density microsatellite map (1 STR/41 kb) in 1.7 Mb on Xp22 spanning the microphthalmia with linear skin defects (MLS) syndrome critical region. *Eur J Hum Genet* 1998;**6**:406-12.
22 Quaderi NA, Schweiger S, Gaudenz K, Franco B, Rugarli EI, Berger W, Feldman GJ, Volta M, Andolfi G, Gilgenkrantz S, Marion RW, Hennekam RC, Opitz JM, Muenke M, Ropers HH, Ballabio A. Opitz G/BBB syndrome, a defect of midline development, is due to mutations in a new RING finger gene on Xp22. *Nat Genet* 1997;**17**:285-91.
23 Allen LR, Suthers GK, Goodwin B, Cox LL, Haan E, Cox TC. Characterisation of seven novel mutations in MID1 provides support for "loss-of-function" as the cause of X-linked Opitz syndrome. (In preparation.)
24 Schaefer L, Ballabio A, Zoghbi HY. Cloning and characterization of a putative human holocytochrome *c*-type synthetase gene (HCCS) isolated from the critical region for microphthalmia with linear skin defects (MLS). *Genomics* 1996;**34**:166-72.
25 Schaefer L, Prakash S, Zoghbi HY. Cloning and characterization of a novel rho-type GTPase-activating protein gene (ARHGAP6) from the critical region for microphthalmia with linear skin defects. *Genomics* 1997;**46**:268-77.
26 Prakash SK, Paylor R, Jenna S, Lamarche-Vane N, Armstrong DL, Xu B, Mancini MA. Zoghbi HY. Functional analysis of ARHGAP6, a novel GTPase activating protein for RhoA. *Hum Mol Genet* 2000;**9**:477-88.

J Med Genet
2001;**38**:417–420

Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy
E Rossi
F Piccini
C Danesino
O Zuffardi

Istituto di Genetica Medica, Università Cattolica, Roma, Italy
M Zollino
G Neri

IRCCS, Policlinico San Matteo, Pavia, Italy
D Caselli

Dipartimento di Pediatria, Università di Padova, Padova, Italy
R Tenconi

Servizio di Consulenza Genetica, Ospedale di Bolzano, Bolzano, Italy
C Castellan

Servizio di Genetica Clinica, Ospedale San Raffaele, Milano, Italy
R Carrozzo

Laboratorio di Patologia Genetica and UO di Pediatria, IRCCS, Oasi Maria SS, Troina, Italy
A Ragusa
L Castiglia
O Galesi
D Greco
C Romano

Laboratorio di Genetica Umana, Ospedale Galliera, Genova, Italy
M Pierluigi
C Perfumo
F Faravelli
F Dagna Bricarelli

II Divisione di Pediatria, Istituto G Gaslini, Genova, Italy
M Di Rocco

IRCCS E Medea, Bosisio Parini, Italy
M Bonaglia
M Bedeschi
R Borgatti

Correspondence to:
Dr Rossi, rossie@unipv.it

Cryptic telomeric rearrangements in subjects with mental retardation associated with dysmorphism and congenital malformations

Elena Rossi, Flavia Piccini, Marcella Zollino, Giovanni Neri, Desirée Caselli, Romano Tenconi, Claudio Castellan, Romeo Carrozzo, Cesare Danesino, Orsetta Zuffardi, Angela Ragusa, Lucia Castiglia, Ornella Galesi, Donatella Greco, Corrado Romano, Mauro Pierluigi, Chiara Perfumo, Maia Di Rocco, Francesca Faravelli, Franca Dagna Bricarelli, MariaClara Bonaglia, MariaFrancesca Bedeschi, Renato Borgatti

EDITOR—Cryptic telomeric rearrangements are a significant cause of idiopathic mental retardation. Knight *et al*[1] found 7.4% of these rearrangements in children with moderate to severe mental retardation. Clinical selection criteria for testing patients with subtelomeric chromosome specific probes are still not clear cut and the importance of other surveys to define this point has been stressed.[2] With this aim, we examined 200 patients with idiopathic mental retardation, either isolated or associated with dysmorphism and/or congenital anomalies, using FISH analysis with subtelomeric chromosome specific probes.

Material and methods

The sample was collected in four Italian Genetic Centres. Patients were selected on the basis of the following criteria: (1) the presence of mental retardation that was classified as mild, moderate, or severe according to DSM IV[3]; (2) patients under 1 year of age, too young for psychological assessment, were selected for the presence of developmental delay; (3) exclusion of pre- or perinatal distress through an accurate evaluation of the pre-, peri-, and postnatal patient history; and (4) exclusion of Mendelian syndromes and of genomic disorders[4 5] for which a specific diagnostic test is available.

The essential elements of evaluation also included family history, a complete physical and neurological examination of the patients with particular attention to the presence of mental retardation and multiple congenital anomalies, and assessment of the behavioural phenotype. Electroencephalograms, brain CT scan, and MRI were performed in specific situations.

Abnormal methylation and expansion at FRAXA and FRAXE[6] were excluded in 52 and 50 males and in 37 and 32 females, respectively.

Routine cytogenetic analysis at the 400-550 band level was performed in all the patients. In those patients in whom a cryptic subtelomeric rearrangement was identified by FISH, prometaphase chromosomes were also analysed to determine if the rearrangement could be detected in retrospect by cytogenetic analysis. Chromosome preparations from peripheral blood or from lymphoblastoid cell lines were used for FISH analysis. The Chromoprobe-T kit with telomere specific clones[7] was used according to the supplier's instructions (Cytocell, UK) with minor modifications. To establish the origin of each rearrangement, FISH and microsatellite analysis with subtelomeric probes were performed in the parents of the patients. FISH experiments with different YACs from each rearranged chromosomal region were performed to define its size.

Results

Among our 200 patients (table 1), 44 had mild mental retardation (IQ 50-70), 62 were moderately retarded (IQ 50-35), and 55 were severely retarded (IQ 35-20). A total of 39 patients had mental retardation not otherwise specified.

Table 1 Classification of the patients according to degree of mental retardation, familial occurrence, and association with malformations/dysmorphism

Mental retardation	*Mild*	*Moderate*	*Severe*	*Unknown**	*Total*
Number	44	62	55	39	200
Familial	15	15	12	11	53
Sporadic	29	47	43	28	147
Isolated	13	8	2		23
Associated	28	53	52	27	160
Unknown	3	1	1	12	17

*Unspecified degree of mental retardation or children below testing age.

Table 2 Prevalence of cryptic telomeric rearrangements in 200 mentally retarded patients

Mental retardation	*Mild*	*Moderate*	*Severe/ profound*	*Unknown*	*Total*
	44	62	55	39	200
Translocations	0	3	2	1	6
Deletions	0	4	3		7
Total	0	7 (11.2%)	5 (9%)	1	13

Mental retardation was familial in 53 and sporadic in 147 subjects; it was isolated in 23 subjects, associated with dysmorphism and/or congenital anomalies in 160 subjects, and unknown in 17.

We identified 13 rearrangements (table 2). Seven rearrangements were de novo deletions. Six rearrangements were derivative chromosomes inherited by a balanced parent in the five cases where the parents were available. Twelve rearrangements were present in patients with moderate (7/62, 11.2%) or severe (5/55, 9%) mental retardation, whereas one rearrangement was found in a 3 month old patient, too young to assess the degree of his psychomotor retardation. No rearrangements were found among the 44 patients with mild mental retardation.

In all the 13 subjects with rearrangements, mental retardation was associated with dysmorphism and, excluding case 12, with at least one serious congenital anomaly (table 3). Cases 1-6 have unbalanced translocations. In the two families (cases 1 and 2) in which we could examine other relatives, we found that the translocation was present in several members, both in a balanced and unbalanced state. In family 1, in which an X linked dominant trait had been suspected,[8] the two translocations' derivatives were present in subjects with different phenotypes and different degrees of mental retardation, whereas in family 2 only one derivative was identified. In this family, the proband's mother received amniocentesis at 19 weeks' gestation because of fetoplacental hydrops. The presence of two paternal uncles with severe mental retardation and multiple congenital anomalies but with a normal karyotype made counselling difficult. After normal cytogenetic results the pregnancy continued. At birth the child showed congenital chylothorax, facial dysmorphism, and bone malformations prompting the counsellor to request chromosome specific telomere testing. The test was then extended to the mentally retarded uncles, who showed the same unbalanced translocation. Also in family 3, the mother received amniocentesis at 18 weeks' gestation after the detection of omphalocele. The cytogenetic results were normal and the pregnancy continued. A previous pregnancy had resulted in a child who died immediately after birth. At necropsy, an atrial septal defect and accessory spleen were recorded. Since accessory spleen has been reported in association with 9p duplication,[9] one could speculate that this child was also unbalanced for the maternal translocation, having the der(13) instead of the der(9) found in the proband. In family 4, a paternal cousin was referred with mental retardation. Cases 7-13 have deletions and all of them are de novo. The origin of the deletion was paternal in cases 7 and 11 and maternal in case 9.

We identified by FISH and PCR analysis the breakpoints in most of the rearrangements. The size of the rearrangements ranged from less than 2 cM to 31 cM (table 4). Some of the rearrangements involving large chromosome regions have not been detected cytogenetically because the translocated region has the same size and overlapping banding pattern of the

Table 3 Ascertainment in the 13 cases with chromosome rearrangements

Case	*Age at request of karyotype/ telomere test*	*Phenotype + family history*
1 der(1q)t(1q;12p)mat	8 y/16 y	Profound MR, pachygyria, seizures, facial dysmorphism, scoliosis, toe syndactyly A cousin with the same phenotype, a sister and another cousin with moderate MR, hypoplastic supraorbital ridges, large mouth, crowding of toes, and talipes equinovarus
2 der(6q)t(6q;19p)pat	Prenat/1 y	Fetoplacental hydrops, MR, chylothorax, absence of sacral vertebral fusion Two uncles with severe MR, facial dysmorphism, short stature
3 der(9p)t(9p;13q)mat	Prenat/1 mth	Omphalocele, trigonocephaly, C syndrome, motor delay Brother dead neonatally with cardiopathy and accessory spleen
4 der(16q)t(16q;19p)pat	3 y/11 y	Moderate MR, facial dysmorphism, precocious puberty, short stature, hypernatraemia, behavioural disturbances
5 der(5p)t(4p;5p)mat	2 y/15 y	Moderate MR, triangular face, gingival hypertrophy, prominent incisors, ogival palate, posteriorly angulated ears, behavioural disturbances
6 der(10q)t(10q;16p)	1 y/2 y	Moderate MR, hypoplasic penis, facial dysmorphism
7 del(2q)de novo	5 y/7 y	Severe MR, short stature, brachymetaphalangism, cone shaped epiphyses
Paternal origin		
8 del(17q)de novo	15 y/15 y	Moderate MR, cardiopathy, extreme thinness
9 del(6q)de novo	8 y/14 y	Moderate MR, hypotonia, skeletal anomalies, epilepsy
Maternal origin		
10 del(3q)de novo	2 mth/1 y	Moderate MR, facial dysmorphism, horseshoe kidney, hypospadias
11 del(1q)de novo	7 mth/7 mth	Severe MR, corpus callosum agenesis, cardiopathy, short stature
Paternal origin		
12 del(9q)de novo	2 y/8 y	Moderate MR, facial dysmorphism
13 del(11p)de novo	6 y/6 y	Severe MR, epilepsy, West syndrome, metabolic acidosis, microcephaly, ogival palate, simplified ears, thick lips, micrognathia

Table 4 Extent of chromosomal rearrangements

Patients	*Abnormality*	*Size of monosomy**	*Size of trisomy**
1	1q monosomy 12p trisomy	14 cM (bp: 962B9)	15 cM (bp: 780D6)
2	6q monosomy 19p trisomy	0 cM (bp: 770E8)	>11 cM (702E11−; 872G3+)
3	9p monosomy 13q trisomy	27 cM (762D7−; 830F3+)	31 cM (bp: 794G5)
4	16q monosomy 19p trisomy	4 cM (792E1+)	<11 cM (702E11+)
5	5p monosomy 4p trisomy	18 cM (bp: 941A2)	25 cM (bp: 776G9)
6	10q monosomy 16q trisomy	16.7 cM (943C4+; 745C10−)	12 cM (bp: 738H1)
7	2q monosomy	12 cM (D2S336+; D2S338−)	
8	17q monosomy	<14 cM (659G5+)	
9	6q monosomy	>20 cM (D6S1581+; D6S305−)	
10	3q monosomy	<2 cM (768C10+)	
11	1q monosomy	<12 cM (bp: 835D8)	
12	9q monosomy	<7 cM (951B12+)	
13	11p monosomy	<3 cM (892G9+; D11S4046+)	

*In brackets are the YACs or the microsatellites flanking the breakpoint or at the breakpoint (bp).

original region. In case 5, when we reanalysed the chromosomes with high resolution banding to verify if the rearrangement could be detected in retrospect, we discovered that the abnormal chromosome 5 appeared to have a longer short arm. In the mother, the translocation was also evident at the 400-550 band level owing to the small size of the der(4). Three rearrangements (cases 1, 5, and 7) involve chromosomal regions whose monosomy is associated with specific syndromes. In case 1, the 9p deletion spans 27 cM. The main anomalies found in the patient at birth were omphalocele, trigonocephaly, and genitourinary abnormalities (right cryptorchidism with micropenis), all of them reported in 9p22 deletions.[10–13] However, based on London Dysmorphology Database suggestions,[14] C syndrome (trigonocephaly, metopic synostosis, strabismus, cryptorchidism) was suspected. These data indicate that some cases diagnosed as C syndrome could have chromosome 9p microdeletion as already hypothesised.[15] We cannot, however, exclude that the trigonocephaly is the result of the 13q duplication since it has been reported in at least three patients with 13q22-qter duplication.[15–18] In case 4, the 18 cM 5p deletion covers the region of the characteristic cat-like cry (which has not been reported by the mother) but not that of the distinct facial features associated with the cri du chat syndrome.[19] In case 7, the Albright-like phenotype associated with distal 2q deletion[20] was in fact present. The breakpoint of the deletion spans the polymorphic markers D2S2253 and D2S338, placing between KW and RA cases of AHO-like syndrome reported by Wilson *et al.*[20] We recently narrowed the AHO-like critical region to 10 cM in the subterminal distal 2q (from D2S338 to D2S2253).[21]

In conclusion, we show that cryptic unbalanced translocations and deletions are the cause of mental retardation and phenotypic anomalies in 13 out of 200 subjects without a previous diagnosis. In five of the cases, the finding of a balanced translocation in one parent makes it possible to monitor future pregnancies for the presence/absence of chromosome anomaly. For the seven de novo deletion cases, although the risk of recurrence is low, prenatal diagnosis of future pregnancies can monitor for the presence/absence of the deletion in case of parental germinal mosaicism.

The finding that cases 2 and 3 were cytogenetically investigated prenatally after the finding of echographic abnormalities and a suspicion of chromosome anomalies suggests extending the telomere test to these situations.

Discussion

The results of our study largely overlap those obtained by Knight *et al.*[1] Putting together the two sets of data one can conclude that 6.5-7.4% of the children with moderate/severe mental retardation have a cryptic telomeric rearrangement, whereas the probability of finding these anomalies among subjects with mild mental retardation is less than 0.4% (1 out 182 children in the English sample, 0 out 44 in the Italian one). In both studies half of the rearrangements are de novo deletions and half are unbalanced translocations inherited in most of the cases. In six out of the 15 families with cryptic translocations, both subjects with one derivative chromosome and subjects with the other one were present. In families with more members affected by mental retardation, differences in the phenotypes associated with the two derivatives should help the clinician to encourage diagnostic laboratories to search for a chromosome anomaly. Knight *et al*[1] concluded that abnormalities that include the ends of chromosomes are the commonest cause of mental retardation in children with undiagnosed moderate to severe mental retardation. In fact, in our patients, subtelomeric rearrangements were always associated not only with moderate or severe mental retardation but also with dysmorphic features and congenital anomalies. Therefore, our conclusion is that once a well defined aetiological cause has been excluded, screening with subtelomeric probes should be applied in subjects with a "chromosomal phenotype". In fact, as stressed by Knight and Flint[2] in their review of subtelomeric probes and their use in clinical diagnosis, all telomere positive cases reported to date have had physical anomalies in addition to mental retardation. Recently, a whole genome microsatellite screening[22] has led to identification of two cases of interstitial aneusomy among 11 children (18%) with multiple congenital anomalies and mental retardation, normal for subtelomeric chromosome analysis. This indicates that among our 147 subjects with mental retardation associated with an abnormal phenotype and with a normal subtelomeric test, roughly 26 of them should have an interstitial rearrangement.

The detection of cryptic telomeric rearrangements through FISH with subtelomeric probes raised the question of whether most of these rearrangements could have been detectable by appropriate high resolution banding,[23] thus saving time and money. The same question had been raised when FISH was introduced to detect the 15q11-q13 deletion in Prader-Willi/Angelman syndrome. The demonstration that both false negative and false positive results occur with high resolution banding[24–26] led to the conclusion that this technique is insufficient for deletion detection.[27] The same

can be extended to telomeric or interstitial rearrangements falling into a size critical for their detection even on high quality chromosome preparations. The setting up of appropriate microarray tests will probably bypass this problem. For the moment, FISH telomere analysis is an excellent support to the clinician faced with a malformed, mentally retarded subject in whom known syndromes have been excluded.

This work was supported by grant ICS.1,1/RF98/81 from the Italian Health Ministry. MP, CP, FF, and FDB were also supported by the CARIGE foundation. DC was supported by grant 390RCR 99/11, IRCCS Policlinico San Matteo, Pavia. FP is a Rusconi Foundation fellow. ER and OZ were also supported by cofin98-MURST. Part of the cell lines have been stored thanks to Telethon projects C.18 and C.42.

1 Knight SJL, Regan R, Nicod A, Horsley SW, Kearney L, Homfray T, Winter RM, Bolton P, Flint J. Subtle chromosomal rearrangements in children with unexplained mental retardation. *Lancet* 1999;**354**:1676-81.
2 Knight SJ, Flint J. Perfect endings: a review of subtelomeric probes and their use in clinical diagnosis. *J Med Genet* 2000;**37**:401-9.
3 American Psychiatric Association. DMS-IV. *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington DC: The American Psychiatric Association, 1994.
4 Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet* 1998;**14**:417-22.
5 Ji Y, Eichler EE, Schwartz S, Nicholls RD. Structure of chromosomal duplicons and their role in mediating human genomic disorders. *Genome Res* 2000;**10**:597-10.
6 Murray A, Youings S, Dennis N, Latsky L, Linehan P, McKechnie N, Macpherson J, Pound M, Jacobs P. Population screening at the FRAXA and FRAXE loci: molecular analyses of boys with learning difficulties and their mothers. *Hum Mol Genet* 1996;**5**:727-35.
7 Knight SJL, Horsley SW, Regan R, Lawrie NM, Maher EJ, Cardy DL, Flint J, Kearney L. Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. *Eur J Hum Genet* 1997;**5**:1-8
8 Zollino M, Mastroiacovo P, Zampino G, Mariotti P, Neri G. New XLMR syndrome with characteristic face, hypogenitalism, congenital hypotonia and pachygyria. *Am J Med Genet* 1992;**43**:452-7.
9 el-Fouly MH, Higgins JV, Kapur S, Sankey BJ, Matisoff DN, Costa-Fox M. DiGeorge anomaly in an infant with deletion of chromosome 22 and dup(9p) due to adjacent type II disjunction. *Am J Med Genet* 1991;**38**:569-73.
10 Young RS, Bader P, Palmer CG, Kaler SG, Hodes ME. Brief clinical report: two children with de novo del(9p). *Am J Med Genet* 1983;**14**:751-7.
11 Chaves-Carballo E, Frank LM, Rary J, Reda AW, Shoaibi A. Neurologic aspects of the 9p- syndrome. *Pediatr Neurol* 1985;**1**:57-9.
12 Nagy E, Bod M, Nemeth I, Timar L. A new case with de novo partial 9p monosomy. *Acta Paediatr Hung* 1991;**31**:407-13.
13 Raymond CS, Parker ED, Kettlewell JR, Brown LG, Page DC, Kusz K, Jaruzelska J, Reinberg Y, Flejter WL, Bardwell VJ, Hirsch B, Zarkower D. A region of human chromosome 9p required for testis development contains two genes related to known sexual regulators. *Hum Mol Genet* 1999;**8**:989-96.
14 Winter RM, Baraitser M, eds. *London dysmorphology database* and *London neurogenetic database*. The Oxford Medical Databases. Oxford: Oxford University Press, 2000.
15 Haaf T, Hofmann R, Schmid M. Opitz trigonocephaly syndrome. *Am J Med Genet* 1991;**40**:444-6.
16 Jotterand M, Juillard E. A new case of trisomy for the distal part of 13q due to maternal translocation, t(9;13)(p21;q21). *Hum Genet* 1976;**33**:213-22.
17 Giraud F, Mattei JF, Mattei MG. Partial trisomy 13 due to maternal translocation t(2;13). *Ann Genet* 1977;**20**:203-8.
18 Chu TW, Teebi AS, Gibson L, Breg WR, Yang-Feng TL. FISH diagnosis of partial trisomy 13 and tetrasomy 13 in a patient with severe trigonocephaly C phenotype. *Am J Med Genet* 1994;**52**:92-6.
19 Gersh M, Grady D, Rojas K, Lovett M, Moyzis R, Overhauser J. Development of diagnostic tools for the analysis of 5p deletions using interphase FISH. *Cytogenet Cell Genet* 1997;**77**:246-51.
20 Wilson LC, Leverton K, Oude Luttikhuis MEM, Oley CA, Flint J, Wolstenholme J, Duckett DP, Barrow MA, Leonard JV, Read AP, Trembath RC. Brachydactyly and mental retardation: an Albright hereditary osteodystrophy-like syndrome localized to 2q37. *Am J Hum Genet* 1995;**56**:400-7.
21 Bonaglia MC, Giorda R, Gerardina Poggi G, Raggi ME, Baroncini A, Rossi E, Giglio S, Borgatti R, Zuffardi O. Inverted duplications are recurrent rearrangements always associated with a distal deletion: description of a new case involving 2q. *Eur J Hum Genet* (in press).
22 Rosenberg MJ, Vaske D, Killoran CE, Ning Y, Wargowski D, Hudgins L, Tifft CJ, Meck J, Blancato JK, Rosenbaum K, Pauli RM, Weber J, Biesecker LG. Detection of chromosomal aberrations by a whole-genome microsatellite screen. *Am J Hum Genet* 2000;**66**:419-27.
23 Browne C, Moore KJ, Dennis NR, Joice CA. High resolution chromosome analysis: a more cost effective approach? *J Med Genet* 1999;**36**:S50.
24 Kuwano A, Mutirangura A, Dittrich B, Buiting K, Horsthemke B, Saitoh S, Niikawa N, Ledbetter SA, Greenberg F, Chinault AC, Ledbetter DH. Molecular dissection of the Prader-Willi/Angelman syndrome region (15q11-13) by YAC cloning and FISH analysis. *Hum Mol Genet* 1992;**1**:417-25.
25 Delach JA, Rosengren SS, Kaplan L, Greenstein RM, Cassidy SB, Benn PA. Comparison of high resolution chromosome banding and fluorescence in situ hybridization (FISH) for the laboratory evaluation of Prader-Willi syndrome and Angelman syndrome. *Am J Med Genet* 1994;**52**:85-91.
26 Butler MG. High resolution chromosome analysis and fluorescence in situ hybridization in patients referred for Prader-Willi or Angelman syndrome. *Am J Med Genet* 1995;**56**:420-2.
27 ASHG/ACMG Report. Diagnostic testing for Prader-Willi and Angelman syndrome: report of the ASHG/ACMG test and technology transfer committee. *Am J Hum Genet* 1996;**58**:1085-88.

Laryngeal atresia, encephalocele, and limb deformities (LEL): a possible new syndrome

Karim D Kalache, Bianca Masturzo, Rosemary J Scott, Charles H Rodeck, Lyn S Chitty

EDITOR—In 1987, Machin *et al*[1] reported a case of laryngeal atresia in association with an asymmetrical parietal encephalocele and limbs anomalies. Other anomalies included a horseshoe kidney and immature, low set ears. We now report a similar case and discuss possible aetiologies and the differential diagnosis.

Case report

This was the second pregnancy of a non-consanguineous, healthy couple with an unremarkable family history; the mother was 26 years old. Ultrasonoraphic findings at 23 weeks' gestation were gross fetal ascites and enlarged echogenic lungs subsequently found to be the result of laryngeal atresia (fig 1). There was a large facial cleft with an anterior encephalocele involving the left orbit (fig 1). There were also flexion deformities at the wrists and hips suggesting bilateral radial and tibial aplasia, respectively (fig 1). In view of the serious nature of the anomalies, the parents requested a termination of the pregnancy. Asystole was induced by intracardiac injection of potassium chloride and labour was induced. Analysis of fetal blood showed a normal karyotype (46,XX).